

AGENCY FOR INTERNATIONAL DEVELOPMENT

RESEARCH ADVISORY COMMITTEE

SEVENTY-THIRD MEETING

NOVEMBER 17-18, 1981

PAN AMERICAN ORGANIZATION BUILDING

CONFERENCE ROOM 'C'

A.I.D. Research Advisory Committee
 Seventy-Third Meeting
 November 17-18, 1981

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A.I.D. Research Advisory Committee

Seventy-Third Meeting

November 17-18, 1981

Pan American Health Organization Building*

Conference Room."C"

AGENDA

November 17

TAB

- 9:00 a.m. Opening Remarks Dr. Ralph Smuckler
(Chairman, RAC)
- Dr. Miloslav Rechcigl
AID Representative, RAC
- 9:10 a.m. Remarks by the Dr. Nyle Brady
Senior Assistant Administrator for SAA/S&T
Science and Technology
- 10:15 a.m. Morning Coffee
- 10:30 a.m. Report on Science Dr. Howard Minners U
Advisor's Program AID/SCI
- 11:00 a.m. Proposal for RAC Review
- Dr. J. P. Carter(Ch) N
Dr. C. C. Black
Dr. B. S. Schweigert
Dr. T. H. Weller
- Sporozoite Induced Immunity in
Simian Malaria - Use of Hybridoma
for the Characterization of
Protective Sporozoite Antigens
- New York University
- Project Manager: Mr. Edgar Smith
S&T/HEA
- 12:15 p.m. Adjournment for Lunch

*Twenty-third Street and Virginia Avenue, N. W.

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1:15 p.m. Proposals for RAC Review

Studies on the Culture
of Malaria - Rockefeller
University

Dr. S. Wishik (Ch)
Dr. E. B. Connell
Dr. D. Pimentel
Dr. T. H. Weller

Project Manager: Mr. Edgar Smith
S&T/HEA

2:30 p.m. In-vitro Cell Culture
of the Exoerythrocytic
Stage of Malaria Para-
sites - Biomedical
Research Institute

Dr. D. Pimentel (Ch)
Dr. J. P. Carter
Dr. T. H. Weller
Dr. S. Wishik

Project Manager: Mr. Edgar Smith
S&T/HEA

3:00 p.m. Afternoon Coffee

3:15 p.m. Proposal for RAC Review

Ticks Immunology Research
- International Centre for
Insect Physiology and
Ecology (ICIPE)

Dr. D. Pimentel (Ch)
Dr. T. R. Greathouse
Dr. M. L. Peterson
Dr. T. H. Weller

Project Manager: Dr. Douglas W. Butchart
S&T/AGR

4:30 p.m. RAC Subcommittee Report

International Fertility
Research Program (IFRP)

Dr. E. B. Connell (Ch)
Dr. F. E. Linder
Dr. E. R. Swanson
Dr. S. Wishik

Project Manager: Dr. James Shelton
S&T/POP

5:30 p.m. Adjournment

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November 18

8:30 a.m. Opening Remarks

Dr. Ralph Smuckler
Chairman, RAC

8:45 a.m. Proposal for RAC Review

In-vitro Cultivation
of Human Malaria -
New York University

Dr. S. Wishik (Ch) Q
Dr. J. P. Carter
Dr. B. S. Schweigert
Dr. T. H. Weller

Project Manager: Mr. Edgar Smith
S&T/HEA

10:00 a.m. Morning Coffee

10:15 a.m. Proposal for RAC Review

Agricultural Development
in Senegal: Prospectives,
Risks and Production Strategies
- Princeton University

Dr. E. O. Heady (Ch) M
Dr. W. P. Falcon
Dr. J. D. Montgomery
Dr. E. P. Skinner

Project Manager: Mr. David Shear, USAID/Senegal
Washington Liaison: Ms. Frances Johnson
AFR/SWA

11:30 a.m. Terminal Evaluation of Project

The Development Potential
of Agricultural Settlement
in New Lands - Institute for
Development Anthropology

Dr. E. P. Skinner (Ch) L
Dr. C. A. Anderson
Dr. M. L. Elmendorf
Dr. J. H. Ehrenreich

Project Manager: Dr. Robert A. Simko
S&T/RAD

12:00 NOON Adjournment for Lunch

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- 1:00 a.m. Terminal Evaluation of Sorghum - Millet Research Projects by RAC Dr. M. L. Peterson (Ch)
Dr. C. C. Black
Dr. D. N. Moss
Dr. E. R. Swanson
Dr. B. S. Schweigert
- Inheritance and Improvement of Protein Quality and Content of Sorghum bicolor -Purdue University H
- Improvement of Grain Sorghum for Developing Countries - Texas A&M University I
- Development of Improved Sorghum Physiology of Yield and Stress Reaction - University of Nebraska J
- Agronomic and Nutritional Improvement of Pearl Millet for Semi-arid Areas - Kansas State University K
- Project Manager: Dr. Robert I. Jackson, S&T/AGR
- 3:00 p.m. Afternoon Coffee
- 3:15 p.m. Terminal Evaluation of Projects by RAC
- Energy Policy and Planning - Resources for the Future Dr. J. A. Hrones (Ch)
Dr. E. O. Heady
Dr. J. D. Montgomery
Dr. D. Pimentel R
- Project Manager: Dr. Pamela L. Baldwin S&T/EY
- 4:15 p.m. International Primary Commodity Markets and Economic Development - University of Pennsylvania Dr. E. Thorbecke (Ch)
Dr. W. P. Falcon
Dr. J. D. Montgomery
Dr. E. R. Swanson S
- Project Manager: Dr. T. Morrison PPC/EA
- 5:30 p.m. Adjournment

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Dr. Maurice L. PETERSON Dept. of Agronomy and Range Science Univ. of California Davis, California 95616 (916) 752-1705 (Office) (916) 756-0339 (Home)	Professor

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9/29/81

RAC Subcommittee Assignments

November 17-18, 1981

Food and Nutrition

Tick Immunology Research

Subcommittee

Dr. D. Pimentel
Dr. T. R. Greathouse
Dr. M. L. Peterson
Dr. T. H. Weller

Terminal Evaluation of Sorghum -
Millet Research Projects by RAC

Dr. M. L. Peterson (Ch)
Dr. C. C. Black
Dr. D. N. Moss
Dr. E. R. Swanson
Dr. B. S. Schweigert

The Development Potential of
Agricultural Settlement in
New Lands

Dr. E. P. Skinner (Ch)
Dr. C. A. Anderson
Dr. M. L. Elmendorf
Dr. J. H. Ehrenreich

Agricultural Development in
Senegal: Prospectives, Risks
and Production Strategies

Dr. E. O. Heady (Ch)
Dr. W. P. Falcon
Dr. J. D. Montgomery
Dr. E. P. Skinner

Health and Population

Sporozoite Induced Immunity in
Simian Malaria - Use of Hybridoma
for the Characterization of
Protective Sporozoite Antigens

Dr. J. P. Carter (Ch)
Dr. C. C. Black
Dr. B. S. Schweigert
Dr. T. H. Weller

Studies on the Culture of Malaria -

Dr. S. Wishik (Ch)
Dr. E. B. Connell
Dr. D. Pimentel
Dr. T. H. Weller

Health and Population (Cont'd)

In-vitro Cell Culture of the
Exoerythrocytic Stage of Malaria
Parasites -

In-vitro Cultivation of Human
Malaria

International Fertility Research
Program

Energy and Natural Resources

Energy Policy and Planning

Selected Development Problems

International Primary Commodity
Markets and Economic Development

Subcommittee

Dr. D. Pimentel
Dr. J. P. Carter
Dr. T. H. Weller
Dr. S. Wishik

Dr. S. Wishik (Ch)
Dr. J. P. Carter
Dr. B. S. Schweigert
Dr. T. H. Weller

Dr. E. B. Connell (Ch)
Dr. F. E. Linder
Dr. E. R. Swanson
Dr. S. Wishik

Dr. J. A. Hrones (Ch)
Dr. E. O. Heady
Dr. J. D. Montgomery
Dr. D. Pimentel

Dr. E. Thorbecke (Ch)
Dr. W. P. Falcon
Dr. J. D. Montgomery
Dr. E. R. Swanson

AGENCY FOR INTERNATIONAL DEVELOPMENT
RESEARCH ADVISORY COMMITTEE

Minutes of the Seventy-first Meeting
March 3-4, 1981

Dr. Smuckler opened the meeting at 9:00 a.m. He introduced Dr. Stephen Joseph, who is the Acting Assistant Administrator for the Development Support Bureau.

Dr. Joseph remarked that this was an interesting period in AID. There was, of course, a change of national administrations and this was creating some changes in AID. In addition a new Science Advisor had been appointed and he would be affecting some of the deliberations of the RAC.

Dr. Joseph indicated that he would sit in on the review of some of the projects as well as the discussion with the AID Administrator scheduled for later in the day.

Dr. Smuckler remarked that Dr. Wishik was writing a more comprehensive response to the Administrator following the discussion on research review and policy from the last meeting. He reminded the RAC members that he had sent to the previous Administrator an executive summary of the RAC's recommendations. The document being prepared by Dr. Wishik and now before them in draft form was a bigger document and provided more detail.

Dr. Smuckler then introduced Dr. Howard Minners, the new Science Advisor.

Dr. Minners told the RAC that there had been a number of changes since the last RAC meeting. His appointment had been completed and he was beginning to get his office established. The Office of the Science Advisor was a line item in the budget appropriations. He would be putting together a small staff as soon as he identified and recruited qualified people. Out of the budget for the Science Advisor he reminded the RAC that six million dollars would be going to the research project in NAS that the RAC had reviewed at its last meeting.

Dr. Minners indicated that the program for the Science Advisor's Office needs discussion inside and out. The emphasis would be on science and technology. He also wanted to move to stress career opportunities in science in LDCs. Beyond the

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strengthening of capability, he indicated that there would be a small program to provide opportunities for investigators to submit small innovative proposals in science and technology. He was having some problem determining how small research should be defined. In addition to his program, Dr. Minners was looking at several other matters. For example, he wanted to consider the question of peer review versus interdisciplinary review. There was some feeling within the Agency that changes could be made so that quality and relevance in the research process could be assured to a greater extent.

He remarked that during the preceeding day, he had met several members of the RAC who were in town for a subcommittee discussion. He went over some of the items in greater detail with those members.

Dr. Minners indicated that he welcomed suggestions from the RAC about the Science Advisor's programs, about peer review and other matters.

Dr. Montgomery suggested that it would be useful to have peer review for mission research largely by scientists from LDCs. He thought that scientists should take a broad view of research and that some kind of network of peer review for missions should be possible.

Dr. Greathouse felt that the review concept used in the CRSPS might be appropriate for all research. He gave as an illustration the small ruminants CRSP which was reviewed by people in the missions, by his co-workers, and by researchers on the site.

Discussion with the A.I.D. Administrator

Dr. Smuckler described the type of review carried out by the Research Advisory Committee, to the Administrator. He pointed out that RAC spends most of its time reviewing specific project proposals. The Committee has existed for many years and met regularly with previous A.I.D. Administrators Hannah and Parker. However, the Committee met on rare occasions with Administrators, Gilligan and Bennet.

Dr. Smuckler expressed appreciation on behalf of the RAC for this chance to get Mr. McPherson's views on matters concerning the RAC.

Dr. Smuckler explained that the RAC membership was drawn from all over the country and from many academic fields. He pointed out that the Committee included a number of medical doctors who were engaged in research, a number of social scientists, economists, political scientists, an anthropologist, several agriculturalist, an engineer, one specialist in animal science, education, and various other specialities.

Recently, the RAC had paid considerable attention to the question of research policy and the research review process. Generally, the Committee was considering how the RAC review could result in the Administrator having greater assurance of good research projects, how the results from these projects could be picked up quickly by field missions and how the projects could be made more relevant to the AID program overseas. All of these topics were addressed in the last meeting of the RAC and the Administrator would be hearing in the near future from the RAC concerning that discussion.

Dr. Smuckler then introduced the new Administrator to the RAC and pointed out that he was a graduate of Michigan State University, had completed law school and had served in the Peace Corps. Mr. McPherson was on the White House staff of President Ford, he later practiced law in the state of Washington, recently he was a part-time member of the BIFAD, and through that broad range of experience has learned much about development. Mr. McPherson was also on the transition team from the Carter Administration to the Reagan Administration.

Dr. Smuckler remarked that it was unusual for A.I.D. to have an Administrator with that range of experience.

Mr. McPherson mentioned a few thoughts that he had formulated on the A.I.D. research program in the few days that he had been the A.I.D. Administrator. He has dug through the program and talked to the A.I.D. staff about institution building and technology transfer. However, he felt that it was not possible to turn the A.I.D. program upside down. Obviously, the creation of new technology and new information is required in the adaptation of technology in LDCs. He expressed confidence that he knew quite well what the JRC and BIFAD should be doing but knew less about the RAC. He thought that the Agency should be taking a look at the CGIAR and the CRSP to see if each development dollar is being spent well. He expressed the opinion that it was not obvious that major technological breakthroughs have been very critical in economic progress through our history. There is much technology available now that could be transferred to LDCs but, on the other hand, it was obvious that new technology is needed.

The RAC had been deeply involved in individual projects. Mr. McPherson felt that perhaps it was necessary to get some broad policy thinking as opposed to project review from the RAC. Maybe the need is for an NIH peer type review system. If the goal is to make major use of technology it is clear that somebody has to have as his primary mission the formulation of broad policy. He pointed out that his thoughts were in the formative stage and that he was open for suggestions and comments.

Dr. Smuckler commented that Dr. Wishik has prepared a summary paper of the RAC discussion on the topic of how AID research should be reviewed. This provides additional information over the executive summary which had been sent to the previous Administrator. He pointed out that the RAC is charged with broad responsibility on research policy in its charter. However, the Committee has not devoted much of its attention to the broader questions of research. Such activities by the RAC would impinge on several zones of AID in which the Committee has little knowledge to function effectively. The same was the case with setting priorities. The malaria network was an example of priority setting by the RAC where RAC's advice has been very effective. However, priority setting is very difficult for the RAC and that function might be better handled within AID.

Dr. Smuckler pointed out that the Committee has emphasized project review such that each project is subjected to interdisciplinary review by the Committee. Comments by the Committee are passed on to the Administrator. He pointed out that one or two projects at every meeting are always taken apart and major changes are recommended and are actually implemented on RAC's advice. In the past, the RAC has been challenged within the Agency on several occasions and these challenges have always been reviewed by outside experts. He pointed that the Committee has always been supported by these independent reviews.

Dr. Montgomery remarked that he wasn't sure that there was a substantial market for research policy in AID. However, RAC has been helping to build a policy in an inductive way. He felt that the best discussion on this topic is grounded in experience. RAC's experience in reviewing in detail specific projects gives it a comparative advantage over policy setting groups without the benefit of this experience.

Dr. Montgomery pointed out that the RAC was successful in helping to establish a policy on malaria research. They have made major contributions in the field of population, and in earlier years in the field of economics, particularly, in economic projects dealing with growth and equity. He pointed out that there are two categories of research which never enters into this scenario: 1) there is some research that's not called research because project managers prefer that it not go to the RAC and 2) there is research carried out by AID regional bureaus and missions overseas. Such research is thought to be generated out of country needs. He doubted that this type of research was entirely site specific. Such research is never subsequently reviewed and cannot be depended on. He pointed out that development policy requires cumulative knowledge building. It requires much more than the experience and review by specialized peer groups.

Dr. Connell was interested in the recent decision by the Administrator that AID would not support abortion research. She pointed out that it is the number one method of family planning and that AID now has two projects which supports some form of abortion research along with other forms of contraception. She asked the Administrator to clarify his position on this topic since there were recent new attacks on the use of IUDs, pills, etc. and there was some danger that these family planning methods could also be defined as a type of abortion. She suggested that if the Administrator would like to defer discussion of this topic that would be fine. However, if he wanted to clarify his position she would appreciate it.

Mr. McPherson stated that his position is that AID will discontinue research on methods of performing abortions. He pointed out that Senator Helms had been interested in the question of abortion type research prior to his appearance before the Senate Budget Committee. He felt that it was important to deal with the issue for the sake of the whole budget and hoped that by stating his position early that a potential issue had been defused. He pointed out that last year \$750,000 had been spent on research on methods of abortion

Dr. Connell pointed out that abortion takes place at many different times. She asked if he was limiting this policy to surgery? She felt that AID should continue to support IUDS.

Mr. McPherson restated the position that AID would not support research on methods of performing abortion. He did not wish to go into greater detail on this.

Dr. Heady posed a general question. He felt that it was necessary to think what the research philosophy should be for the next twenty years. How will AID research contribute to development? How is AID to control its research? Recently, there has been considerable fragmentation of the research effort in AID. He asked if this research effort is under AID control? Dozens of institutions appear to be defining and reviewing research for AID. He was of the opinion that the situation was becoming increasingly complex and that it was now necessary to think through the AID research program very carefully.

Dr. Smuckler added that recently much research had been contracted to intermediaries who were making decisions on AID matters. He felt that AID must have a way of dealing with research directly and a strong internal group of researchers is needed.

Dr. Greathouse remarked that from his experience as a member of both BIFAD and RAC, the approach to research was indeed very diverse. There was the Title XII approach; There are strengthening grants; and there is the ISM approach. In one sector the issues are technical while in another sector they are not. And he feels that he is in two different worlds. He feels the Agency needs an opportunity to attract top qualified persons.

Dr. Anderson pointed out that while the Committee's decisions focused on the merits of particular projects, the decisions reflect on the extent to what the RAC knows is going on in the Agency. It would be desirable too if there were better linkage between what goes on the Agency and what comes to the RAC. The Committee does not hear much about the activities of the Agency.

Dr. Falcon pointed out that the role of the RAC is in part that of memory bank for the Agency. There was more continuity on the RAC in some matters than in AID itself. This is both good and bad. The good part is the continuity and the bad part is the frequent change in the AID staff. He also commented on the limited technical capabilities in the Agency and questioned whether the Agency should hire more people with strong qualifications.

Dr. Falcon thought that this would depend to a large extent where the Agency is going. He asked Mr. McPherson if he saw any new directions on the horizon for AID.

Mr. McPherson responded that he has been aware for some time of the lack of technical expertise in the Agency. He thought that perhaps this could be overcome to some extent through better contracting methods, through joint appointments of people by both AID and other institutions. He described how he persuaded the President, that the Agency should have its work force reduced only by 300 and not 500 in FY 82 as was proposed by the Budget Office. The fact that the President agreed with him, he took as a sign that Mr. Reagan is attaching a lot of importance to the Agency. He was aware of the fact that AID is becoming an Agency of generalists and this is a factor that will have to be dealt with in the future as personnel changes are made. With regard to the AID budget, Mr. McPherson pointed out that the food and nutrition sectors will continue to be heavy and that population would continue to grow. Initially many countries were not interested in the population program of AID. There are many interested now and he questioned why there were not other donors in this area.

He pointed out that it would appear that the Agency will be using, as much as possible, the private voluntary organizations to deliver its program. This is a reflection of Congressional interest as well as the budget cuts. He asked whether contractors could provide monitoring for the PVOs. And he asked also whether there should be more host country contracting. He thought that might be important for technology transferring. He pointed out that technicians cost a lot to send overseas.

On a different subject Mr. McPherson pointed out that if a RIF is to be avoided, AID will have to reduce the level of its employment of host country nationals.

Dr. Tanter suggested that the system used by the Department of Defense in many of its research activities might be applicable in AID. He pointed out how at the Department of Defense a single group would take a project through the entire funding cycle, including research, testing, and evaluation. AID, on the other hand, has separate research and development groups.

He thought that AID consider looking at the procedure at DOD to see how they assure use of research products.

Dr. Elmendorf thought that there was a need for coordination of the operations research, mission research, etc. She thought that there was a lack of operationalization of pure research. If the operationalization is done to show usefulness resistance of AID missions to research could be overcome. She pointed out that a strong central research unit would be needed to register the research projects to abstract them and to see that the products were made available for use.

Mr. McPherson asked if it is feasible to have a strong central unit and yet have projects run out of missions? He pointed out that as we move in the direction of technology transfer the process cannot be U.S. based, ISTIC is an example of this problem. If we are to be better aware of what's needed then the technology transfer must be done from overseas.

Dr. Elmendorf agreed that most of the technology transfer should be done overseas. However, much research is done in one country that is applicable in other countries and AID should have a system which takes advantage of this.

Dr. Carter pointed out that it was impossible to divorce AID from the political process. In the absence of ISTIC and also under conditions where we cannot build up a technical staff it is imperative to create an island of objectivity. This is one reason for peer review. He is not sure how this would be done that if missions function in a political context.

The Administrator had to leave the RAC meeting. Dr. Smuckler thanked him for coming on behalf of the entire RAC and expressed hope that he would be able to return to future meetings.

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Review of Malaria Research Workshop and Revision of Malaria Vaccine Strategy

Dr. Weller, Chairman of the RAC malaria subcommittee, reported on the workshop which had been held in January in Washington.

I. SUBCOMMITTEE REPORT

As summarized in the minutes of the 69th meeting of RAC (June 17-18, 1980) the malaria subcommittee recommended at that time that two or three meetings be convened that would: a) promote integration of research activities and identify high priority targets within the network and b) separately identify a small group of consultants knowledgeable in the area of genetic engineering, who would work with AID supported investigators, the RAC subcommittee, and AID staff to suggest ways of applying this new technology to the malaria vaccine field.

It was emphasized that application of the techniques of genetic engineering might well by-pass the laborious antigenic purification and concentration processes that are the primary goals of several investigators in the network.

When RAC met in November, 1980, plans for a meeting scheduled for January, 1981 had been developed. This was held on January 14-16, 1981 at the Uniformed Services University of the Health Sciences School of Medicine, Bethesda, Maryland. More than 90 scientists participated. These included eleven principal investigators of AID supported projects and their associates; a group of scientific consultants; Dr. Peter Twrigg, Secretary of the WHO Scientific Working Group on Malaria; and scientists working on malaria from WRAIR, the Navy, NIH and CDC. RAC was represented by Drs. Schweigert and Pimentel, Wishik and Weller who attended throughout.

As to format, after opening remarks, the first day and the first part of the second morning was devoted to reports by the principal investigators. Then Mr. Miller and Dr. Contacos presented an updated Pert Chart. On the second afternoon, the group split for two mini-workshops; one on "hybridoma technology and engineering" and the other on "in-vitro culture systems." This format was continued on the third morning with two workshops; one on "antigen characterization and purification" and the other on "Aotus biology." On the final afternoon the AID principal investigators met with Dr. Erickson to discuss administrative matters and concurrently the consultants were

assigned the task of updating priorities. The consultant meeting suffered from early departures to catch planes, however, a concensus was reached on some priorities. The final scheduled session, a gathering of all participants to update the AID strategy did not materialize. A post meeting session was held on Saturday morning, January 17th, at which Mr. Smith, Dr. Erickson, Dr. Rechcigl, Dr. Wishik, and the speaker met to review new proposals.

In general, the three day session was a highly constructive meeting. Of particular value was the presence of the co-investigators and junior investigators who profitted from the scientific interactions. The principal investigators acknowledge the need for integration and standardization of technical approaches. The transcript of the meeting is not yet available. However, a summary may be of interest to RAC.

In an excellent introductory statement Dr. Stephen Joseph traced the development of the AID malaria network. He emphasized that the next five years would be a period of great budgetary stringency, that the AID network should be incorporated with the WHO malaria immunology program, and that as soon as possible applied research in the field should be featured.

Dr. Peter Trigg, of WHO, summarized administrative aspects of the WHO program. The WHO malaria component is now budgeted at a level of \$900,000 a year, and for next year \$1.3 million is requested. However, the WHO special program has fallen short of its fund-raising goal, and increased funding for malaria research is probably unrealistic. Dr. Trigg did not outline WHO's progress or priorities. As a consequence, although the meeting which provided a good overview of AID and non-AID funded malaria research in the United States, it failed to provide background information on the large amount of research being carried out in other parts of the world. This objective should be addressed.

Their findings and directions were numerous. Several workers are now using rabbits for the production of anti-sera against various malarial antigens; however, both Jensen and Green have demonstrated cross antigenic reactivity between coccidia that commonly infect rabbits and the malarial parasites. Therefore, coccidia-free rabbits must be used.

While Siddiqui's group has concentrated on the use of saponin to release merozoites from infected red cells, Trager believes that such merozoites may have an antifactual antigenic structure, and that only spontaneously released merozoites should be employed in vaccines.

That parasites may change on prolonged cultivation, and that different strains may be heterogeneous antigenically was repeatedly indicated. Therefore, if results from different laboratories, or within a laboratory, are to be compared then parasite lines must be cloned and working stock stabilates prepared. Cloning of parasites is now underway in Trager's and in Green's laboratory. Green has developed four different cloned strains from a Trager cultured line. These vary as to presence or absence of knobs, rapidity of growth in-vitro, as to pathogenicity for Aotus monkeys, and as to patterns of recrudescence in inoculated monkeys.

The hybridoma technique has been widely adopted by investigators within and without the AID network. Nussenzweig and her NIH collaborators now have nine hybridoma lines producing monoclonal rodent malaria antisporozoite antibody; these have a range of activities. Mention was made of the availability of P.f. and P.v. sporozoite monoclonal antibodies and protective test done in collaboration with CDC. A different NIH group has antigenic monoclonal antibody. Several laboratories have anti-schizont, anti-merozoite, or infected cell membrane hybridoma-produced antibodies including several groups of workers at the NIH, Dr. John David's group at Harvard, the group at WRAIR, and Reickmann's group at Albuquerque. An obvious next step will be to select and standardize hybridoma reference sera for distribution to the network. The protective capacity of such sera will be a key determinant, and further work is needed to correlate in-vivo and in-vitro assays of parasite inhibition and protectiveness.

Several "breakthroughs" or advances were reported at the meeting. Vandenberg claims to have solved the problem of producing infective P. f. gametocytes by using a combination of aged culture media and the incorporation of hypoxanthine in the medium. Hollindale reported marked progress on growth of preerythrocytic stages of P. berghei using human lung cell cultures. Intra-cellular schizonts develop. However, while young merozoites harvested at 24-48 hours apparently are infective for mice, no evidence was presented indicating that the cycle had been completed in-vitro, with infectivity at 65-90 hours.

In the discussion of priorities, issues arose that deserve immediate attention by AID. Foremost is the consideration of patent policies and the propriety of AID investigators establishing relationships with commercial companies in the rapidly expanding area of genetic engineering. This matter is emphasized by press reports that one investigator supported by AID

is negotiating with a recently founded company in the field of genetic engineering. While an Inter-Agency Coordinating Committee is tentatively scheduled to meet in June to consider matters of patent rights for malaria vaccines, the current events indicate that all malaria network investigators should be made aware of AID patent policies inherent in signed contracts.

The recommendations for action and for priorities stemming from the January meeting were considered at a RAC malaria subcommittee meeting held yesterday. Working with Mr. Smith and Dr. Erickson the following statement was developed:

Priorities:

A. Procedural

- 1) Standardize techniques:
 - a) agreement on selection of strain; cloning thereof; working pool & stabilates
 - b) develop standardized monkey challenge
- 2) Should emphasize limited development of breeding for Aotus

B. Scientific

- 1) To identify specific antigens of the several stages of the parasites that are responsible for the development of protective immunity. This may well require the comparison and standardization of assays of protective immunity by in-vitro methods. Once identified, studies should be initiated to determine if the antigens can be replicated by biogenetic engineering.
- 2) To develop the methodology for culture of other species of human malaria with emphasis on P vivax.
- 3) Continue present level of effort on erythrocytic, sporozoite, and exoerythrocytic stages.
- 4) Defer studies on adjuvants for present time.
- 5) The culture of parasites per se has developed to a point where further efforts to increase yields, or to achieve synchrony are not indicated at present.

Dr. Wishik referred to the conference in Bethesda where the subject of patenting was discussed. He felt that AID should encourage its principal investigators to take out patents. The matter is likely to be in court for years and the fact an AID principal investigator takes out a patent does not mean that he will profit from it. It will, however, prevent an idea developed through AID research from being preempted by outsiders.

Dr. Wishik pointed out that several members of the network had already taken advantage of the meeting of the network investigators by changing their protocols. He traced the history of the network to some extent. Initially the scientific world was convinced that a malaria vaccine was not possible. AID gambled and picked one institution to begin work in this area but that institution did not succeed due to the complexity of the problem. So AID decided to go another route to disburse the effort but at the same time to maintain relationships among the various investigators, thus the network was born.

In the early stages it was necessary for the network to choose among mosquitoes, animal models, etc. each one of which had certain types of specificity. For example, once the type was selected it was necessary to choose the stage of development of the malaria parasite. The decision was therefore made to take certain parallel routes. There was for example work in the sporozoite stage of the mosquito as well as the merozoite. The early emphasis was that as things changed new research topics can be added and old ones can be dropped. Now there are about four parallel tracks. The network function has changed from that of simply providing a quick exchange of information to contractual where different members of the network are contracted to pursue different lines of investigation. AID needs applied research moving hopefully to a rapid conclusion. Now the network approaches can be standardized and consequently members in the network can become specialists.

Dr. Wishik suggested that we should give full recognition to the network. It has been very successful. No one could ever have hoped to have been so far along in the vaccine research as the program is today.

Dr. Pimentel remarked that he was not involved at the formation of the network, however, he complimented the RAC for making the suggestion. He thought the network was extremely effective in reducing duplication of research and adding a quick and effective exchange of information. He also complimented the project managers on their management of the program.

Dr. Joseph remarked that he had attended part of the workshop and was impressed with the flow of information and the ability of the researchers to work together. He listed some issues about which he was concerned and thought the RAC's advice on these would be useful: (1) the technical aspects of the research are becoming increasingly complex, (2) the technical capacity of AID to handle the research, and (3) lack of dissemination of information. He also indicated that the network was indeed a very valuable tool and came at an excellent point in time.

Dr. Weller pointed out that the problems which caused concern to Dr. Joseph were not alien to the thinking process of the RAC. He agreed that the situation is becoming increasingly complex. He felt that the technical capacity of AID and RAC to deal with the issues was too thin. The subcommittee method now in effect is a good one. However, he recommended a small ad hoc group on the subject of genetic engineering. The question of the interchange of information was not a concern to him since the malaria network involved a few people and was highly interlocked.

Dr. Hrones summarized an article in the New Times which described malaria as being wiped out. In discussion with lay people he also had the impression the feeling was prevalent that malaria was beaten. He warned that this could lead to difficulty in funding if such impressions are left unchallenged.

Dr. Wishik agreed that there was a need to think ahead on the issues suggested by Dr. Joseph. He described a personal experience along these lines where he had been responsible for establishing a new population unit. The first person hired was a philosopher, who was given the task of listing the issues surrounding the subject of population. He suggested that perhaps a short contract to list the issues surrounding malaria might be feasible. He also pointed out that computerized data and an information system might be useful.

Malaria Immunity and Vaccination - University of Hawaii

Dr. Carter, chairman of the subcommittee, which also included Drs. Schweigert, Weller and Wishik reported as follows:

I. SUBCOMMITTEE REPORT

At the Malaria Workshop, in the final session, the working group discussed priorities for future research. It was decided that "the technology for tissue cultivation had progressed to a satisfactory point and that this facet of the research should have a low priority."

By definition, therefore, a significant portion of Dr. Siddiqui's proposal falls into this category. Dr. Siddiqui has forwarded an appended proposal dated January 30, 1981, and written after the Workshop, in which he proposes more sophisticated immunology analysis of parasitic antigens. However, he and his group lack prior experience in this area, i.e., hybridoma technology.

In addition, while Siddiqui et al have been consistently and moderately productive, there has always been a subtle mistrust of his findings on the part of many of his colleagues.

Since the funding situation is critical, it would seem to be more cost-effective to go with the aspects of the proposal which fit in with the recently established priorities. They are: antigenic analyses of parasite fractions, use of sonicated particulate parasite material, and examination of long-term cultured parasites as immunogens.

However, the proposal makes no mention of the use of clones or of the desirability of comparing stabilates of different passage levels as to possible loss or acquisition of new antigens during cultivation.

Other aspects of the proposal, such as:

- 1) large scale production of parasites
- 2) increased yield and synchronization of parasites
- 3) release and purification of schizonts with saponin
(comment was made at the Malaria Workshop that saponin may destroy essential immunogens)

are less relevant to producing a vaccine at this point in time, and of a lower priority according to what was discussed at the Malaria Workshop.

In addition, Siddiqui et al have also been involved in the immunization and challenge of Aotus. This work should not be funded unless it is integrated into the network and the techniques for immunization and challenge of Aotus are standardized.

Also, cloned isolates and preserved stabilates should be used in these vaccination studies.

The new study, proposed by Siddiqui, of the nature of Aotus immunoglobulins appears peripheral to the main objectives. Also, Siddiqui et al appear to be the only ones placing emphasis on the study of adjuvants. This work should be supported and continued.

With regard to staffing and budgetary matters, the following comments have been made about the Siddiqui application:

- 1) Dr. Ono, a new 100% time appointee has had no prior experience with malaria. This position should perhaps be deleted.
- 2) Mr. Kramer, also 100% time, is a candidate for the MPH degree at Hawaii. How can he do both?
- 3) Dr. Kan, who is Dr. Siddiqui's associate, made a favorable impression on several members of the sub-committee.
- 4) Do the few monkeys require 75% time of an animal man?
- 5) After several years of support, they are still buying major pieces of equipment.
- 6) The Coulter counter should be deleted. It has been requested and rejected on past applications.
- 7) Another tissue culture hood is not essential.
- 8) The request for \$8500 for permanent equipment in year two should not be approved without specific justification.

In summary, therefore, the proposal should be revised. Those aspects which fit in with the overall strategy and newly established priorities at the Malaria Workshop, should be encouraged and funded. The others should be deleted. For example,

"other laboratories are heavily involved in hybridoma work; Siddiqui should not be encouraged to do likewise."

This will amount to funding at a reduced rate. A completely revised proposal should be submitted first.

II. SUBCOMMITTEE COMMENTS

Dr. Schweigert noted that the University of Hawaii malaria team had made significant contributions to malaria research. He felt that the present proposal had components which could be developed, revised and rewritten proposal which could continue to contribute to this research.

Dr. Weller suggested that redirection of the project was probably not the appropriate approach to take. He was concerned that the research staff at the University of Hawaii did not have the know-how and experience to redirect it. He thought it better to pick from the strengths of the research team and ask them to continue their research in these areas. In particular, he thought the purification of parasite cells might deserve further exploration. In addition, he thought that if Dr. Siddiqui and his team could work out his technique more completely and compare his results with those of the Trager method. He specifically suggested that Dr. Siddiqui not get into hybridoma research.

Dr. Wishik supported the comments by the other subcommittee members. Over the past several years Dr. Siddiqui and his research team have made consistent and valuable contributions to the state of the art in malaria research. At first, he was involved in animal inoculation. Since then he has used monkey vaccines in different ways. Dr. Siddiqui has taken up some new techniques developed by other members of the network, but has not taken up some others. The tissue culture work is an example. Dr. Siddiqui was trying to learn about parasite culture, but has not considered the work that has been done in Germany. His directions have now been bypassed by events in other places. He has a tendency to go off on tangents in his research.

OTHER COMMENTS

Mr. Smith, of the AID staff, provided a few background comments for perspective on this proposal. He reminded the RAC that the University of Hawaii project came into the network with the first request for proposal. The project was approved then on the basis that AID needed many people working in the area of malaria research. He had on his team several young professional persons. After Dr. Trager's breakthrough, Dr. Siddiqui reproduced Trager's work. Later AID asked for proposals for developing methods for culturing parasites. Dr. Siddiqui responded. He was the first to immunize Aotus monkeys. He learned quickly after getting

into the subject. In his current proposal he has adapted and prepared his research plans around the priorities of two years ago. The priorities have changed only in the past January. Dr. Siddiqui was quite understandably upset at the new priorities since they gave the work that he was doing a lower priority. However, he was perfectly willing to take on the work that had been listed as high priority and prepared an amendment to his project.

Dr. Erickson, of AID staff, pointed out that Dr. Siddiqui had achieved several successes in his lab. Dr. Kahn, who is a member of his team, has purified antigens which appear to work well. He is continuing work on the sapon issue. His project proposal does not mention clones but neither do other project proposals since cloning is a relatively new development and standardizations came up only at the recent workshop. Dr. Ted Green, at the University of Missouri, was the first to clone.

Dr. Erickson was sure that the principal investigator would get into cloning quickly.

Dr. Erickson pointed out that percol has been used by many others to purify antigenic material. However, he indicated that percol residue creates a problem. The researcher does not know what effect it may have upon the vaccine material.

Dr. Siddiqui has already begun work on the hybridoma technique. Perhaps it is duplicative but it may also be useful. He has experience working with antigens and is interested in using the hybridoma technique as a tool. He is also interested in a variety of other techniques. With regard to the issue of the experience of Dr. Ono, has a Ph.D. from Japan in Immunology. He has worked with Dr. Contanie as a Post-Doctoral Fellow. A second member of the staff, Mr. Kramer, works 100% of the time as a technician on the project.

The University of Hawaii encourages technicians, such as Mr. Kramer, to continue their education and Mr. Kramer is enrolled as a candidate for a masters degree in public health. All the hours that he misses from the laboratory are made up after hours. The caretaker for the monkeys is fully occupied also. Dr. Siddiqui has 40 monkeys on hand and plus rabbits, etc.

III. MOTION: That the project be approved subject to revision. That it be focused on those elements that fit in with the newly established priorities of the malaria vaccine network. For example, purification and antigenic analysis of saponized cells and comparison with preparations using the Trager technique. The revised application should be submitted to the malaria subcommittee. Dr. Siddiqui should deemphasize mass production of the parasite. He should not be encouraged to go into hybridoma technology. Inevitably, a reduction in funding level should result.

Dr. Rechcigl asked for clarification of the motion. It would appear from the motion that the project would have to come back to the RAC at its next meeting. However, the Agency was experiencing a shortage in travel funds and there was a possibility that the next RAC meeting will not be held until October. That would be too long a delay for the project.

Mr. Smith pointed out that the current project ends in June and unless the project is continued at that time Dr. Siddiqui's research team would lose personnel as has happened in prior occasions.

Dr. Wishik proposed a friendly amendment. He suggested that the malaria subcommittee be designated to work with the AID staff to review the revision of this project and that RAC delegate authority to the subcommittee to recommend approval.

Dr. Carter pointed out that the intent of the motion is to allow funding to continue but with realignment of the work. He agreed that the subcommittee could handle this.

Dr. Weller thought that the amendment by Dr. Wishik was not approval of the amount of money requested by the principal investigator. He pointed out that with the realignment of work there could be substantial savings in the budget.

AID staff found both the motion and the friendly amendment to be acceptable.

Vote: Unanimous for approval.

Antigenic Analysis of Cultured Plasmodium falciparum - Michigan State University

Dr. Smuckler turned the chair over to Dr. Montgomery during the discussion of this project and left the room since he is from Michigan State University.

I. SUBCOMMITTEE REPORT

Dr. Schweigert, chairman of the subcommittee, that also included Drs. Carter, Weller and Wishik reported as follows:

Dr. Jensen has submitted a project for a three year period as a follow-up to support for a small project initiated during the past year (approximately \$34,000 in support) with the objective of developing an analysis of the antigenic properties of cultures of Plasmodium falciparum. It will be recalled he is the scientific associate with Dr. Trager of Rockefeller University that developed the continuous culture system for this parasite.

Dr. Jensen and his colleagues propose to identify, isolate and characterize soluble antigens in the exhausted culture medium used in growing Plasmodium falciparum. They plan to use rabbits, guinea pigs and monkeys to test the cultured antigenic preparations and to ascertain if the soluble parasite antigens obtained from the medium will neutralize the inhibitory activity of serum from humans determined to be functionally immune to malaria from the Sudan. They have an associated project investigating malaria in the Sudan. They plan to follow-up with the antigens that show promise as potential vaccine candidates to analyze and characterize these antigens.

The importance of developing highly purified preparations of key antigens is emphasized in that extraneous material associated with contaminating erythrocytes has most likely caused severe anemia not associated with the properties of the malaria antigen active components. Further, there is of course some evidence that other contaminating proteins induce an immune response completely unrelated with the malaria circumstances.

Purification techniques anticipated include column chromatography, DEAE ion exchange chromatography, and preparative electrophoretic methods such as disc-gel electrophoresis and isoelectro-focusing.

It should be emphasized that starting these methods and developing the sophisticated laboratory procedures and controls is a challenge in any laboratory.

As the work progresses, they will be interested to determine if any of the soluble parasite antigens are possible vaccine candidates. Hopefully, by using animal models and comparing with human immune sera, a sound basis for proceeding will be developed. They anticipate in the latter phase of the program determining the subcellular position in the parasite where the soluble parasite antigens are located. This, of course, is considerably in the future, and the priority and design for these experiments may well be in need of major review and redefinition.

Dr. Jensen has submitted a proposed budget approximating \$100,000 a year for a three year period. All of his salary is to be paid by Michigan State University. He has an extensive list of equipment proposed for purchase in the first year, and he has provided justification for each item. While it is surprising to me that his laboratory is still not well equipped, it is noted that he is not proposing any additional new equipment in the second and third year.

Dr. Jensen has established a new program of research support at Michigan State University in very rapid fashion after leaving the Rockefeller University. He has three other major research grants--all dealing with malaria including the studies on resistance of Plasmodium falciparum to the drug 4-amino quinoline in the Sudan, a study on erythrocyte requirements supported by WHO, and the small grant program for antigenic analysis supported by AID. It can be assumed that the new contract, if approved, would be a follow-on to the latter grant so that actually he would have two other continuing grants.

Dr. Jensen is very impressive on the basis of his excellent training and publication record and achievements to date. He certainly appears to be attacking the key problem of characterizing antigens that may be potential vaccine candidates for malaria. The project is of modest size and scope, and is "do-able" even though he has two other major research grants. The key evaluation of this project will center around the priorities and planning developed for future strategies in the malaria program.

II. SUBCOMMITTEE COMMENTS

Dr. Carter stated that he would like to see some statement to the principal investigator that he should be looking at a strain of malaria that is specific to the Sudan.

Dr. Weller remarked that he would like the motion to include a directive to the principal investigator that the focus of research be on the exoantigen studies and limited to that. He was not sure why the principal investigator needed a \$3,000 scale. The budget was not broken down sufficiently to show that.

Dr. Wishik pointed out that this was a three year project that would be following a one year small project. The summary report of that small project appeared in the appendix. It was useful in assessing the potential of this new project. He felt the one year project had achieved an acceptable level of analysis and that several accomplishments had been achieved.

Dr. Wishik pointed out that there are three parallel tracks in the malaria research approach now and that this project represents one of those three tracks. It deals with the approach involving soluble exocellular antigens. He pointed out that this project adds a whole new dimension to malaria research, an important dimension.

The principal investigator has used information gained from the January conference in his proposal.

Dr. Wishik continued to explain that the Sudan work is funded from other sources than AID. He was surprised that the principal investigator was careless about where he obtained the malaria parasite and the sera with which he worked. This was surprising because in previous work with rabbits he had carefully saved the rabbits until later in the experiment where he used the same rabbits for testing to avoid any complications that would be caused by using different rabbits.

Dr. Wishik thought the project should be supported.

III. OTHER COMMENTS

Mr. Smith, of AID staff, pointed out that Dr. Jensen's original proposal was considerably larger. He wanted to do some work in the Sudan but was asked to cut this from his proposal.

Dr. Erickson thanked the subcommittee for providing him with written comments in advance and described the written comments as being very important in preparing for the meeting.

He pointed out that the work that Dr. Jensen had proposed to do in the Sudan had been cut out because previous RAC discussion (U. of Missouri) felt it was premature to go into Africa.

Dr. Erickson also explained that Dr. Jensen was requesting a normal laboratory scale and that the cost was entirely in line with todays prices.

IV. MOTION

That the project be approved with two provisos: (1) the research be focused on the study of exoantigens and (2) the budgetary and other items be reexamined by AID staff in light of RAC's discussion.

V. VOTE

Unanimous in favor.

Antigens of Plasmodium falciparum - Scripps Clinic and Research Foundation

I. SUBCOMMITTEE REPORT

Dr. Wishik, chairman of the subcommittee, that also included Drs. Pimentel, Schweigert and Weller reported as follows:

The project has two major aims: To identify immuno-effective membrane antigens for P. falciparum parasites and for infected erythrocytes; and secondly, to clone the essential proteins by hybridoma technic. These are high priority components of the AID malaria network objectives.

The principal investigator presents a confidential detailed description of steps that would be taken. The purposes, hypotheses, procedures and sequential attack are logically laid out. Although the subject is technically complex and highly specialized, the relevance of the plan is evident. The principal investigator's primary strengths are in biochemistry, with particular attention to malaria in his recent years at the Rockefeller University. He is familiarizing himself with hybridoma work.

Dr. Robert Reese will join the Scripps Research Institute in April of this year. Dr. Wishik visited the Institute, interviewed the Director, Department Head, and Dr. Reese and looked over the facilities.

The clinic is an outstanding biological research center with a high reputation, new buildings and an annual research budget of 25 million dollars. It has no direct university affiliation, though some staff share time. There is a high level Scientific Advisory Board and each department has a full external program review every five years. There are five departments - clinical, biochemistry, developmental and cellular immunology, molecular immunology, cellular biology and immuno-pathology - which would offer a strong supporting scientific context for the proposed project, especially in the closely relevant fields of biochemistry and immunology.

Fourteen-hundred square feet of laboratory space are immediately available for Dr. Reese. Although this is said to accommodate 8 to 10 persons, Dr. Reese thinks that would be crowded. Large equipment are located in adjacent space. The Institute furnishes investigators with centralized supporting services, on pro-rated charges to each project budget. These include: protein labeling and protein sequencing units, electron microscope unit, photography, animal facilities and care, workshop and electronic repair shop and dishwashing.

AB

All transfer problems from Rockefeller University to Scripps have not been resolved, especially with respect to equipment purchased on Dr. Reese's past contracts and grants. Neither Rockefeller nor NIH officials seem willing to initiate necessary correspondence about the listed items attached to the project proposal. There seems to be a bureaucratic impasse. It is also likely that Dr. Trager will continue to work at Rockefeller after the previously scheduled termination date of December, 1981. He seems to want to keep the thirty monkeys. These considerations have a bearing on the project budget.

II. SUBCOMMITTEE COMMENTS

Dr. Pimentel remarked that Dr. Reese appeared to be an outstanding scientist who had submitted a very well written project. He suggested that if Dr. Reese's thesis is correct then the project would contribute very much to malaria research.

Dr. Pimentel thought the budget appeared to be a little high but was unable to comment in detail on this.

Dr. Schweigert commented that he was reassured by Dr. Wishik's visit. He felt that Dr. Reese, the principal investigator, had not been fully responsive on budget items but felt that the project should be supported.

III. OTHER COMMENTS

Dr. Erickson, of AID staff, pointed out that the Office of Health had the proposal reviewed by several expert malariologists. With regard to the move from Rockefeller University to Scripps, Dr. Erickson pointed out that Rockefeller University has no problem transferring equipment that were purchased by Dr. Reese at Rockefeller. Similarly, WHO and the Department of Army have no problems with regard to equipment that Dr. Reese was using which had been purchased by those two agencies and which now would be shifted to Scripps. Dr. Reese was still negotiating with NIH with regard to moving NIH purchased equipment to Scripps. Dr. Trager, of Rockefeller University, has written a letter stating that he would support the equipment transfer to the Scripps.

With regard to the monkeys Dr. Trager has indicated that he has no problem with the transfer of the monkeys. However, it is necessary to wait for animal facilities to be prepared at the Scripps Foundation.

Dr. Wishik noted that while the principal investigator from the University of Hawaii had simply made reference to carrying out work using the hybridoma technique, Dr. Reese in his proposal had given a detailed protocol of how the hybridoma technique would be used. He felt that this difference should not be lost on the RAC.

IV. MOTION

That the project be approved as proposed.

V. VOTE

Unanimous in favor.

Biological Medical Institute

Dr. Weller brought up for RAC consideration a very preliminary proposal that had come in from the Biological Medical Institute. This proposal proposes to do some work on Plasmodium falciparum and Plasmodium vivax. The cost of the proposal was \$257,600 and that included \$100,000 overhead.

Dr. Weller pointed out that the work in this area indicated that progress thus far in this area was not great and it appeared to the subcommittee that it was premature to move the experiments which the Biological Medical Institute was doing to human systems until it was demonstrated beyond doubt in rodents.

Recommendation: That the request be disapproved.

Dr. Carter concurred.

The recommendation passed by a voice vote and it was requested that the minutes record RAC's consideration of the project.

Discussion on Setting a Date for the Next RAC Meeting

Dr. Smuckler pointed out that there was a strong desire within AID that the Committee not meet until the next fiscal year. His personal view was that given the small cost of a RAC meeting (\$12,000) that this was primarily a bureaucratic problem that should be resolved within AID.

Dr. Weller thought that the cost of a RAC meeting was also very small in comparison with the importance of the meeting. He felt that the Science Advisor should perhaps discuss this problem informally but he would like to extend the RAC suggestion that the meeting should be held as scheduled to include site-visit travel, which Dr. Weller felt was important.

Dr. Smuckler then asked if the consensus was that if there was research to be reviewed the meeting should be held. He and other members of the RAC pointed out that site-visit money is usually from project funds and not from operating funds and should be separated from this. However, the consensus was that the RAC should be on record that adequate site-visit money should be available.

Dr. Connell also suggested that evaluations of individual projects be made known as a line item in the budget.

Dr. Erven Long, of AID, explained that the Agency had overspent its travel budget and that the need to curtail RAC travel was simply a reflection of this rather than any decision not to have a RAC meeting.

Dr. Rechcigl described the items which might be included on the RAC agenda for the next meeting. There were three or four extensions of projects and two new projects as well as several terminal reviews.

The Committee set a tentative date of June 1-2, 1981 for the next meeting if it is held and October 21-22 and possibly 23 for the following meeting.

Man and the Biosphere (MAB) II - Consortium for the Study of Man's Relationship with the Global Environment

I. SUBCOMMITTEE REPORT

Dr. Pimentel, chairman of the subcommittee, that also included Drs. Ehrenreich, Hrones and Swanson reported as follows:

From AID's perspective, the goals of this proposal are to improve environmental protection and natural resources management in LDCs and to increase sustainable use of those resources to better satisfy basic human needs.

The objective of the Consortium to Study Man and the Biosphere is to develop the basis within the natural and social sciences for the rational use and conservation of the biosphere and for the improvement of the relationship between man and the environment.

The primary project areas of MAB are: (1) tropical forests, (2) temperate forest, (3) grazing lands, (4) arid and semiarid zones, (5) fresh and coastal waters, (6) mountains and tundra, (7) islands, (8) biosphere reserves (conservation of natural diversity), (9) pesticides and fertilizers, (10) major engineering works, (11) urban ecosystems, (12) demography, (13) perception of environmental quality and (14) pollution.

Clearly many important environmental resource management problems are identified in these subject area headings of the MAB program. AID specifically identified several items that it stated were "most important problems." These are:

- Billions of tons of soil are lost every year as a result of deforestation and poor land management.
- Deforestation is forcing people to switch to burning dung and crop residues badly needed to regenerate cropland.
- Tropical forests are disappearing at an alarming rate and this deforestation relates to flooding siltation, and desertification.
- Deforestation is causing the rapid extinction of many plant and animal species.
- Aquatic systems, both fresh and saline, are suffering widespread pollution.

All of the areas mentioned by the MAB program and those five related areas mentioned by AID are important environmental and resource management problems. Most of the areas deserve the high priority for research and education and will help LDCs to strengthen their capability to solve their problems.

However, if one of the major concerns in the MAB II program is as stated soil erosion, siltation, and flooding, then the great emphasis on forest ecosystems is misplaced. Most soil erosion, siltation, and flooding problems are associated with agricultural ecosystems. For example, in the United States three-quarters of the soil erosion and siltation problem is associated with agriculture while the remaining one-quarter is associated with forests and construction projects.

If indeed our concerns in the MAB II program are for the environment and for the basic human needs of people in the LDCs, then environmental protection and natural resource management should include land and water resources for agriculture at the top of the list. Soil erosion rates in LDC agriculture are estimated to be much more severe than in the United States--reports from India suggest soil erosion from agriculture is three times more severe than in the United States.

Soil erosion has serious effects on land productivity, food supplies, and resource needs. For example, for each inch of topsoil lost crop productivity may be reduced 5%. In addition, valuable nutrients of nitrogen and phosphorus are removed with the eroded soil. With each metric ton of soil eroded, about 5 kg of nitrogen and 1 kg of phosphorus are removed from the land. With soil erosion rates ranging from 20 to 40 tons per hectare in row crops grown on slopes over 2%, the impact is important. Nitrogen and phosphorus are essential fertilizers and are costly to LDC farmers especially with high energy costs.

These fertilizers and pesticides washed into aquatic ecosystems cause problems and this was mentioned in the MABII program. This problem is important but not as important as maintaining the productivity of agricultural lands for sustained food production.

Water associated with fresh and coastal aquatic ecosystems was a major topic, but water resource management for agriculture and other human needs was hardly mentioned. Water is the major limiting factor for crop production and the demands for irrigated crops are immense. Under arid conditions, over one million gallons of water per acre are required for the growing season. During the season the crop will transpire about 500,000 gal per acre.

Not only are there serious water shortages in most regions of the world, but agriculture is suffering from serious environmental degradation due to poor water resource management. It is estimated that about 60% are suffering from salinization and other irrigated lands suffer from waterlogging due to poor drainage.

When the specific investigations are being carried out, this applied research would benefit if the investigators also considered the social, cultural, economic, and health aspects directly benefitting development. Efforts should also be made for the principal investigators to collaborate with the LDC scientists.

The average grant of \$50,000 for research in developing countries appears too small to make substantial contributions when travel, per diem, and overhead are subtracted. It would appear desirable to at least double the maximum size of the grants.

Recommendations

(1) Instead of listing urban ecosystems, perception of environmental quality, and pollution, as major environmental problems in the LDCs, it would be immensely more helpful for development to focus on agricultural land and water resource management. Most of the LDCs have serious food shortages now and anything that can be done to protect the environmental and agricultural resources of the rural poor should receive top priority.

(2) The applied research projects are urged to also consider the social, cultural, economic, and health aspects that directly benefit development. Because most actions to improve the environment and to manage natural resources are public policy in nature, recognition of the LDC context for policy decision is crucial in the implementation of any research results. Collaboration with LDC scientists should be encouraged.

(3) The contribution of the past two years of the Consortium should be evaluated and reported to RAC.

II. SUBCOMMITTEE COMMENTS

Dr. Ehrenreich strongly supported the proposal and felt that AID should be involved in this multi-agency program. He pointed out, however, that many of the particular projects of the MAB II Consortium have not always met their goals. Some of the MAB II projects were not either applied or basic research but were more in the form of technical assistance. Other projects were on topics which had already been extensively researched.

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Dr. Ehrenreich felt that \$50,000 average amount to be spent on each project was not likely to be enough money to permit adding much to the total bank of knowledge. He would like to see AID participate in only a few of the areas listed by the Consortium. And these, he felt, should be in the areas of high interest to the Agency. He reiterated that he supported variable size of funding so that some of the projects at least would have a greater amount of money than \$50,000 to support their research. He hoped that the Agency would not fall into the management trap that other projects with this Consortium had fallen into. He felt that AID participation could help clean up management problems when they occur.

Dr. Ehrenreich pointed out that in the proposal there was considerable over-dramatization of the problem. For example, many ecosystems are very resilient and after destructive events have occurred bounce back to recover very quickly.

Dr. Hrones thought the proposed areas of research were very important. He agreed that the average amount of funding was too small. He thought AID participation in the program should be more sharply focused. In almost every project he felt the researchers were attempting too much for the amount of money available. The instructions for preparing project proposals encourage prospective proposers to cover all areas and thus reduce the sharpness of the focus of the proposals

Dr. Swanson commented on the review process that the Consortium used to select projects. He thought that technically and scientifically that process would be strong. However, he wondered if a different set of standards would be used for projects in the U.S. as compared to those in LDCs. He pointed out that of the sixteen funded projects, only four were in LDC sites. He would like to see more specificity in project proposals. It appears that the project proposals would not include the topic of public policy.

Dr. Swanson felt that this should be reviewed and clarified.

Dr. Falcon did not particularly like being asked to approve a procedure. He had quite a problem with the project. He pointed out that the issue was not the importance of the topic but whether or not AID would continue to handle research in the manner proposed here. He thought that the procedures were not reasonable for a \$50,000 funding limit. He thought it would be much more useful to focus on one or two issues and spend more money on each of the issues.

Dr. Heady pointed out that other organizations would carry out research on questions on erosion etc. He wondered why the Soil Conservation Service had been left out of the Consortium.

Dr. Pimentel pointed out that AID had not contributed to the selection of projects in the past. The subcommittee was quite concerned about the size of the grants.

Dr. Pimentel was of the opinion that even \$100,000 would not be enough to have much effect on the amount of knowledge produced from these small research projects. However, if the proposals were tailored to fit the funds available, the projects could make a contribution. The problem being addressed by this program is immense and of tremendous concern to everyone.

Dr. Wishik asked whether there was an option for the AID contribution to be kept separate from that of other contributors. What would be the implications of handling the AID funds separately?

Dr. Ehrenreich replied that the procedure would be that the project proposals would go first to AID project managers, then to peer review and then return to the AID project manager.

Dr. Greathouse had questions about this particular procedure. He felt that the Agency was asking for trouble by handling its research in this manner.

Mr. Roseborough, of AID staff, pointed out that the program would not ignore erosion and agriculture. In the draft project paper he had only indicated some problems. Erosion happened to be the first one cited. He stated that the researchers are finding that population is a driving force to find more ways to increase production. Now the trend is to move into infertile land, sloping land, and other types of lands that previously were not used for agriculture. Erosion of these lands tend to be excessive. He mentioned the firewood problem and pointed out that in developing countries 90% of the fuel come from firewood and this percentage was accelerating. For this reason he did not feel the program had an over emphasis on forestry. He hoped that, however, more projects in AID areas would be incorporated into the program.

Mr. Roseborough felt that \$50,000 projects as described in the program looked good to him. Mr. Roseborough thought that AID should not address all project areas in the MAB II program. Out of the fourteen programs AID has cited only six. Project proposals will be received in only some of these six.

Mr. Roseborough then described the review procedure used by the Consortium.

Dr. Pimentel pointed out that the U.S. Forest Service and the Fish and Wildlife Service and other members of the Consortium had not had significant experience in the international area. He thought that the researchers could accomplish quite a lot with a small sum of money.

III. MOTION

That the Science Advisor encourage research efforts on agricultural land degradation and reforestation. That this consortium project be approved with three suggestions: (1) Instead of listing urban ecosystems, perception of environmental quality; and pollution, as major environmental problems in the LDCs, it would be immensely more helpful development to focus on agricultural land and water resource management. Most of the LDCs have serious food shortages now and anything that can be done to protect the environmental and agricultural resources of the rural poor should receive top priority; (2) The applied research projects should also include social, cultural, economic, and health topics that directly benefit development and to manage natural resources are public policy in nature, and recognition of the LDC context of any research results. Collaboration with LDC scientists should be encouraged; (3) The contribution of the past two years of the consortium should be evaluated and reported to RAC.

IV. VOTE

Approval with four abstentions.

The RAC members who abstained were asked to explain why. One member pointed out that he felt the project needed more focus, the size of the money for each project was not enough and that he was being asked to judge a procedure rather than to pass judgment on research projects. Another pointed out that the open ended wholesaling of research projects was not to the benefit of AID. The third and fourth also had reservations about the wholesaling out of research projects.

Program for Applied Research on Fertility (PARFR) -
Northwestern University

I. SUBCOMMITTEE REPORT

Dr. Wishik, chairman of the subcommittee, that also included Drs. Carter, Linder and Weller reported as follows:

The PARFR was reviewed by RAC at its last meeting. It was approved with the proviso that the funding would be continued with a 10% inflation factor, at the rate of \$3.6 million per year. The first year funding would be made available and would be at a somewhat smaller amount than \$3.6 million. The proviso provided for a review of the projects to be carried out shortly after the November RAC meeting and the continuation of the PARFR project was made conditional on the outcome of the review.

Team members on the review team were as follows: Dr. Patinelli, of NIH; Dr. Mishell, of USC; and Dr. Wishik. Dr. Wishik pointed out that the team members were careful and critical in their review and very balanced in their approach. The review team came to the general conclusion that the PARFR project was worthy of continuation.

Dr. Wishik commented that the staff at Northwestern University appeared to be too small for the magnitude of the project. He pointed out that every small grant made under this program was visited once per year by the contractor. He indicated that the sub-projects were subjected to a very good level of selectivity. The program has been reasonably successful at stimulating research in the biomedical area. It has, however, only been moderately effective at dissemination of results. There have been many efforts to publish data from results. However, publication is very often such that the information does not get into the mainstream of information of the biomedical field. The review team recommended that attempts be made to publish some of the findings of the research in standard medical journals.

At the November RAC meeting a list of questions was raised by RAC members, but most of these were covered by the site visit report: (1) the size of the staff at PARFR is too small and it should be enlarged. There should also be a corresponding enlargement of office space, (2) there is a question of intermediaries carrying out AID research. However, although it is justified that this research be carried out by intermediaries, the PARFR program should be allowed to have adequate staff and (3) although the Scientific Advisory Committee was composed of

outstanding scientists, a few had dual roles as administrators and implementors of the PARFR program and advisors on the Scientific Committee. It was recommended that the chairman of the committee be removed from the SAC.

Dr. Wishik attended a full day of the Scientific Advisory Committee for PARFR and was impressed with the quality and objectivity of the Committee's discussion.

Dr. Carter pointed out that he was satisfied that the review of the human experimentation aspects was more than adequate. He was concerned about the possibility of a conflict of interest with the head of PARFR also sitting on the Scientific Advisory Committee. He pointed out that the budget is twice what it was previously and it is very difficult to justify such an increase. He felt that it might be necessary to select some of the PARFR projects and carry them through to the end while delaying other aspects of this research.

Dr. Carter was generally satisfied with the review and the quality of science in the PARFR project.

Dr. Linder remarked that he generally liked the report from the review committee. The project appears to be small and tightly managed with good attention to scientific standards. He raised the question of the increase in funding. He pointed out that in the first nine years of the PARFR project the average cost per year was one million dollars and that it had suddenly jumped to \$3.5 million. He questioned whether the organization was able to absorb such a quick increase and asked whether or not such an increase should be phased in.

Dr. Weller remarked that the review report was very reassuring. He was pleased that the possibility of conflict of interest had been looked into. He was concerned that there is not enough management staff at AID and at Northwestern to manage the project carefully. He thought that if more staff were available the end result would improve.

Dr. Weller asked again for clarification on the level of funding. He questioned why the PARFR committee needed a computer expert, why it couldn't be tied into the computer network at Northwestern University. He pointed out that the workshops carried out by this project were well written and appeared to have a lot of scientific merit. On the other hand, this material almost never got into scientific journals.

Dr. Wishik then pointed out that, at the present, PARFR prioritizes projects and in fact could fund more projects were the money available.

Dr. Carter agreed. He did not think the project was unmonitored, if anything it was overmonitored. He pointed out that in the opinion of the reviewing committee, the scientific monitoring was of very high caliber and was quite adequate. The monitoring of the human experimentation aspect of the program was very adequate and monitoring by AID staff was being done relatively effectively. However, the monitoring workload was very heavy.

Dr. Speidel, of AID staff, reminded the RAC that this was the third year of a continuing resolution for the Agency. This year there was some indication that there would be a modest increase in the budget for the Population Office which would give the office some latitude to increase funds for the PARFR project. Unless there was a substantial increase it was very unlikely that the Population Office would have the money to increase expenditures in this program very much. However, the office wanted the authority to do so should the money be available. He pointed out that the proposal for a separate computer had been shelved. The Population Office was also concerned somewhat about the lack of published papers in standard journals. The workshop publications were very good and were often of journal quality as an alternative to being published in the more common biomedical journals.

Dr. Speidel pointed out that Popline, a computerized information system, is up and running at the National Library of Medicine and all the findings of the PARFR program are available from that source. He remarked that it was not very likely that the workload for the AID staff in monitoring this program will change very much since staff increases were not contemplated.

With regard to the question of conflict of interest, Dr. Shelton pointed out that there was considerable question as to whether or not you could get the best people in this field on the Scientific Advisory Committee and still have people available to actually carry out the research projects. AID would make every effort to comply with the recommendation to avoid any conflict of interest.

Dr. Wishik pointed out that one of several recommendations about the Scientific Advisory Committee was that the chairman should be changed. At present the principal investigator is the chairman. Several people were disturbed about this.

Dr. Shelton stated that Dr. Wishik had watched the Scientific Advisory Committee in action. It should have been fairly obvious that the chairman, who is the principal investigator of the project, can run the meeting without voting.

Dr. Speidel pointed out that the chairman is extremely effective and unbiased in the way he runs the meeting. He ties a knowledge of staff into the meeting. Dr. Speidel would like to defer on the question of changing the chairmanship of the Committee. At present they are trying to stabilize the budget and it would be difficult for AID to commit themselves to making such a change.

Dr. Wishik pointed out that one device that may be used to stabilize the budget is to use a three year roll over system where one year's budget is added each year.

Dr. Speidel replied that if there is a large increase that this would actually add to the PARFR budget. He did not see how the rollover would work. It would not be different than RAC giving additional authority to increase the budget.

IV. MOTION

Dr. Wishik then proposed the following motion:

(1) That the previous RAC recommendation of approval and funding for the first year be extended to the remainder of the project period and at the requested budget level; (2) That copies of annual progress reports be made available to RAC; (3) That there be a thorough project review and site visit two years after the last one, including RAC representation.

V. Vote

Unanimous in favor.

International Fertility Research Program (IFRP) - International Research Program

I. SUBCOMMITTEE REPORT

Dr. Connell, chairman of the subcommittee, that also included Drs. Linder Montgomery and Wishik reported as follows:

IFRP was established on July 1, 1971. Its goal was to conduct comparative field trials on new means of fertility regulation primarily in developing countries. In order to accomplish this, IFRP developed an international network of more than 250 collaborating investigators working in over 30 countries. Data collection was preceded by the establishment of standard methods of gathering and reporting clinical data. The earliest work by IFRP documented the short-term safety and effectiveness of a number of fertility regulation methods. It also pointed out the relative appropriateness of the different methods and procedures for people living in a variety of cultural and medical environments. Finally, IFRP disseminated the information generated by these trials, and helped to improve developing-country research capabilities.

IFRP has conducted straight and comparative field trials in six major areas: 1) systematic contraception including oral preparations, 2) intrauterine contraception, 3) menstrual regulation, 4) pregnancy termination, 5) male sterilization, and 6) female sterilization. The emphasis has shifted from time to time, but support to continue work in these same general categories is currently being sought. In March, 1977, a renewal proposal was reviewed by RAC. A number of serious issues were raised and funding was not approved pending further study. In September, 1977, a RAC subcommittee visited IFRP. During the site visit, the subcommittee looking into the validity and reliability of methods of data collection and analysis. It concluded that while the data were generally satisfactory, they had not been subjected to more than rudimentary analysis.

The subcommittee also considered proposals which would have expanded IFRP's functions to include studies of alternative community-based delivery systems, program evaluation, program administration and implementation strategies. In the view of the subcommittee, IFRP's capability for doing this type of research was extremely limited and thus it felt that these activities would require procedures and skills quite different from those which IFRP had developed to date and thus should not

be undertaken. Moreover, such a change, it concluded, would take IFRP away from its original and unique role in research on family planning technology. At the conclusion of its site visit, the RAC subcommittee made a number of recommendations which included the following.

1. IFRP should set a ceiling on the number of participating centers, the amount of data be generated, and the duration of data collection.
2. IFRP should be more selective in maintaining its repository of data for future use; it should develop more complex study designs and use more sophisticated analytical techniques in processing existing data.
3. IFRP should reassess its staffing pattern and the makeup of its consultative groups.
4. IFRP should consider the addition of a senior staff member with wide prior training and experience in basic research in reproductive biology regulation techniques.
5. IFRP should continue to place its greatest emphasis on clinical trials and the services needed for the implementation of the various fertility regulation techniques.
6. IFRP should not attempt to cover the much broader administrative community and social aspects of planning program development.
7. In the future, only the research components of the IFRP program should be reviewed by RAC.

Following receipt of the report of the RAC subcommittee and consultation with AID staff, a number of changes were made by IFRP in both staffing and administrative procedures, a number of them recommended by RAC. Those areas which contained primarily the research elements of the program were identified and a new contract was written to cover them. Thus, IFRP has support to continue their biomedical research in the area of fertility regulation as well as related research in the social sciences.

IFRP also applied for and received funds under a new grant which allowed it to carry out certain other programmatic activities. Under the grant, a considerably broader range of

activities are funded in the area of institutional development for fertility research and programmatic support for family planning services.

In September, 1980, at the request of AID, an American Public Health Association (APHA) Evaluation Team was selected. It consisted of Dr. Elizabeth B. Connell, Chairman, Dr. Donald Lauro, Sociologist/Demographer, REsearch Associate, Columbia University Center for Population and Dr. Nicholas Wright, Epidemiologist, Chief of the Contraceptive Evaluation Branch, NICHD, NIH. The team first reviewed a number of documents including annual contract and grant reports for 1978, 1979, and 1980, the current IFRP table of organization, project descriptions, research forms procedure, a publications list, minutes of the Board meetings, and copies of trip reports. After reading this material, the team made a site visit to IFRP where interviews were carried out with members of the various divisions and with individual staff members to hold more in-depth discussions relevant to their particular areas of interest. The team then met on several occasions to discuss their findings. Notwithstanding the considerable differences in the academic backgrounds and work experiences of the various team members, there was complete unanimity of opinion on all of the major issues under review and the report sent to the RAC members represents the unanimous conclusions and recommendations of all its members.

Following this extensive review and deliberation, the team concluded that IFRP was continuing to make progress in dealing with the recommendations of the RAC subcommittee but that there were areas in organization, staffing and research which could be further strengthened. Therefore, a series of recommendations were made dealing with the following areas:

1. Structure: IFRP is currently being funded by AID under two mechanisms, a contract and a grant. This has had both advantages and disadvantages for the overall IFRP program. At the present time, projects funded under the contract are almost entirely in the research category. However, there are a number of studies supported by grant monies which are also research in nature but are not reviewed by RAC. Additionally, there are a number of small projects which the team felt were handled as research projects but were best called program introductions.

2. Focus: The team felt that more attempts should be made to move beyond short-term clinical trials into field and clinical studies of the long-term impact of fertility regulation techniques in developing countries. Moreover, it is believed that grant activities, though necessarily at times deviating significantly from contract efforts, should focus in the same general directions as the contract-supported work. It felt that to use the flexibility built into the grant as a mandate to go off in a number of new directions would be a mistake.
3. Staff: The team noted the changes in staffing over the past year, particularly the shift to lower level positions. It, like RAC, recommended the early recruitment of a senior scientist with broad biostatistical and epidemiological training. The team further recommended the establishment of contractual links with local universities, including the UNCSPH, to increase the skills necessary to IFRP's research program. It felt that the presence of scientists from neighboring institutions might help to re-establish prior links with these academic groups.
4. Projects: The team made a number of specific technical recommendations regarding individual projects, as noted in their final report.
5. Technology Transfer and Training: The team believed that IFRP's interest in providing mini-computers and the necessary software packaging and training required for their effective use to LDC's was worthwhile and recommended that IFRP conduct more short-term formal, training courses.
6. Technical Advisory Committee: The team felt that, as presently constituted and utilized, the TAC did not sufficiently serve the needs of IFRP for systematic review of particular research proposals and projects, providing only an overview function. Thus, it strongly recommended that all research proposals be subject to thorough and rigorous review by a revitalized and re-oriented TAC.

This is a large, complicated and expensive project.

II. SUBCOMMITTEE COMMENTS

Dr. Linder thought there were many points about the project that could be criticized. However, at this point he preferred not to discuss the details of the project in depth. He personally thought it was a poor project of low grade science. He would go along with this review committee providing that the review is not a whitewashing of what is really being done at IFRP.

Dr. Linder pointed out that the relationship with University of North Carolina which was described in the project documents doesn't exist. It is quite clear from the previous reviews that the IFRP is professionally isolated.

Dr. Montgomery asked with so many flaws in the program, why should the PARFR program continue? Or better yet, why not continue only those activities in the program that are essential to AID's Population program? Even though the data base is not what it should be the information that has been provided by IFRP has been valuable and if this organization did not exist it would have to be invented because such an organization is necessary.

Portions of the portfolio are supervised and standardized. There is some research done in that portion. There are documents describing the program, one is describing research and one describing a technical assistance component.

Dr. Montgomery could not distinguish between the two in terms of the activities that were undertaken. He pointed out that there were reports of tension among the staff between those in research and those on technical assistance activity. Research activities are reviewed; the technical assistance activities are not. Why is IFRP in research? The leaders in the program say they need to establish credibility among scientists in the Population field. Dr. Montgomery was not sure that they added to their credibility with statements of this type.

Dr. Wishik commented that there was a need for a thorough review. Even if there were no technical reservations about the program the fact that it is carried out at great expense to AID would warrant a special indepth review. The review, in Dr. Wishik's opinion, should include an overseas trip to observe activities in LDCs.

Dr. Wishik thought the intensive review should include a statistical look at the material in the data file at North Carolina. He was wondering if just any type of data can be put into the IFRP data bank.

II. OTHER COMMENTS

Dr. Carter asked why are there no other donors to the IFRP? The program presently is almost totally funded by AID. Second, he wondered why it wasn't associated with the University. During an earlier review there was some suspicion because the IFRP printed their own journal.

Dr. Carter pointed out that it appeared to him that an enormous amount of funding was being spent and the management of that funding was somewhat out of control.

Dr. Connell did not think that the IFRP was such a monster. She pointed out that IFRP had, itself sold the journal that it published. For economic reasons there are elements of the program that were out of control but not irreversibly so.

Dr. Anderson asked how did we get into this program? Did not RAC want to look at the project carefully?

Dr. Moss said the project was extremely difficult to deal with. He asked for some of the RAC members to point out some of the excellent things that the project had accomplished.

Dr. Connell remarked that there were enough good points. There are many projects that are well conceived and well carried out. The problems in many of the areas of the IFRP program were not insurmountable.

Dr. Carter asked if IFRP had a continuing relationship with outsiders.

Dr. Montgomery pointed out that when the project was site-visited the last time it came to RAC the review committee looked at some new research proposals. New research was discouraged then because the staff expertise at IFRP was not sufficiently strong. He did not think that the IFRP had structured a process for continual appraisal of research since then.

Dr. Wishik asked what are the merits of the program? He pointed out that the breakthrough in contraceptive techniques was accomplished after the disassociation of contraception and sex. Contraceptive technology which the IFRP is field testing

can't breakthrough some of the obstacles to these family planning methods. There are only two organizations, WHO and IFRP carrying out research on contraceptive methods. WHO does not subject new methods to Phase III testing, IFRP does.

Dr. Wishik pointed out that IFRP has made sufficient contributions but it is necessary to learn more about what these contributions are. The program needs to be narrowed and tightened up and controlled better. He felt that the ongoing relationship with a RAC subcommittee was necessary. Finally, Dr. Wishik thought that the Population Office should act to set up a strategy plan for contraceptive research.

Dr. Elmendorf remarked that as a field worker who has worked for a large organization such as IFRP, she feels that this program is important. Better organization is needed but the importance of the work is beyond question. With regard to the data bank, Dr. Elmendorf felt the great need was for following through on new prospective contraceptive techniques rather than obtaining quick results. She pointed out that the IFRP had no women on its research staff.

Dr. Speidel, of AID staff, remarked that the project plays a key role in AID's population program. As an example, IFRP has provided a reliable set of guidelines on more than one occasion which were used to make decisions on the purchase and distribution of IUDs and other contraceptive devices. However, he also felt some frustration since the program was not living up to its initial promise. It has not become the outstanding field research unit that it was intended to be. Steps had been taken to resolve problems in the organization management of IFRP but the reforms were not fully realized.

Dr. Speidel pointed out that the University relationship with IFRP didn't really work out as it was planned. Initially, the field work was to be carried out with IFRP being located within the University of North Carolina. This did not work out and it was necessary to establish a separate unit. As for the funding of IFRP it was true that most of their funds come from AID. However they do have one \$600,000 grant from the National Institutes of Health. The organization is being encouraged to seek diversified support.

With regard to the research versus the technical assistance or grant part of the AID support for IFRP, Dr. Speidel remarked that AID was keeping the relatively rigorous research under contract. Grants had proven to be better for the other type of

work that the IFRP does for AID. As for a contraceptive stragtey program, Dr. Speidel did not wish to address that issue off the top of his head.

Dr. Shelton, also of AID staff, pointed out that apparently everybody agrees that some good is coming from IFRP as are some accomplishments that are not so good. He personally thought that the contributions of the IFRP are very good. It's true that almost every decision the Agency has made in the population field has relied very heavily on IFRP data. Perhaps we are making decisions on not so good data but there has been good correlation with other work. He agrees that they lack some senior people and that they need to improve the role of the technical advisor. He didn't agree with the report on the differences between the grant and research. There are definitely differences in the type of activities carried out under each. He did not think the organization was out of control. There are only 99 people working and the yearly budget is smaller than previously (2.0 million last year as compared to 2.3 million for the previous years.) A lot of these issues were much worse in 1977 when the project came to the RAC and progress has been made in resolving the problems.

III. MOTION

That the proposal be approved with a three-year authorization but only a one year approval for financing at the present time; (2) That the chairman of RAC appoint a RAC subcommittee which will have an overview function for the duration of the project; (3) That a review team be constituted. The team should have representation from RAC, AID and the IFRP Board of Directors. The team should have expertise in management, biostatistics, medicine, epidemiology and demography. Selection of the team members should be carried out jointly by the AID Population Office and RAC; (4) That the team conduct an in-depth review of the entire IFRP program funded by AID, both the grant and the contract. This on-site review should encompass the activities in North Carolina and a representative number of the overseas projects; (5) That the team write a comprehensive report of its findings and make a series of concrete detailed recommendations; (6) That this report be completed as rapidly as possible, consistent with thoroughness, but, in any event, within one year of this date; (7) That a status report be sent to RAC by the Population Office prior to its fall meeting in 1981; (8) That a final report covering the team's recommendations be made available to RAC prior to its winter

meeting early in 1982; (9) That decisions regarding recommendations for future funding and program content beyond one year be made at the time of that winter RAC meeting.

Dr. Wishik wanted to make a friendly amendment to the motion. He thought the scope of the review should include both the grant and research under activities under IFRP.

Dr. Rechcigl asked about the role of the contractor in the review.

Dr. Speidel pointed out that the contractor will work with the review team.

Dr. Montgomery asked about a continuing relationship between IFRP and the RAC.

Dr. Connell reported that she did not see this as a single time involvement of the RAC. She felt that the relationship between the RAC and IFRP would continue.

Dr. Wishik pointed out that there were two ways of having a continuing relationship with the RAC, one would be to have a subcommittee within RAC to review the project continuously, the second approach would be to have outside experts plus a RAC subcommittee that would continue for the life of the project.

Dr. Anderson asked Dr. Linder why wasn't the project eliciting a collegial relationship with the University of North Carolina? Why aren't other professionals talking to them? How much of their work shows up in publication, etc.?

Dr. Linder sketched the history of the relationship between the University of North Carolina and IFRP. Originally, the project was in the medical school of the University. There were constant conflicts between the project and the University administration. The project was impatient with administrative procedures at the University. The IFRP engaged in human experiments using devices that may or may not have had the necessary approval of the FDA and others. The University was afraid of reactions to this. They did not have a formal relationship with the Biostatistics Department of the University of North Carolina. Finally, the relationship dissolved because the IFRP did not accept the advice of the members of the statistical faculty and others in the University. It was a messy sort of divorce. However, this did not prevent consultation from members of the University faculty to IFRP sponsored projects.

IV. VOTE

The motion was approved with two abstentions.

The chairman of the RAC interpreted the motion to mean that a permanent subcommittee of the RAC would be established to work with IFRP in keeping the program under review.

Dr. Smuckler will advise the Committee of the permanent chairman who would be appointed shortly.

Forest Surveys - Contractor(s) to be Selected

I. SUBCOMMITTEE REPORT

Dr. Ehrenreich, chairman of the subcommittee, that also included Drs. Hrones, Moss and Pimentel reported as follows:

The proposal, "Forest Surveys", a research proposal to monitor tropical deforestation by remote sensing, is a very timely, appropriate and important one considering the importance to the world community of the world's tropical forests. This project would be a logical first step in developing a global research strategy in helping to solve the problems of tropical deforestation.

The research proposed would address five principal areas of forest survey activities: (1) delineation of forest types from images (stratification), (2) automatic classification, (3) change detection, (4) sampling, and (5) area measurement. The research is being designed to interrelate these five topics into a systems approach to forest survey. Dr. Paul, the project manager, is to be congratulated on producing a fine draft proposal. He is obviously up-to-date on the status of tropical forestry and the problems associated with Landsat computer--aided processing. The only important omission appears to be the work done by Dr. Donald Stellingwerf in Indonesia and reported in the The Manual of Remote Sensing and in ITC reports.

The RAC subcommittee assignment is to review this draft proposal and offer comments for improvement. Hopefully, these comments will be helpful to Dr. Paul in polishing his excellent draft proposal.

Page i and Page 1: The statement "to effectively exhaust the world's supply of tropical forests" may be a bit exaggerated, and would suggest picking an example from some country or region to illustrate the point that the tropical forests are being depleted.

Pages 1-4: The Introduction and Background is generally convincing of the need for the proposed project although some of the subcommittee members have a few comments which are worth Dr. Paul's consideration.

Pages 4-6: The case is well made here that the tropical forests have never been accurately inventoried and it is indeed essential that a baseline study such as the one proposed here be made to assess extent and type of tropical forests as well as rates and directions of change. Remote sensing (including low and medium altitude photography) followed by ground true

measurements is the only practical way such baseline information may be obtained. As Dr. Hrones points out, this is particularly true for tropical zones in LDCs where there are huge roadless expanses of unsurveyed land.

Page 6, line 9: aerial or area?

Page 7 last sentence: After a time there

Page 7-17: An excellent description of tropical forest parameters, problems in measuring them and the need for this information.

Page 12, line 5: ...or natural disasters such as stress, insect or disease damage ...

Page 15, line 6: ... Landsat

Page 16, line 15: ...contrast ratios ...

Page 18-50: Again the section on Review of Current Remote Sensing Techniques in relation to forest stratification, classification, change detection, sampling strategy and area measurement is very well done although there are a number of specific comments listed below.

Page 24, line 15: In forestry ...

Page 26, line 10: each of the Landsat ...

Page 26, line 15: drawn Landsat ... discrete ...

Page 27, line 16 & 17: Strahler et al ... technique

Page 28, line 13: assigned to classes ...

Page 29: Page 29 is missing.

Page 30: On page 30, there is still disagreement about combining pixels into groups of 9 pixels and using the new values in classification. For one thing, it lowers resolution and the shifting cultivation fields are usually of the order of 2-3 pixels.

Page 31: On page 31, principal component (PC) analysis appears more fruitful than linear discriminant analysis. The International Training Center in Enschede, The Netherlands has done a good bit of work on this (Mulder et al.).

on line 12 should be LDA is a ...
on line 18 respectively:

Page 34, line 12: of Landsat ...

Page 35, line 11: (1980), a

Page 36, line 7 & 8: is required
patterns of change, ...

Page 37, line 1: approach ...

Page 38, line 14: Thus, a second iteration of ...

Page 39, lines 2 & 13: ... in equation 6.
of this proposal. This will ...

Also concerning page 39: to get at seasonal aspects or change detection over years, you may use optical combiners as was done to detect newly irrigated fields (Heller & Johnson, 1979; last 2 pages).

Page 40:

Under a) basal area

Under c) can identify tree species only at extremely large scales.

Under d) All of these could only be obtained from the ground or from very large scale.

Page 41, line 14: Langley

Page 41 & 42: The 2nd equation on nested probabilities is not shown. The equation on the bottom of page 42 is the equation for the Coefficient of Variation.

Page 44, line 7: ... inventories (Langley, 1975).

Page 46, line 11: However, in situations ...

Page 48, line 4: Landsat

Page 49, lines 5 & 17: Circular
(and, if necessary, K₂)

Page 50, last sentence: This study will not prevent depletion, but merely provide some baseline information necessary for formulating a plan to prevent depletion.

Page 55, line 14: ... combine fine spatial ...

There are seven other general comments on the proposal which may be of use to Dr. Paul:

65

- 1) There is no doubt that a large research institute such as ERIM in Michigan, LARS at Purdue, Remote Sensing Institute at Brookings, or a large consulting company such as Lockheed, Earthsat, Electromagnetic Systems Laboratory, etc. could address such a proposal. Upon completion of the research, could the developing countries with lack of trained personnel, adequate computer facilities and software programs handle such a program? Would it be more realistic to use Landsat images optically with the accompanying aircraft (multistage) photographs and ground data? The developing countries could handle this with a minimum of specially trained people and lower cost equipment.
- 2) Although there are highly qualified people to undertake such research, it is obvious that increased training programs for LDC personnel are also needed. Should these training programs be part of this proposal?
- 3) If shifting cultivation advances into the forest in bands, could not these bands or lines be the stratification on which most research is centered?
- 4) Cloud cover in the moist tropics accompanied by the small size of newly cleared forest areas may make Landsat a poor source of basic data.
- 5) The Landsat program is in temporary trouble. Landsat 1 is out of commission, Landsat 3 has line-start problems and is now only recording 16 gray scales instead of 127, and Landsat 2 has not video tape recorders operating. In other words, only Landsat 2 is a currently reliable satellite and then when in line-of-sight of a ground receiving station. The study could use old satellite data for the past land cover condition but the new data may be difficult to acquire. Landsat D is now scheduled for late 1982 and with the Thematic Mapper still later. Finally, in making change detection assessments with two date images, registration problems both optically and with the computer are difficult. Pixel registration is difficult for 8 channels of data even with the same satellite. Detecting 2-10 pixel land cover changes under these conditions is problematical.
- 6) There is general agreement with Dr. Pimentel's comment:

"The proposal points out why tropical forests are valuable and why they should be assessed (monitored), but little or no mention is made of how these data will be used to conserve the world forests for the benefit of mankind. One might legitimately ask, why assess the problem when we already know that it is bad? Why not spend any money that is available for reforestation?"

7) This proposal, if carried out, would be of immense value to the LDCs and the entire world. AID would receive tremendous recognition for such an effort and it is predicted that these research results would be some of the most often used throughout the world for decades to come.

II. SUBCOMMITTEE COMMENTS

Dr. Hrones' comments are summarized as follows:

1. The use of remote sensing is essential to the management of natural resources. There is no other practical way to monitor on a continuing basis the state and rate of change of those resources. This is particularly true of tropical zones in LDCs where there are huge expanses of unsurveyed land.
2. The results of the proposed research can be used worldwide.
3. The launching of Landsat-D will provide important additional data.
4. To develop the much needed forest survey system, research of the nature indicated in the preliminary project statement is essential.
5. There are highly qualified interested people available to undertake the research.
6. Extensive training programs for LDCs personnel are needed.

Dr. Moss remarked that he didn't know much about tropical rain forests. He read some of the language in the RFP that seemed to dramatize the need for forest measurements somewhat. He thought the RFP was a bit judgmental. The remark that tropical forests produce a large proportion of the oxygen in the atmosphere is an example of the proposal's exaggeration. He felt the oxygen there would be produced no matter what type of vegetation was growing there.

Dr. Moss thought that based on the present evidence it would be difficult to use satellite imagery when the land was sloping.

Despite these observations, Dr. Moss thought the proposal was sound and should be supported.

Dr. Pimentel thought the RFP required a paragraph or two to say how the data from the surveys would be used. He also asked, why not simply proceed with reforestation rather than spend large sums of money measuring changes in the tropical forest? He thought that this particular point should be discussed in the proposal.

Dr. Pimentel then provided some specific comments on the proposal. First, he thought that a specific region should be used as an example to help strengthen the proposal. He then pointed out some inaccuracies in the text of the proposal. For example, the proposal states that only five nations have coniferous forests. Also the statement that 40-50 percent of the earth's species of plant life exist in the tropical is an exaggeration. These should be checked.

He thought the proposal should discuss more of the large quantities of plant and animal food harvested from the tropical forest. Such forests are used for much more than conserving water by reducing runoff and helping to stabilize world climate.

Like other members of the subcommittee, Dr. Pimentel was very much in favor of the proposal, but made suggestions for improvement, since the proposal was in a relatively early stage of development.

III. OTHER COMMENTS

Dr. Falcon was less enthusiastic about the proposal than were other RAC members who commented on it. His primary concern was with the project as part of the overall research strategy of the Agency. On the previous day of this RAC meeting the Committee had discussed a small grants program which delegated important research decisions to outsiders. Today this particular project appears to be a hardware program without a well defined purpose.

Dr. Falcon wondered where the Agency is going with regard to its research program. As a research strategy in forestry the Agency would not start with this project. He felt that since the project was basically a hardware project that perhaps NASA should be doing this particular piece of work.

Dr. Falcon felt the fundamental problem was not to measure the tropical forest but to determine public policy with regard to the tropical forest. He feared that the project would produce maps and data that would not be interpreted without some need for public policy behind it.

Dr. Heady asked if one of the major goals of the project should not be concerned with the potential for food production from land that is cleared in the tropical forests? He recognized that the program would give a good inventory of slash and burn type agriculture and this had great long run importance. However, the measurement of change in the forest should be tied in some way to food production potential.

Dr. Elmendorf suggested that the Agency look at the people who live in the forested areas as they relate to reforestation and other activities that might result from the forest survey data. She suggested that if there were indigenous people in the area, that those people could reveal secrets for effective use of the areas surveyed that might not be obvious to outsiders.

Dr. Carter pointed out that there was an opportunity here to internationalize this project. There is an opportunity to cross national borders and move into the international sphere. This opportunity was not available with many AID projects.

Dr. Hrones pointed out that to an engineer this would not be a hardware project. It would be very much an application or software project. With regard to the issue of priorities, Dr. Hrones remarked that reforestation as well as depletion of the forests are long range problems. An investment of such an amount as that proposed for this project would not contribute significantly to solving the depletion of the forest nor to reforestation. Reliable survey methods to detect forest changes would be a significant contribution to what will certainly be a continuous problem that requires close monitoring.

Dr. Ehrenreich agreed that there was a need to see a general forest research strategy. There was now a number of forestry projects in AID, including some in the regional bureaus. The Agency should be encouraged to develop an overall strategy in this area.

Dr. Elmendorf asked again if it would be possible to get information on the people as well as the forests as part of the total natural and social resources picture. And she asked a second question. What was the relationship of this project with the Man and Biosphere project which was also reviewed by the RAC?

Dr. Feldman, of AID staff, commented that AID has formulated a policy statement on forestry. Policy Determination No. 74 is a sort of a declaration of general intent to go everywhere and do everything in the area of forestry. The policy is intended to make it possible for missions to attack specific forestry problems and yet allow the Agency to do other things. One central theme of the policy concentrates on the fact that people live in the forests and because of this there is degradation of agriculture soil. There is a question whether the tropical forests should be retained and conserved or whether some removal of the forest should be permitted to permit more agriculture. That part of the forest which is yet undisturbed is now in the process of being disturbed and it's important that the Agency try to monitor the changes that are occurring.

As far as public policy is concerned, Dr. Feldman pointed out that there are other projects which get to this issue. The Agency approach is sensitive to the fact that even though some things are technically possible, unless methods for the collection and use of data are developed public studies will not be carried out.

Dr. Feldman pointed out that the Agency has some \$200 million dollars in forestry projects. DSB projects are supposed to provide the cutting edge in support of this investment. He commented that the proposal would cost \$4 million dollars for six years. In the view of most people who reviewed it, the proposal is for a software or application project that will support the larger AID effort in forestry.

Dr. Feldman remarked that the Policy Determination No. 74 would be sent to the RAC members.

Dr. Paul, of AID staff, expressed appreciation for the RAC's comments. He pointed that he is very sensitive to the issue of how data from the surveys will be used. The research proposal does not say anything about implementation of the project beyond the collection of data. There is a need for ground sampling methodology which was not described in the proposal. He commented that the Agency is in constant communication with the Food and Agriculture Organization of the United Nations (FAO) and the positions of AID and FAO are compatible on the subject of forest inventory. The data collected as a result of this project should be of tremendous help to FAO in carrying out its charges in the area of forestry.

With regard to the question about NASA doing this work, Dr. Paul pointed out that NASA has no international responsibilities except for some work they had done in the area of grain crop measurement in China and the Soviet Union. NASA has no international clients and therefore projects such as this one must be supported by organizations such as AID. He pointed out that training might have been overlooked in the RFP and this would be corrected. It was intended to bring professionals from LDCs to the United States for two years of study in U.S. universities.

Mr. Bob Otto, of the LAC Bureau, commented that the LAC Bureau had serious questions about the project. He felt that it was time to look at the payoff from such a project at a cost of \$2 million dollars. He felt the cost benefit ratio was not very high.

Dr. Rechcigl pointed out that it was not the usual procedure for regional bureau representatives to comment on projects before the RAC. Such comments were usually taken into consideration prior to a project coming to the RAC.

IV. MOTION

That RAC go on record as recognizing the importance of this proposed research and further encourage AID to proceed with the necessary steps leading to funding and implementation of this program.

V. VOTE

Unanimous in favor.

Nitrogen Fixation by Tropical Legumes (NifTAL) - University of Hawaii

I. SUBCOMMITTEE REPORT

Dr. Moss, chairman of the subcommittee, which also included Drs. Black, Greathouse, Peterson and Swanson reported as follows:

This is a second extension of an original three year contract previously extended for three years. This project has been identified by DS/AGR as being of high priority, and its extension for five years has been included in DS budget plans. The plan of action presented in the proposal may be expected to provide substantial benefits to LDC countries who must increase food production with minimum inputs. The cost estimates are appropriate for the planned level of effort; reduction of budgets will result in reduced level of effort.

The project apparently will be funded as a "cooperative agreement." Perhaps Dr. Frederick could comment on the implication for RAC if that contractual arrangement is indeed used.

Rationale for Extension

The main rationale for extension of the NifTAL project is that the facets of the food/fertilizer/energy scenario which justified the original (1975) proposal and its extension in 1978 have persisted and intensified. The project is on target in its projected ten-year life and the in-country outreach programs have been welcomed to an extent that could not have been anticipated. The investment of time and resources in forming a highly qualified team of scientists, developing the methods and assembling the materials needed for in-country field trials with legumes and rhizobia is now paying off. There is demand for NifTAL's services worldwide and there is active participation by a host of institutions (at their own expense) in the biological nitrogen fixation programs coordinated and backstopped by NifTAL. Other international BNF programs, some funded largely through USAID, draw on NifTAL for support in areas in which they do not have specific expertise (training/-inoculand production), for materials, and to extend their testing to regions in which NifTAL has strong institutional linkages.

With increasing frequency, the success stories of the "green revolution" are being reappraised as the limitations of production systems that depend so heavily on high inputs become apparent. Sustained productivity with low inputs is now recognized as a more valid objective than high yields in the short term. Thus biological nitrogen fixation is being resorted to increasingly for sustained production of high-protein crops with minimal dependence on nitrogen fertilizers.

Given the multi-facted needs of the urban rural poor in developing countries, legumes have a potential importance not only for food production but also to provide timber for shelter and fuel for cooking.

Given the protfolio of BNF programs supported by USAID at the International Agricultural Research Centers that deal wthi the world's major commodity legumes, an ability by NifTAL to give attention to innovative technologies with less-known or minor legume species is the key to provision of balanced, comprehensive development support to countries seeking to exploit BNF to the full in their agriculture. NifTAL, more than any of the IARCs, has the flexibility to be responsive in addressing innovative technologies because of: the size and expertise of NifTAL's research team which specializes exclusively in BNF; the range of tropical soils and climates accessible within Hawaii for field-testing legume production systems for specific regions of the world; and also because NifTAL is not so rigidly tied to the highly focused commodity and geographic mandates that have emerged as a characteristic of the IARC management regimes. Thus, although several other BNF programs have emerged as a logical consequence of political and economic pressures to perfect agrotechnologies that use less nitrogen fertilizers, the NifTAL project continues to have a unique and major international role to play.

Specific rationales for this proposed extension of the NifTAL project are:

- 1) that the three-stage program of developing country field trials, begun in 1979, should be completed;
- 2) that a capability for provision of BNF services should be generated in the regions from 1983 and within developing countries by 1986;
- 3) that agrotechnologies developed in the U.S. and Australia and modified in Hawaii should be fine-tuned to regional (by 1983) and national (by 1986) circumstances; and
- 4) that the project be permitted to arrive at its projected conclusion, its objectives having been accomplished.

PROJECT MANAGEMENT

The project is the exclusive occupant of a suite of spacious buildings on the island of Maui. Linkages with the main University of Hwasii campus at Manoa are assured through location there of the NifTAL Information Office, Communications Center

and laboratory, two key professional personnel and the NIFTAL Graduate Assistants.

Two of the project's objectives will be undertaken through subcontracts to institutes with predominant expertise in those subject areas.

The overseas operations (INLIT) are coordinated on a regional basis with project staff assigned as Regional Coordinators for Africa, Asia, and the Americas.

Training courses will continue to be mounted in developing countries with cooperating institutions.

Over the period of the extension, the hub of project activities will transfer from Hawaii to the Regional BNF Resource Centers.

TECHNICAL ASPECTS OF PROJECT BACKGROUND

The NIFTAL project recognizes that the major constraint to fuller implementation of legume-based BNF technology in developing countries is inadequate understanding of the interactions between host legumes, rhizobial strains, and the environments in which they are grown. As a result it is impossible to predict whether a legume will respond to inoculation in a particular region.

The NIFTAL project has been designed to complement other related projects sponsored by national and international agencies in tackling the constraints to fuller implementation of legume-based agrotechnologies in a comprehensive and balanced manner.

The NIFTAL project has assembled, and will preserve in perpetuity, a major genetic resource housing strains of Rhizobium of worldwide origin. Technicians and professional researchers have been trained.

PURPOSE

The main purposes of this proposed extension of the project are: to permit completion of the three-year sequence of developing country field trials implicit in the INLIT concept; to provide further technical and professional training; to develop methodologies for quantifying the nitrogen balance in legume-based technologies through selection of superior rhizobia for tropical soils and simplification of the inoculant production process; development of appropriate inoculation methods for use by small farmers in developing countries, and to establish

regionally located BNF Resource Centers for provision of technical assistance in inoculant production, developing training programs for extensionists, performing regionally-specific adaptive research, and quantifying the nitrogen balance in legume-based cropping system of the region.

In 1986, it is intended that NifTAL will have catalyzed a capability within developing countries for implementation and further development of the BNF component in legume production systems. The support in the form of research services and technical assistance that is currently provided centrally by the University of Hawaii NifTAL project will become increasingly available on a regional basis after 1983 through the bnf Resource Centers that are proposed for Asia, Africa, and the Americas. By 1986 the scenario in which legume-based BNF technologies are being implemented will be such that the needs of developing countries for this research can best be met nationally and regionally, and it is therefore proposed to terminate the NifTAL project in 1986.

SPECIFIC OBJECTIVES

Objective 1

Assemble a Rhizobium Germplasm Resource comprised of authenticated, characterized rhizobial strains from a wide array of plant hosts and diverse geographic origins, from which to select strains which effectively nodulate agriculturally important legumes under the conditions prevailing in developing countries.

- a) Augment Rhizobium Germplasm Resources (currently have collected and maintain 1500 strains)
- b) Identify three Rhizobium strains for each potentially important legume (now have 3 for 13 priority legumes)
- c) Produce legume inoculants on a pilot scale (now do this in small quantities in Hawaii)

Objective 2

Establish a serum bank housing antisera for identifying the strains of Rhizobium used in inoculants for the International Network of Legume Inoculation Trials (INLIT). This will be a new activity to develop rabbit and anti-Rhizobium antisera for identification of strains and make this available to researchers.

Objective 3

Determine the economic yield benefit resulting from the inoculation of local varieties of agriculturally important legumes under the soil and climatic conditions of the regions in the tropics where they are, or will be, grown.

This will include continuation of worldwide trials (100 now being conducted), providing materials and technical advice to cooperators (a procedural manual was published in 1979), performing statistical analyses for cooperators, and distributing information. NifTAL has cooperated in organizing an International BNF workshop at CIAT in Cali, Colombia, March 9-13, 1981.

Objective 4

Develop systems for improved inoculant delivery, and for ensuring dependable, effective nodulation in the field.

This activity was postponed, to be initiated during the extension.

Objective 5

Develop improved techniques and cultural practices for increasing the nitrogen fixed and the contribution of nitrogen to companion or following non-leguminous crops in tropical cropping systems.

This would include developing methodologies for quantifying the nitrogen balance in tropical cropping systems and employing it to study nitrogen balance in cropping systems. This would include evaluation of leguminous trees for improvement of grazing and wood supplying capacity of non-agricultural land.

Objective 6

Establish a Legume Germplasm Resource comprised of seed of those legumes that have an agricultural potential in the tropics but which are not readily available to researchers in developing countries from commercial sources, nor from the world's major Germplasm Resource Centers.

Currently NifTAL gets about 100 requests a year for seed of special promising legumes which they have catalogued.

Objective 7

Study the effects of soil infertility on symbiotic performance of legumes in order to define tolerances, guide choice of

legumes for agricultural systems, improve performance, and lower costs of soil management.

This would be a subcontract to the University of California, Davis, and would include a search for phosphorus efficient species. It would also test validity of current greenhouse screening methods for classifying Rhizobium strain activity.

Objective 8

Provide economic information on the relative costs of BNF technology and of fertilizer nitrogen technology through an economic analysis of the demand for legume inoculants.

This would be a subcontract to the University of Minnesota to be completed in 18 months. Dr. Swanson will have specific comments on this objective.

Objective 9

Provide for regional and in-country support to legume programs in developing countries.

This includes identifying a capable cooperative institution in each of the three developing countries prepared to host a Regional BNF Resource Center to provide for research, technical assistance and training support to the African, Asian and Latin American regions. They will locate an outreach scientist specializing in BNF technology at each Regional BNF Center by the end of 1983. They will install a pilot-scale inoculant production plant at each Center by the end of 1984. Also, they plan to assist former NifTAL trainees now located in developing countries to design relevant production-oriented research programs by providing \$10,000 competitive grant support to 15 NifTAL trainees.

Objective 10

Train 14 professional researchers, 140 trainees, 50 technicians, and in a new activity, train extension workers for their respective roles in the development and implementation of legume-based BNF technologies. They would also provide 12 internships at NifTAL.

Dr. Black, a member of the subcommittee, who is not present submitted his written comments on the project as follows:

Last July I was the RAC representative on the evaluation team for the NifTAL project in Davis, California and in Hawaii. I admit to being somewhat skeptical regarding the use of legume

inoculants in agriculture; but these workers have convinced me that it is worthwhile, particularly when introducing new legumes.

This project obtained excellent leadership in Jake Halliday. He is young, vigorous, and has a "no bureaucratic nonsense", "let's-get-the-job-done" sort of personality. RAC can be confident that Halliday will push everyone on the project (and no doubt ruffle a few feathers in the process). The clarity of the project write-up for RAC members to read is an example of Dr. Halliday's ability to provide clear directions.

The project has been successful. They have a fine culture collection. They have means for testing field inoculation through fluorescent antisera. They have a network of legume inoculation trials operating in tropical agricultural situations now. They have developed a carrier system (peat primarily but they are looking at other locally available vegetative carriers) which can be readily used in LDCs to deliver the inoculants. They have small pilot plants for growing the bacteria which work. They realize the diversity of environmental problems in tropical agriculture which may influence successful inoculation (i.e., temperature, competition, pH, Al, etc.) and are addressing these. This is an area where the California basic research input is particularly useful.

They have and are developing a delivery system in LDCs. This was particularly impressive--the training of people, then the pilot-plants, then the peat carrier, then application and field testing.

They are covering the breadth of uses for tropical legumes - not just food or bean type legumes. For example, they have work on tree/shrub type legumes for use in soil conservation--erosion control, or for use as livestock browse, or for use as wood.

In an overall view, certainly the staff is fine, the facilities are ok, and the training is working. In other words, the project is operational with a "head-of-steam."

Dr. Black was surprised at the large budget. But since RAC tells itself it can't set budgets, AID staff is expected to support the budget. Personally, he would favor a reduction but have not studied the budget closely. The detailed budget in Appendix C does not show much "slack" where he could suggest cuts.

Finally, he likes the fact that the project predicts a termination date (on page 4) with its work done and the results turned over to LDCs to carry on.

Dr. Swanson reported as follows:

Objective III (page 6) - This objective deals with the determination of the "economic yield benefit resulting from inoculation of local varieties..." It is not clear until one reads about the planned output that the purpose of this economic yield benefit analysis is apparently the selection of site and legume crop by the principal investigator and his staff. The initial impression is that the determination of the yield benefit is to assist the producer to determine if inoculation should take place.

Objective V (page 7) - This is a very important objective and without information on the contribution of nitrogen to companion and/or following non-leguminous crops one cannot really evaluate the "economic yield benefit" in Objective III. There is a danger that an economic yield benefit calculated in Objective III may not correspond to the economic benefit when the particular crop is placed in the context of the total cropping system.

Objective VII (page 8) - This objective proposes to study the effects of soil infertility in order to "lower costs of soil management." It should be emphasized that lowering costs of soil management is a means to an end (increasing yield) and not an end in itself. It may not always be optimal from the standpoint of the total farm business to lower the cost of soil management. These efforts might better be applied somewhere else in the crop enterprise in order to increase crop production and to lower total costs of producing crops rather than the costs of soil management.

Objective VIII (page 9, Subcontract B pages 36-39)

The subcontract calls for three separate components: (1) specified farm model, (2) inventory available data, and (3) estimation of demand.

First of all, given the regional variation within countries and among countries, it is not likely that a single farm model can usefully capture the essentials. Consequently it will be necessary to choose from among areas and this will require some judgements made in close collaboration with the principal investigator.

In the section on inventory of available data it is indicated that the analysis will be restricted to the single region. This is somewhat disappointing in that the results are not likely to give the desired generality.

The third component "estimation of demand" seemed to be somewhat obscure. The stated intent is to estimate an aggregate demand function. The time for such an estimation should have been before this project started. After all, most of the efforts in providing the inoculation technology that will eventually be available for a very low cost (approximately one percent of the seed costs) is from public funds. Presumably the research effort has assumed from its beginning that there will be a substantial demand from an aggregate view.

It is important that the analysis of the selected farm situations indicate how the inoculated legumes will fit into the cropping sequences. This is more important than studying the impact of such variables that are mentioned on page 36, especially the value of carryover nitrogen to the succeeding crops. The latter is an implicit component of an optimal solution. Although I would not want to generalize from U.S. Corn Belt conditions we found that with alternative cropping systems including corn, soybeans, wheat with a legume catch crop, and meadow, the elimination of nitrogen fertilizer as a source for nitrogen caused soybeans to become the most profitable crop. Meadow could be forced into rotation only if we required the farm to have a positive nitrogen balance. If negative nitrogen balances were permitted the rotation of corn and soybeans, although less profitable than continuous soybeans, at least in short run, was optimal.

Parameterizing the yield response to the previous inoculated legume crop would be an important aspect of determining just how much impact on the succeeding crop is necessary before it becomes economically optimal to shift to another rotation or to forego the use of nitrogen fertilizer. Are the experiments designed in such a way that this yield response does not have to be parameterized?

In any event, the need for an aggregate demand function for legume inoculants is not readily apparent. If a price analysis is done the companion supply function would also be needed. However, the private cost of inoculants is so small that this may not be a very interesting exercise.

All in all, this is a good proposal and its extension should be supported.

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Dr. Greathouse had the following comments:

The Project Paper is very well written. The SOTA and Technical Review is excellent but sober. The author is to be commended for not overglorifying Biological Nitrogen Fixation (BNF).

An analysis should be made of projected BNF research (type and magnitude) to be conducted in the Bean/Cowpea, Peanut, and Soil Management Collaborative Research Support Programs (CRSPs).

RE: Objective 9. It is probably overly optimistic to try to establish three BNF centers within the next five years. The three regions (Africa, Asia and Latin America) should be prioritized and only one selected to see: 1) if the concept will work, and 2) if a bnf center will truly reach and benefit all LDCs in the region.

RE: Objective 2. How does the current Rhizobium Germplasm Bank compare with others that evidently exist (such as Australia)? Has this been thoroughly analyzed for duplication of effort?

Considering current budget constraints, this seems to be an over commitment of research dollars when compared with other well-defined needs. A more thorough cost/benefit analysis is needed, with specific attention paid to the connection between BNF research and actual farm use. Some specific budget considerations are:

- a. Why isn't this project handled like a CRSP? Why isn't the University of Hawaii more directly involved administratively and on a cost-sharing basis? The UH contribution is about 2% compared to the 25% matching in CRSPs.
- b. From 1975-81 there were no facility lease and utility costs, but the 1981-86 budget shows a \$471,000 allocation for these two items. Why?
- c. What is the significance of a Cooperative agreement versus Research Contract (see cover letter Holliday to Frederick)?

Objective 8 should have occurred much earlier! An economic analysis should precede the decision to fund a major extension of this contract. Hidden constraints probably exist and may be nearly insurmountable. These must be identified before a major outreach program is initiated.

In Appendix E (p. E-12) of the PP, the author states "...the principal contribution of biological nitrogen fixation to human nutrition will continue to be a via the protein in legume grains." The conclusion of the author is that N contribution of a grain legume crop to a following cereal grain is small but significant (3-6 kg/ha). Studies conducted in the wet/dry tropics show an accumulation of up to 80 kg/ha of N in the A-horizons of soils at the end of the dry season ("Birch effect"). To use this illustration the need for soil management research focussed on NO₃ dynamics in soils as a major contribution to cereal grain nutrition. Cereal grain yields per unit area and per year are still much higher than for grain legumes. Also, the author's statement that "the priority given in legume breeding programs to improving their harvest index, i.e., maximizing the fraction of each plant's total production that is removed as grain should be called into question" is very controversial. The Egyptian plow is not a very good tool for incorporating legume residues and few farmers in the tropics have sufficient acreage or labor to allow land to remain out of production. To support the author's statement above, an intensive effort to increase the yield potential of grain legumes is urgently needed. Also, a greater effort to introduce improved higher yielding grain legumes to LDCs, along with consumer education programs, is needed.

In summary, Dr. Greathouse cannot support the extension of this project as presented. He recommended that an economic impact analysis be conducted to evaluate the cost/benefit ratio of a continued project in relation to LDC needs. He believes a RAC analysis is inadequate for sufficient long-term feasibility approximations. He further recommended the investment in an in-depth review of the degree of integration of this project with current and planned CRSPs involving this area of research.

II. OTHER COMMENTS

Dr. Heady pointed out that the University of Hawaii is contributing significantly to technology transfer. The University collected data and an economist had been hired to analyze the data for policy considerations. He would agree that the analysis of demand probably is impossible and maybe should be deleted from the project.

Dr. Ehrenreich remarked that under subcontract A the experimental design was not shown and included many vague statements such as "we shall compare." He noted the overhead charges, which were quite sizeable, and then separate charges for some items which would be normally covered under overhead.

Dr. Wishik asked what portion of the nitrogen would be supplied in a cropping system that included inoculated seed for legumes?

Dr. Frederick, of AID, remarked that that would depend on the type of system that was set up. If the crop is legumes, then all the nitrogen costs are taken care of through nitrogen fixation. With cereals about 20% of the cost could be supplied through nitrogen fixation.

Dr. Frederick commented that he usually uses a figure of 20% of nitrogen in a farming system which included nitrogen fixation. He pointed out that some 65 million tons of nitrogen fertilizers are used in the developing world every year. The cost of that nitrogen varied in the United States from anywhere from \$500 a ton to \$800 a ton. The cost to LDCs therefore, could be estimated to be at least \$50 billion dollars per year. Twenty percent, the saving of a significant proportion of this amount of money would be an important contribution to development.

Dr. Carter asked Dr. Greathouse to elaborate on his comment that BNF was overrated.

Dr. Greathouse responded that his concern was the cost/benefit ratio. Secondly, the concept of one university carrying out the research was not something he agreed with. He felt that the work should be carried out by a consortium of universities.

Dr. Frederick commented on the budget, why it was so high. He said that at the present time international cooperative trials would be carried out and airfares for people to participate in these trials was another item added to the cost. In general there were three or four new activities in the proposed list compared to the last time. There was the economic analysis that had been added and then the resource centers themselves had added cost.

Dr. Frederick commented on the importance of serology. He described an experiment where when the researchers finally learned to identify the bacteria that was fixing nitrogen, they discovered that there were no nodules of nitrogen when the soil already contained some nodules. This is why Hawaii is proposing to look at this particular problem. This particular phenomena added some work to the research. It is now necessary to identify strains of bacteria in the soil. He answered the question, why an economic analysis? He felt that only now is the project getting data that is reliable and only now could an economic analysis begin. He pointed out that Brazil in particular has developed a sizable inoculant program over 30 years and the work in that country was proceeding rapidly. He thought a five year extension was needed in order to prevent interruptions in the work.

Dr. Frederick answered the question of how this project interacted with the CRSP. He thought that this was an area for consideration by the Joint Research Committee of BIFAD. They did not want to include BNF in their priority area of research. He pointed out that the funding crisis prevented including BNF research in crop activities with one exception. There was a small BNF project in the beans and cowpeas project. The Agency does need a core activity in this area.

Dr. Frederick pointed out that he is constantly trying to get the project management to integrate more completely into the University of Hawaii. He noted that there had been important contributions from the University of Hawaii even though the project itself is located in buildings just off campus from the University.

Dr. Wishik asked, in view of the recent appreciation of differences in strains, could one have confidence that the effectiveness of the inoculant is stable?

Dr. Frederick replied that this varied with the legume.

III. MOTION

That the project be approved with consideration by AID staff of the suggestions made by RAC.

IV. VOTE

Unanimous for approval.

Dr. Greathouse then commented on the difference between the cooperative agreement as compared to a contract. He felt that this project has opportunities which may be missed under a contract, that the contract could not specify exactly what product should be produced in a project where new opportunities are occurring with new research findings.

Mr. Babb, of AID, pointed out that the field missions could easily access a contractor. The cooperative agreement was much more flexible for this purpose. There was no need to go through complicated changes in a contract when a cooperative agreement is in effect.

Dr. Wishik asked about when the project would be reviewed within the five year period?

Dr. Frederick responded in the third year.

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Research on Nonformal Education for Preliterate Adults - World Education, Inc.

I. SUBCOMMITTEE REPORT

Dr. Anderson, chairman of the subcommittee, which also included Drs. Elmendorf, Skinner and Tanter reported as follows:

The subcommittee is not asked to assess a proposal preliminary to approval or disapproval by RAC. Our task is to evaluate the activities that were funded. The request for extension of time (without additional funds) to complete the final report calls for no action by RAC.

Documentation is quite full. Additional information to be expected in the final report---especially details about individual villages--are available now only in brief form. But the preliminary final report (and supplementary material) is long and candid. There are serious methodological problems about the procedure; these are discussed below.

The contractor did what he proposed within the limitations of their understanding of criteria for evaluation, and these criteria can be debated. Whether the outcomes of the work are commensurate with the \$400,000 disbursed must be judged in terms of one's position on the methodological issues.

A genuine merit of the program is that it does not presuppose literacy of the people - particularly women--with whom it works. However, the coordinators from a village and the facilitators from outside are supposed to be literate. This program faces a difficulty common to all instruction (or "animation"): does it select its pupils for particular potentialities? The same question must be asked with respect to choice of villages. World Education or other users of this kind of program do not need to decide that question, but any evaluation data are strongly affected by this ambiguity.

Evaluation requires that one identify project outcomes. One is expected also to decide whether these outcomes serve development needs of individual and community. This task is compromised by multicollinearity. And, if the tasks or activities are chosen by the women of a village, who is to decide whether they facilitate development?

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The program is supposed to integrate the concerns of the women (as for health) "as (they are integrated) in daily life" (final report p.1). Each project is to be integrated. How does one identify this integration?

Women's aims and activities are to be linked to the economy, not something separate from or even superior to mere economic affairs--a highly commendable aim.

The program is supposed not only to teach certain skills but to also teach women how to learn. The report concedes that learning aides work well because they are used well, not because of their own features (Tototo report p. 30). Here again one confronts the difficulty that what works is determined in some part by the characteristics of the village social climate. Collecting data for that evaluation is difficult.

To identify women's principal needs (final report p. 2) and make women conscious of these needs is commendable. Women are supposed to be shown how their own goals relate to broader aims in development (Ibid p. 2). Both these tasks presuppose deeper knowledge of life in the villages than the outside "facilitators" are likely to have and presuppose more insight about the larger scene than the local coordinator can have. If a program works well, was it not a main need?

One often reads that planners must understand the social context for their programs. They must know what will be acceptable in each village. This is very difficult and in some degree is what this program is to demonstrate. Most judgments of "fit" or congruity in societal matters are post hoc. The backlash that accompanies almost every effort at ameliorating social conditions should tell us how rarely can sponsors of programs have this knowledge. One infers that this program relies on trial and error to find out what a village wants to do--and that is something that answers to a need of the village people. Knowledge of the social context in advance will be rare.

It is said that this program will study "learning processes" to see if they are carried out. How does one collect the pertinent data? How to structure the design so as to obtain such answers? The learning processes in community development are inchoate (perhaps fortunately); they involve many crisscrossing loops; they are directed often toward vaguely phrased aims. In particular, to decide whether unintended events have influenced the outcome of a program surely will rarely be possible.

The foregoing queries go to the foundation of the program--only makes its evaluation impossible. It is difficult to formulate aims, difficult to judge what different individuals will have done during the activities, difficult to identify influences at work. Seldom can one judge what were the crucial conditions that must be replicated in order to carry out similar sequences of activities elsewhere. Where this sort of program does work, the results can be impressive; in many or most situations success will be limited. Can odds be estimated in advance?

The AID in-house report by Barnes for Kenya lists the steps in a village program:

1. village leaders are consulted
2. villagers choose a local person to be trained as coordinator
3. these are given training
4. how a program works is explained to villagers (mainly women)
5. villagers are stimulated to express needs
6. villagers set priorities among their needs
7. learning sessions "pursue a specific interest"
8. villagers agree on how to proceed with task
9. central project administrators counsel local leaders
10. the local coordinator takes over and outside advisers drop out
11. an outside evaluation team makes its survey.

Relationships with government for one of these self-actualizing projects are ambiguous--as also often has been true for community have a range of services from government. In some places emphasis is put on the "unofficial" activities in the program and detachment from the central government's agenda of programs is recommended. World Education apparently prefers to work with nongovernmental agencies (proposal p. 10). But at several points it is stated that approval by the local village leader or local representative of government is a preliminary step in getting a program underway. There are sufficient instances mentioned of undue influence by local leaders (whether officials or not) to make one uneasy on this point.

Of nonformal education generally one can point out two variants. A NFE program can be deliberate; sponsorship or initiative can in varying degree be official or not, local in origin or dependent upon permission or support or cooperation by supra-village agencies. On the other hand, one can prefer when speaking of NFE to emphasize the situation in which it emerges due to the absence, deficiency, or malfunctioning of central

agencies. The degree of responsiveness to "local needs" will depend upon the particular mixture of these circumstances relating to nonformal program.

The project was supposed to have certain outputs (proposal p. 5):

- evaluation data (including estimates of cost & effective-
-ness)
- develop a research method for learning what gains are made
- provide guides for subsequent use in another village
- develop organizational and administrative patterns
- demonstrate how to shift responsibility to residents of
the village and away from the outside stimulators.

In the nature of the case, a "community development" approach cannot generate firm principles or generalizations in a form useful to new users of the method. Good field workers or leaders in this sort of an approach acquire lore; they often become sensitive and skillful advisors. By apprenticeship other individuals may absorb that lore, but they do this by working with successful leaders.

The judgments on this program are much the same as would be offered on a program in community development. The present findings add little to the literature or lore on community development. The "self-actualizing" procedure has been a basic feature of that earlier sort of program.

However, this program definitely is more free of anti-economics views such as characterized many (most?) advocates of community development. Art Mosher (once Chariman of RAC) was skeptical about using community development techniques for programs directly related to economic change.

These "self-actualizing" procedures could be made part of normal extension programs. Some readers now would say that it is less important to insure that solid research results are available for extension than they would have said before this self-actualizing method had become known.

There is no separate reason why the self-actualizing method should not be built into extension programs. Its advocates would contend that their approach emphasized freedom of villagers to choose and to carry out activities irrespective of whether the activities are aligned with programs sponsored by specialized agencies (health, agriculture, etc.). This procedure is a very labor-intensive way of stimulating local

groups to undertake self-help activities. Some observers would say also that provision of money to in-village coordinators subverts some important principles.

This procedure seems especially suited to programs conducted by religious and philanthropic organizations, as with many programs, it promises to flourish more vigorously when it is unconnected with officials.

When all its steps or stages are considered, this self-actualizing approach entails a very large amount of stimulation by outside personnel. But when one reads all the activities embraced in a successful village endeavor, one has to ask (paradoxically) whether the programming or training of these semi-professional personnel is sufficient.

It is difficult to decide that question. The reason is that villages in any society will vary greatly in their degree of integration, cohesion, or capacity for self-direction. This fact complicates any assessment of the procedures advocated in the project of World Education. When one places this method against the larger background of community efforts, one has to ask will it inhibit tendencies toward family enterprise or diligence. Surely the participants would be disproportionately drawn from the more change-prone residents.

Dr. Rechcigl posed four questions in his letter to the subcommittee. On the basis of the foregoing discussion terse replies are given here.

1. Did the project accomplish all of its objectives described in the project paper?

The project demonstrated a set of procedures by carrying them out (to varying extent) in several villages in Kenya and in the Philippines. Whether the accomplishments were novel can be questioned. There are formidable difficulties about finding cogent evidence.

2. What well-defined end products resulted from the result?

On the basis of evidence so far supplied, one cannot test the completeness of the protocols. The procedures intended are comparatively definite; to a varying degree in different villages these procedures for arousing and guiding participation by villagers were carried out; rather unsystematic comments about factors in success or deficiency of the procedures were offered. Due to the logical questions raised in previous comments, "well-defined" should perhaps not be applied.

3. Do the results have potential to be generally useful to LDCs or to AID's development assistance program?

As would be true of practices in "community development" (were such nowadays in fashion), the procedures could be used elsewhere in the world and could become auxiliary to many AID programs. But the procedures are inherently inchoate. Whether they are used and to what degree are questions not susceptible of precise conclusions.

4. Are there any precautions that AID should take in making use of the research results?

This is a procedure that could be recommended to many kinds of participatory programs. Whether it would be effective must depend on local capability in carrying through the operating steps. Effectiveness must also depend on the estimated correlation of the procedure with particular characteristics of the villages and villagers who would be included. But selecting the villages or estimating those correlations will be difficult, and this difficulty precludes prior estimates of likely success or of benefit/cost ratios for its use.

Some operations (including this one) can be evaluated only impressionistically because of the inherent complexity of the work.

If the AID staff had reviewed the literature on community development, they could have known that useful evidence would be very difficult to procure---irrespective of how effective "community development" may be in favorable circumstances.

We do not say that everyone know what the contractors would discover. We say rather that evaluating these kinds of operations is an intractable task.

II. SUBCOMMITTEE COMMENTS

Dr. Skinner commented on the project as follows:

The partial draft-final report needs to be completed if one would assess the conclusions of the study: "Education for Adults." If indeed there is no more to be added, then I fear that I must consider this project to have been a simplistic waste of time.

The view that the less-developed countries lack critical ingredients in their socio-cultural systems, or they would have developed, papers over a range of complex factors. That "education" is one of these factors is conceded. Yet, we are still far from any agreement as to what kind of education is useful to the development process.

The views expressed in the paradigm used in this study, and the methodology used to educate illiterate women for development are highly questionable. It is really true that "ultimately development workers and rural residents have similar objectives?" In this case no effort was made to find out. There are no data about what the rural Kenyan and Philippino women view as the proper role of women in their societies. Is cleanliness, friendliness, cooperation, listening to the ideas of others, and arriving at consensus, pertinent to development? There is no background data that suggest that the women subjects lacked "self-reliance." Did they not have an "n" or "m" or "ps and gs" factors in order to survive in their own societies? Must people "have fun while they learn?" Did the women have to be told to learn to do for themselves and not have people do things for them? Is it not just possible that cooperation and not individualism may be the way to develop at this specific period in the world's history?

The methodologies used to select the villages and the personnel for the study are simply bad. The training sessions were juvenile. In fact one sensed that the project designers believed that preliterate or "non-literate" people are children.

Although Dr. Carolyn Barnes concluded that this project was a success, she correctly highlighted its major shortcomings. If as she pointed out the participants learned not to fear government officials, what does this mean in an environment where deference is necessary for dealing with officials?

Dr. Barnes' comment that it was unrealistic to expect the villagers to pay for the services of the coordinators raises the most troubling problem of them all--and one foreseen by RAC. True, it does take some risks for people to develop, but why expect people to pay for services whose need they were invited to want? In other words, this appears to me to be form of exploitation of the local people. The people in the underdeveloped world have few resources to begin with, and yet outsiders come and attempt to rechannel these sources in novel, and untested ways. Why should not these outsiders bear all the cost until the projects are well tested?

Dr. Barnes left too many unanswered questions: Why did one coordinator drop out midway? Why did the sixth village group not complete any project and was disbanded after midpoint? I suspect that data on these and other topics might have provided the necessary information for future projects. In summary, then, I do not believe that the project accomplished its objectives. The projected research was not good, nor the results generalizable. Professional bias aside, the Agency should ensure that such projects have a good anthropological data base before they are tried.

Dr. Elmendorf had the following comments on the project:

Objectives

The purpose of this project was to design and test an innovative approach for educating preliterate*/ rural adults which would draw upon their inherent interests and skills to develop problem solving methods to be applied to everyday life.

Specifically, the project put to test the following assumptions:

- 1) that the Self-Actualization Method (SAM)**/ will have an important effect on behavioural attitudes and learning of preliterate rural adults;
- 2) that indicators may be generated to show whether such an approach leads to improved quality of life, fuller contribution to and participation in community and family development, and better integration into preliterate rural adults' national economies;

*/ It is important that a distinction be made between "pre-literate" and "illiterate"... preliterate being the anthropological term used to signify an interest and willingness to acquire literacy and modernization.

**/ The Self-Actualization-Method (SAM) accepts Abraham Maslow's theory that human needs are hierachical. An individual must satisfy one category of need, real or preceived, in order to traverse to a higher developmental phase in the journey toward full human potential; that given the opportunity and ability to remove obstacles, it is the natural condition of people to grow toward their human capability.

3) that SAM may be used within the context of a program resulting in increased local participation with program responsibility being maintained at the community level without significant outside support.

The project paper lists the anticipated research products as:

1. comprehensive evaluation data on impact
2. research methodology and instruments for determining learning gains related to the goals of rural adults engaged in the self-actualization method
3. guidelines for replication.

(1) Did the project accomplish all of its objectives as described in the project paper?

As was noted in the RAC meeting of March-April 1977 (p. 46-47), there are many similar efforts in non-formal education and community development - and have been since the 1950s: There are, however, some specific differences in this approach which enhance both the conceptual and real contributions to education and development. The approach is nearer to being truly integrated and interdisciplinary than most. Approaches which are holistic in their integration of a variety of issues and problems seem unmanageable to planners and agencies who are used to implementing programs representing specific disciplines, such as family planning, improved nutrition and agriculture and health. For example, in attempts to meet the 1990 target for providing all people with safe drinking water*/ and improved sanitation, and "health for all by the year 2000,"**/ increased emphasis must be given to their interdependence if these laudable goals for enhancing the quality of life are to be achieved. Both of these efforts depend on mobilizing community

*/ UN Conference on Water in Mar del Plata, Argentina, 1979

**/ WHO/UNICEF Primary Health Conference at Alma Ata, Russia, 1978

participation and resources. But how to generate broad-based community participation is a key problem for all agencies. World Education in these two programs show how, working with groups of preliterate women, support for projects can be engendered in a community if learners identify priorities and use an "active" learning approach (Clark, 1979, p.46). Women are seen in their multiple roles, as co-participants with men in the community development process. If we look at the seven features outlined in the proposal and discussed at the last RAC meeting, we can evaluate briefly the strong and weak points of each.

1) The need-based structure of the curriculum

The materials and activities used to create dialogue and solve problems were not entirely unstructured but were hierarchial and integrated, just as the social and economic factors are interrelated in the villages. We saw in the report that the learners did move toward solving problems of their own choosing and gradually experienced successes which they had brought about. Interestingly, they took hold of small fragments of knowledge and moved to other needs - ones planners might have considered more basic. As they learned decision-making techniques and new problem solving skills, they applied them to new ways to improve the quality of life. The techniques and learning materials for identifying needs were outstanding contributions of the programs.

2) Local, flexible teaching designs

The cultural differences in priorities in Kenya and the Philippines were interesting, and the facilitators/coordinators responded to them differently, using various educational materials as they fit the needs of each group. For example, in Kenya 30 percent of the participants saw a session dealing with income-related activities as valuable and enjoyable versus only six percent in the Philippines. Projects chosen by the groups reflect their interests.

The organizational differences in the two programs, as well as the ages and experience of the coordinators/frontliners, make comparisons difficult, but it is obvious that the groups were flexible in their teaching designs. At the same time, these differences point out the usefulness of the approach in various situations if flexibility is maintained.

- The extensive field training in the learner-centered methodologies, including techniques for development of appropriate materials for stimulating dialogue and problem solving, is undoubtedly a key to the success of these programs.
- The "tested participatory activities for trainers" From the Field, World Education, Crone and Hunter, 1980, based on experiences from Kenya and the Philippines, as well as other countries, is a related contribution from these projects and one which allows for creativity and flexibility in trainers and learners in various situations.
- The monthly meetings of the field workers with support staff provided an opportunity to evaluate the various approaches and to discuss changes as noted in the village.

3) Periodic tests of change

The idea of having several checks of changes of knowledge, attitudes and behaviour was carried out as planned in the proposal. The ongoing assessment, which started at the beginning of the project, included:

- a weekly log kept by the field teams in which careful observations and reactions to each group meeting were recorded, including participation of individual members, techniques and materials used;
- records kept by the field work supervisor and project director of their frequent field visits for monitoring and observation;
- progress on the project as reported by randomly selected villagers whose individual participation and changes were also noted.

Even though details on the baseline survey on village conditions were not included, data from these, along with a needs assessment documenting participants' goals and objectives at the outset, were used to determine change. Specific evaluations by outsiders were made to determine outcomes of the project at two specific times, but the evaluation by the participants and the coordinators is probably the most valuable contribution since it records the process of the project. The analysis by outsiders of the total process and the participatory evaluation as summarized in the draft of the final report is an extremely useful document.

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4) The use of behavioural indicators

As noted in the RAC Minutes (1977, p. 45) the most important element in the program is probably behavioural change and not just new attitudes and knowledge. It was noted that anecdotal material in the field logs and reports referred to numerous changes in personal hygiene and sanitation, but details of these changes were not always included. Numbers and timing related to presentations and projects are extremely valuable for the always difficult task of analysis of such materials, as were given, for instance, in connection with the bakery project in Kenya where many behavioural changes were noted. The building of latrines and taking of inoculations to meet health requirements for opening the bakery had village-wide implications. By mid-point it was established that 23% of the 60 women who baked and sold the bread had already been screened and certified fit by the regional health officer and further screenings were being made. All of the women who were baking had had the required inoculations. Equally important is the fact that 90% of the women interviewed at mid-point were purchasing bread, which would be particularly beneficial for children, for instance, who had some distance to travel to school. Similar benefits were noted with the poultry-raising scheme.

The child-care center in village four, Kenya, also met broader community development goals in that it insured supervision of children while freeing parents for a time to pursue other activities. Health needs such as sanitation were addressed since latrines for basic hygiene must, by Kenyan law, be installed for such group projects as nursery schools, bakeries and the like.

The various successful projects -- bakeries, poultry and pig raising, mushroom culture -- are described in detail, but other equally important behavioural changes, such as increased confidence in approaching government agencies, or in participating in meetings or discussions with friends and family were not as fully reported in the available materials. It was further noted that many women dressed more neatly, carried themselves with more pride, and took more interest in community affairs.

5) Use of paraprofessional staff

The village-level workers, "coordinators" or "frontliners", played key roles in the project, but there was some conflict with lowest level career helpers, the "facilitators." There was not a one-to-one mentor relationship as originally proposed, but more of a semi-supervisory one. The difference in

salary was resented by the village-level workers, who are being faced with lack of any income, since few villages can sustain the programs yet. One recommendation which seems worth pursuing is to eliminate the facilitator role except as a training/supervisory person with say 10 or 12 villages.

6) Use of non-literate teaching methods, or non-formal education for preliterates was a valuable part of the project since it opened participation to the total community, literate or illiterate, without shame or discrimination. Interestingly enough, even though literacy was not one of the goals, improved attendance at literacy classes, or establishment of such classes, has been reported by coordinators in Kenya and seems to be related to the groups' interest to earn more income.

Less motivation for literacy in the Philippines is probably due to the fact that more participants had schooling, but with low-skill level. The teaching methods were appropriate for the Filipinas, who enjoyed particularly the group dynamics and problem solving sessions.

7) The use of non-governmental organizations instead of the Ministry of Education was a very important part of the project design. To have worked within the Ministry of Education would have limited flexibility and process. The Kenyan project was easier to operationalize since the cooperating agency did not have village-level workers and understood the basic goals of the project. In the Philippines there was difficulty in maintaining harmony between the field personnel of the cooperating agency and the project. Such a partnership is difficult at any time and, in a bottom-up approach, extremely complicated. This part of the evaluation should be carefully analyzed for use in developing a cooperative mechanism before replication.

In fact, the group approach within the village was an important key to the success of the project. It was interesting to note that when risk and uncertainty were present, the

group dwindled - or when giveaways were not offered - but peer support in the risk takers kept the momentum going and others rejoined. For instance, in Kenya the 48-member group dropped to 11 as they struggled to raise money for the nursery school. When the risk takers showed that the goal could be achieved, the group grew to 34.

(2) What well defined end products resulted from the result?

The final evaluation will undoubtedly answer some of the questions raised in this brief review, but the results as already reported have great potential to be generally useful to LDCs and AID.

The statement made by Dr. Carolyn Barnes, after her February 1980 field trip to Kenya, reflects my feelings after reading the evaluations of the projects and related literature:

The project process based on the self-actualization method has resulted in a very impressive outcome whereby women organize and obtain some technical guidance to launch and operate local-level development activities identified by them. The basically low-cost approach has been proven to be effective in the Kenya experiment and is worthy of serious attention and replication.

The project's success and usefulness to others can be measured in part by the fact that requests have been received "a) by new members to join existing groups, b) for training to be given to agents of other organizations, c) to form new groups (one request came from the men in village one), and d) for the project to take over flagging groups currently under other organizations."

In fact, the two projects, with some reservations noted in the following comments, showed clearly in the mid-point evaluation in July 1979 that "it is possible to organize and facilitate education to address the real interests of village residents and that learning objectives of the groups are consistent with generally accepted development goals. Furthermore, it is clear that in those villages where the learning approach comes closest to the "ideal" one characterized as the self-actualizing method, success has been most apparent."

Based on reports and my observations in various countries, the impact of techniques and methods used by World Education have already had widespread application in successful projects in Thailand, Honduras and Indonesia. A longitudinal study of the ways in which the self-actualizing approach has been incorporated into various projects could help AID and LDCs find ways to:

- achieve more effective and efficient integration among the many fragmented services and programs designed to meet basic needs;
- incorporate planning, research, training and evaluation into an iterative learning process where "users" and "providers" are in a continuing dialogue of problem solving.

(4) Are there any precautions that the Agency should take in making use of the research results?

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Care must be taken:

- that the learning materials do not become too standardized;
- that flexibility be maintained in relation to needs as defined by the villagers;
- that local coordinators/fronliners are selected by their communities and have some financial support and continuing prestige;
- that there be monitoring and supervision, which should be more like continuing learning, sharing and evaluation of the process with upward mobility for some coordinators to keep up motivation;
- that some seed money, long-term loans or a revolving fund be available to get the self-help projects going, where needed;
- that the emphasis be kept on women in their multiple roles, but that men not be excluded from the process;
- that relationships with other agencies - non-governmental and governmental - be explored to expand the impact and prevent politization.

In closing, it is recommended that the requested extension be granted to World Education and urge that extra funds be made available, if needed, to prepare and publish a training manual and guidelines for replication.

Dr. Tanter reported as follows:

The most important aspect in this project is behavioral change. The project was quite innovative in recording information from field logs that documented changes in personal hygiene and sanitation. It would have been helpful, however, if the project had had a more systematic method for reporting this information.

There seems to be a made off between the rich descriptions presented and the external validity or generalizability of the results. Successful projects concerning poultry, pig raising, etc., are described in rich detail. The generality of such descriptions, however, is unclear.

The materials developed may be the most useful end products. Again, however, the generality or applicability across time and space is unclear. The self-actualization method itself has been validated under various conditions, and the present work may be considered further validation.

With respect to internal validity, moreover, how can one be sure that the effects attributed to nonformal educational techniques are due to them rather than to other factors? Indeed, the problem is even more difficult because of the probable intercorrelation among factors that help determine behavioral change.

It is extremely difficult to get a handle on this type of project. It is intended to be outside the mainstream of traditional approaches and achieves this result!

Dr. Montgomery asked if any efforts to put indicators into the final report had been made.

Dr. Anderson responded that there was both a lack of indicators and an apparent inability to use indicators. This was the main weakness of the report.

Dr. Elmendorf felt that the report did use some indicators. She suggested that perhaps longitudinal indicators were more appropriate than those used.

Dr. Sprague, of AID staff, expressed appreciation for the comments, especially the range and the diversity of comments that were received. He remarked that the Office of Education likes to adhere to as much rigor as possible in education research projects, but did not have available the means for creative evaluations of those projects.

Mr. Hoxeng, also of AID staff, said that in the beginning the project was not an experiment. A log that was kept on the activities allowed some experimental control of the project. The evaluation that later became a part of the project was not in the initial project design. The log will be in the final report.

Mr. Hoxeng went over some of the results of the experiment to indicate the type of findings that appeared in the report. For example, in Losotho there were some 800 different groups identified that were providing nonformal education. The techniques that were being used by these groups should be useful. In the Philippines and in Kenya people were denied change. He pointed out that the project included a broad spectrum of countries and the results should be useful. Some Moslem countries were included.

Distribution of Gains, Wealth and Income for Economic and National Development - Rice University

I. SUBCOMMITTEE REPORT

Dr. Falcon, chairman of the subcommittee, which also included Drs. Montgomery, Tanter and Thorbecke reported as follows:

Background - The Rice project coincided almost perfectly with the decade of the 1970s. The initial grant of nearly \$600,000 covered fiscal years 1971 through 1973. The original grant was extended into 1975 when a major project supplement totalling an additional \$500,000 was again approved by AID. In short, this was a large project covering about \$1 million over an 8 year period, 1971-78.

The Rice project contains a number of important features that need specific assessment. However, it is also an appropriate general vehicle with which RAC and AID can assess where the profession of development economics has come (or gone) during the 1970s. Moreover, in terms of the task of the Committee, understanding the milieu of 1971 is extremely important for evaluating this project. The central review question on this project is not whether RAC would recommend funding for the same topic if it were to come up for review at the present time. Rather the issue is whether the support of the project was a sensible action in 1971 and 1975. Although the Committee is critical of several elements of the project, particularly during the 1975-78 period, RAC generally agreed overall that it was a useful program of research for AID to support.

To help set the stage for this review, it is appropriate to remind all RAC members about the nature of development economics in the late 1960s. Based to a considerable extent on the research of Simon Kuznets--work that helped him to win a Nobel Prize--the prevailing (if caricatured) view in the 1960s was that capital was a limiting factor of economic growth, that growth was the best way to solve development problems, and that a worsening of income distribution was almost inevitable during the process of development. The argument took various forms, but centrally at issue was what the poor did with the incomes that they earned. Part of the argument had to do with the nature of their consumption expenditures and part had to do with their rates of saving. Most people believed the poor would not save and/or they would consume in ways that were conductive to the long run growth of the economy. The Rice project was among the first to challenge these assumptions and

it is to their credit (and AID's) that they were among the leaders in looking at the question of whether greater income growth and improved income distribution were compatible during the development process.

The "style" of the Rice project also deserves mention. Much of the economic research on development being conducted and supported in the late 1960s and early 1970s was more of a program variety, rather than a tightly defined project with more limited objectives. At Yale, the group under Llyod Reynolds was working on a series of country growth studies with AID support. Similarly, Hollis Chenery and his associates at Harvard were also working on an extensive array of issues on structural change and development planning. Thus, the early funding of the Rice project appears to have been very much in the style of the day. That is, the research was on an important broad topic at a major academic center. The assumption was that this was the best way to mobilize a substantial number of good people in a flexible way to work on a central topic.

The breadth, the style and the substance of economics research has changed dramatically in the late 70s and early 80s--probably all for the better. Nevertheless, the two points that have just been made underscore the appropriateness of RAC and AID behaving as they did in 1971. By 1975, however, the general situation had changed and one can certainly raise more doubts about the funding of the last half of the project.

Objectives of the Research - The research at Rice had several major themes. Most importantly, it was concerned with income distribution and the structure of demand. It asked the empirical question of how poorer groups spent their money and whether their savings rates were different from upper income groups.

These questions were of central interest to development theory. First, if savings rate of the poor could be shown to be similar to the rich, a major blow could be struck against the idea that inequality of income was necessary for development. The traditional argument was that rich capitalists were needed to provide the savings and investment required for future growth. If low income groups had reasonable actual or potential savings rates, the implications for development were enormous.

A second major issue involved the factor intensity of the goods which the poor consumed. A key question of the Land/Soligo model was whether the consumption bundle of the poor contained items with relatively high labor to capita ratios.

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If this proved true empirically, and if more income could be directed towards the poor, a development mechanism could be started whereby income was made more rather than less equal. More specifically, if the poor consumed goods with high labor ratios, such consumption patterns would add to employment. And additional employment, as everyone might guess, was crucial in leveling the distribution of income within most of the developing world. Moreover, since capital was still thought to be the critical impediment to growth, the low-capital component in the consumption basket of the poor would be of further benefit by reducing the capital needs of a society.

A third part of the program had to do with indirect employment effects. In housing, for example, (which was a controversial element of the project during the second review process) the Rice group hypothesized that the indirect employment and income distribution effects might outweigh the direct component. Even though housing would be demanded by the upper income groups, the labor involved in the construction of housing might make income distribution within a society more egalitarian. (it is important to recognize that the Rice project had a heavy Colombia association, and that housing issues in that country were then a major feature of economic policy). The foregoing list of specific topics could be expanded but the three examples just presented provide a flavor of the theory, hypotheses, and empirical content of Rice's work on income distribution.

A second broad part of the Rice project included an analysis of how government policies might be used to assure that the poor were not disadvantaged during the development process. The final part of the project was designed to determine how ethnicity and other social variables might either enhance or retard a more egalitarian distribution of income.

Contributions of the Project - The Rice project was successful in developing more than 50 working papers and published articles on this set of topics. The work contributed significantly to a series of review articles on income distribution in the development process. Various later projects, e.g., the Princeton project on Korea, several volumes on Colombia, the Brookings volume on Latin America, and the World Bank volume on Redistribution with Growth, drew somewhat on the general findings of the Rice group. At the same time, there are few essays from the project that can now be regarded as classics.

The empirical findings of the project, which perhaps should have been anticipated from the outset, contained no universal truths. In general they showed that policies to alter income distributions in mixed economies were often frustrated. They showed that what the poor do with their income varies importantly in different countries, and that conclusions on this topic cannot yet be educated from first principles. The results showed further that the data required for making such analyses were extremely demanding and often governments which had such data unwilling to release them for analysis. (Part of the difficulties of the project was the lack of real data and the need to resort continually to simulation.) More generally, the Rice project added to the mainstream of development economics by showing that income distribution issues were topics that required analysis rather than assumptions. The fact that they came to no universal theory is less a matter of the project, and more a matter of the nastiness of the problem. Sometimes even the answer, "it depends," is a useful step forward.

One specific study on savings is illustrative of the foregoing point. The Rice group had hoped to show that low income groups were high savers or potential savers. Yet in fact, in one of their key studies on Colombia, certain poor groups were shown to have different rates of time preference than the rich, and that even when the poor were faced with the same savings options, they saved less. Studies in a number of other countries by other groups have shown contrary results. The important point, however, is that the work at Rice helped demonstrate that income-distribution situations differed by country and perhaps by stage of development. The Rice project was thus part of a process that took development theorizing about distribution out of the armchair and into the nitty-gritty of specific-country settings.

While the Committee is impressed with the work of the Rice project in general, and my colleagues will speak to the specific contributions as they see them, it is clear that the 1975 project extension was less useful than the original grant. The differences were several. The central idea of the Land/Soligo model was probably strong enough to carry the group for four years, but not enough for eight in light of the varying empirical results. The return of Professor Land to the land--he became a rancher--was a significant blow to the project. The failure of the government of Malaysia to release the income distribution data precisely because it might indicate differences by ethnic group was a serious setback as well as to the Rice study on ethnicity. Finally, the changed role of the

AID mission in Colombia, plus the changed set of outside advisors in Bogota', had an important negative impact on providing the data base that was quite essential to the second phase of the project. With a different scenerio in Colombia, the research and policy contributions of the project could have been quite different.

Conclusions - The overall reading of the project is that it had modest success, with the project more successful than the later part. The major contribution was in helping AID and others to think about a broader set of questions that linked income growth and income distribution. Few of their results can be linked with specific AID actions, and yet how the Agency thought about many specific policies changed drastically during the decade, presumably in part because of the work at Rice.

Two strategy conclusions can be drawn from the project that may be relevant for the 1980s. First, work on income distribution should still remain an important topic for empirical research in economics, but the days of broadly based program research on this topic are probably over. What is needed instead is a much more specific set of projects geared to particular questions, sectors, policies and countries. Second, at least for social-science research, the Rice project probably points up the danger of trying to support individual projects for more than five years. Because of the importance of the general topic, and because of the quality of the staff at Rice, the benefits of their total effort exceeded the costs. It is suspected, however, that the ratio would have been substantially higher if the project had been terminated in 1975.

Dr. Tanter who was also on the subcommittee to review the project reported as follows:

The part of the Rice project concerned with political science is not nearly as tight as the economics portions. The Minutes of the April 1971 RAC meeting suggested that the proposed analysis dealing with political phenomena needed to be strengthened. Specifically, the efforts of quantitatively oriented political scientists could complement the statistical work of the economists at Rice.

The ostensible interdisciplinary nature of the Rice project breaks down due to the lack of complementarity between the economists and political scientists. In addition, there is an absence of a theoretical synthesis, across disciplines and subprojects.

The analysis of low government policies might be used to assure that the poor were not disadvantaged during the development process could have been more systematic than it if sophisticated tools had been used. Similarly, the work on ethnicity and other social variables lacked methodological sophistication, and hence this work does not say as much as it could concerning the ways to enhance equity in income distribution for given levels of growth.

A principal finding is that income growth distribution situations differ by country and by stage of development. This finding is of potential policy relevance, especially if the Rice analysts could specify the characteristics of the situations themselves in general terms.

Dr. Montgomery agreed with Dr. Tanter's comments. He felt that when the project was reviewed the Land/Soligo model was presented to the RAC. The results that were being reviewed were an over simplification of this model. This proved the theory that growth required unequal distribution. Price supports usually redistribute from poor to the rich. Rice continued to work after the conclusion of the project. The reports that come out after this evaluation would lead to more positive comments on the final project.

Dr. Falcon pointed out that all the reviewers had reservations about the project and particularly the last phase of the project. There were fewer reservations for the political science aspect.

Dr. Swanson asked if there was anything in the agriculture sector in this project that was dealing with income distribution and the agriculture sector. Specifically, he wanted to know if there was anything that was specific by policy and by country, and second whether or not there was anything on how price policy affects those people with low incomes.

Dr. Montgomery thought the comparative analysis of cross countries was useful. The hypothesis is that cross-country analysis of policies is necessary.

Dr. Heady commented that his university had a project going on in Southeast Asia and he wondered if there were lessons to come out of the project that might be used elsewhere such as in Southeast Asia.

Dr. Falcon responded that nothing classic was produced.

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Mr. Silverstone, of AID staff, expressed appreciation for the review. He thought that the project in the beginning was very hopeful and exciting. He was disappointed, however, in the end result. He thought that there was a failure in conceptualization in the project.

Mr. Silverstone suggested that while Rice did some interesting things they were, however, handicapped by the fact that AID did not allow them to go to the countries involved in the study. Ethnicity cause a problem in the study. Mr. Silverstone indicated that he was personally interested in that subject. He was also fascinated with the attempt to view the quality of data. Data for the assessment of social-economic situations have proven to be extremely difficult to obtain and data have been poor in all projects. He thinks the delphi technique that was used in the project was very interesting. He commented on the findings of the project in the political science area. He thought, for example, the differential effects of expenditures as presented in the project were very interesting.

Mr. Silverstone felt that the thinking in this field had been advanced a great deal by a lot of work that included this particular project. He pointed to some research being done at Princeton University also in the area of policy and income and that particular study hopefully, is more productive than this one.

Dr. Elmendorf pointed out that there is a network of women looking at women's income distribution and this type of information might be used to supplement that in the report.

Inheritance and Improvement of Protein Quality and Content in Maize - Purdue University

I. SUBCOMMITTEE REPORT

Dr. Moss, chairman of the subcommittee, that also included Drs. Carter, Greathouse and Heady reported as follows:

The Purdue maize project began in 1970. It was funded to take advantage of what was considered a significant breakthrough in nutritional quality of maize. Two mutants had been discovered which showed a significantly improved balance of amino acids in their kernel protein--particularly a higher percentage of lysine. These two single-gene mutants, given the names Opaque-2 and Floury-2 from the appearance of the kernel endosperm, were the first indication that the value of maize protein for human nutrition could be improved. These two mutants, discovered at Purdue, were the only known sources of an improved protein quality in maize. It was hoped to transfer quickly the mutant genes into locally adopted maize inbreds around the world and cause a major improvement worldwide in the nutritional quality of maize for man and monogastric animals.

That hope proved disappointingly illusive. These genes proved to have pleo-tropic properties (or to be tightly linked to other genes), which caused significant yield reductions compared to normal lines.

At a RAC meeting on March 20, 1975, at which time the project was extended for three years, the following summary of the problems and potential was given:

The primary objectives of this project was to utilize the Opaque-2, Floury-2 or any other genes of maize still to be discovered to improve protein content and quality of maize. The principle quality limitations of maize nutritionally are lysine and tryptophane. Transfer of these genes to superior hybrids is a simple matter. The difficulties which led to the research project are the characteristics associated with these genes that are undesirable and that are transferred along with the Opaque-2 and Floury-2 genes.

II. SUBCOMMITTEE COMMENTS

Dr. Carter pointed out the difficulty of separating yield and protein as content of maize is increased yields tends to be reduced. He felt that the people who wrote on this topic were optimistic because of emphasis on national adaptation. He personally hoped that the yield problem might be overcome with local adaptation.

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Dr. Greathouse agreed with the report given by Dr. Moss. He did not feel that the project reached its objectives but nevertheless, made significant contributions in the field of maize breeding.

Dr. Heady pointed out that when the project was initiated it was thought that there was a need to increase the protein content of maize. Given this objective the outcome was not as great as was expected. However, it should be pointed out that the task was very complex and that the project should not be judged badly by not having reached that objective.

Dr. Falcon asked for more information in one point made in Dr. Moss's report. He sits on the Board of CIMMYT and members of this Board think that the yields of high protein maize is much more nearly equal to those of traditional maize varieties than has been stated in the project report.

He wondered if the committee had access to latest data on this subject. He personally thought the progress in this area was exordinary.

Dr. Rechcigl, of AID staff, remarked the project goes back to prior to 1970 and was instigated by the discovery of opaque 2 corn. He pointed out that cooking properties of high protein maize was a problem. It was initially tested by chemical means and this didn't say very much about the biological utilization of the protein in the new maize.

Dr. Moss remarked that he was aware that opaque 2 corn and varieties were in the corn breeding program in various places although there were no commercial varieties produced by this project. The various breeding programs were still using the genes from this corn apparently without any problem of low yield.

Dr. Wishik felt that the project had tried to improve genetically the protein content of the corn and had failed.

RESEARCH ADVISORY COMMITTEE MEETING
March 3-4, 1981
ATTENDANCE

RAC Members

Dr. Ralph Smuckler
Dr. C. Arnold Anderson
Dr. James P. Carter
Dr. Elizabeth B. Connell
Dr. John H. Ehrenreich
Dr. Walter P. Falcon
Dr. Mary L. Elmendorf
Dr. Terrence R. Greathouse
Dr. Earl O. Heady
Dr. John A. Hrones
Dr. Forrest E. Linder
Dr. John D. Montgomery
Dr. Dale N. Moss
Dr. David Pimentel
Dr. B. S. Schweigert
Dr. Earl R. Swanson
Dr. Raymond Tanter
Dr. Thomas H. Weller
Dr. Samuel Wishik

Other

Phelps-Stokes Fund, V.M. Turner
MAB (IO/UCS), L Whitmore
MAB (IO/UCS), J. Blowers
Black & Veatch Int'l, W. Weiss

A.I.D. Staff

A/AID, M. P. McPherson
SCI, H. Minners
AA/DS, S. Joseph
DS/DAA/FN, T. Babb
DS/PO/RES, M. Rechcigl
DS/PO/RES, F. O'Quinn
DS/PO, B. Chapnick
DS/PO, A. Morales
DS/PO, E. Roche
DS/AGR, L. Frederick
DS/AGR, R. Sutter
DS/HEA, C. Pease
DS/HEA, E. Smith
DS/HEA, J. Erickson
DS/XII, I. Long
DS/XII, C. Barker
DS/UD, W. Miner
DS/RAD, K. Kornher
DS/POP, J. Speidel
DS/POP, J. Shelton
DS/POP, M. Mamlouk
DS/FNR, W. Feldman
DS/FNR, W. Roseborough
DS/FNR, C. Paul
DS/FNR, M. Kux
DS/IT, D. Clark
SER/CM, H. White
PPC/PDPR, J. Eriksson
PPC/PDPR, K. Replueier
PPC/PDPR, J. Silverstone
LAC/DR, J. F. Smith
LAC/DR, R. Otto

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AGENCY FOR INTERNATIONAL DEVELOPMENT
RESEARCH ADVISORY COMMITTEE

Minutes of the Seventy-Second Meeting
July 21-23, 1981

July 21

The meeting was opened promptly at 9:00 a.m. by the chairman, Dr. Smuckler.

Dr. Rechcigl then was asked to introduce the ad hoc members of the RAC, four people who had been invited to participate as experts in this RAC meeting.

Dr. Rechcigl introduced Dr. Clarence Idyll, Dr. J. H. Farrell, Dr. C. Sloger and Dr. J. Sanderson. The ad hoc members were added to the RAC for this meeting to add expertise in particular areas in projects which had been received.

Dr. Minners, the Science Advisor, then introduced his assistant, Dr. Asher, and Mr. Robert Meehan, who had been detailed to the Science Advisor's office during this period in order to expedite the funding of projects in the new program.

Dr. Minners pointed out that the Science Advisor's program was going to take time to develop. He was trying out a competitive program where researchers were invited to submit proposals and then AID would fund only the best of these proposals. He remarked that his office had not advertised his program or the availability of funding from his program at all, that the word had gotten about from word of mouth and that he had received numerous proposals. It was his intention to identify the best ones and then hold some other projects for wider competition.

He pointed out that there had been an internal review of the projects within AID and the results of that review had not been made available to RAC because he wanted the RAC review to be an independent review. He did not promise the internal reviewers that the projects would be funded if approved by RAC. He mentioned that he himself does not have the broad kind of wisdom to determine what we should do in every field since the research program will be covering a great many fields. He had asked for small proposals on innovative topics. Originally, the Science Advisor's budget was set at \$12 million but he reminded the RAC that \$6 million of that had been obligated for

the National Academy of Sciences project that had gone through the RAC earlier. Currently there is about \$ 5.25 million dollars yet to be obligated. He did not want to fund with this money any research that is not of very high quality.

Dr. Wishik thought that it should be put on the record that this was a special meeting of the RAC. He pointed out that in all previous meetings the RAC had needed recommendations to the Administrator. This was very different now in that these projects would not be sent for the Administrator's approval. He also pointed out that because of the way in which the materials were received that the RAC members were not able to review the materials as carefully as they normally do.

Dr. Smuckler then explained that with the appointment of Dr. Brady, as the Assistant Administrator for the Science and Technology Bureau, there will be created three directorates headed by people with broad expertise in their areas of responsibility. The existence of these directorates will somewhat change the nature of the central core talent in AID. The nature of central research is under the scrutiny of Dr. Brady and others in AID and the RAC perhaps will find itself with a new role as a result of this scrutiny.

Dr. Long remarked that out of this experience over the next couple of days AID and the RAC may gain experience which is useful in establishing a new system of review for the central research program.

Dr. Wishik noted that the innovativeness of the research proposals before the RAC was very apparent. Reviewing these proposals and making recommendations on them is much more like gambling than usual.

Dr. Peterson then pointed out that the real strength of the RAC is in the debate that takes place with each project. If in the future there is a large number of projects, such as there is at this meeting, then each project cannot be closely reviewed and debated.

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To Assess the Ergot Contamination Problems in India by
Determining the Levels of Ergot Alkaloids in Grain and
Foodstuffs and Obtaining Corresponding Epidemiological Data -
Food and Drug Administration

I. SUBCOMMITTEE REPORT

The report of Dr. Schweigert, chairman of the subcommittee which also included Drs. Connell and Moss follows:

The investigator in coordination with Dr. Samuel Page of the Bureau of Foods of the U.S. Food and Drug Administration has developed a research proposal to assess the ergot contamination problem in India. The approach is to determine the level of ergot alkaloids in grains and other foodstuffs and to obtain a corresponding epidemiological data from the regions concerned.

Toxic syndromes associated with the ingestion of ergot alkaloids produced by various fungi growing on various grains including pearl millet in India have been described. This is a major concern with respect to the public health safety of these foods which are vital to providing important cereal grain foods to the population. It is further brought out in the research proposal that the toxicities and alkaloid content of affected grains may vary widely and in certain instances, methods for estimating quantitatively the ergot content of such foods may need further development. The ergot alkaloids in millet are apparently different than those that develop from fungal infections in rye and wheat.

The research plan is to isolate alkaloids from contaminated pearl millet and to establish the chemical characteristics of the alkaloids. Up to date, seven alkaloids have been detected by thin layer chromatographic techniques. It is in this phase that coordination with the Bureau of Foods of the FDA would be particularly valuable in providing analytical support for these studies.

The investigators then plan to utilize high pressure liquid chromatographic methods for separation and quantitative analysis of ergot alkaloids produced by the major fungal species (*Claviceps fusiformis*). After evaluation of the method and improvement in extraction techniques they plan to develop and evaluate sampling techniques for obtaining representative samples. They then anticipate surveying samples of pearl millet and millet products in areas where ergot contamination has been known to have occurred. In this connection, it is significant to note that they anticipate needing one thousand

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samples to assure statistical validity. The basis for this estimate was not presented. They plan to conduct epidemiological studies in areas where there is high incidents of ergot contamination and further analyses of foods may be needed if toxic manifestations are noted in the population.

The National Institute of Nutrition in Hyderabad has had considerable experience in working in the general area of food toxicology with emphasis on mycotoxins. The above project is developed to more specifically define the potential toxicological problems with ergot contamination in millet. No background was provided in the brief proposal relative to methods of preventing the growth of the fungi and thereby developing some technique to eliminate or minimize the presence of these materials. Obviously, after they are produced it is important to know they are there and if they have biological significance. Even more important is attention to prevention. This issue was not addressed. It is further recognized that even when such toxins are present in a foodstuff when food is extremely scarce we have a difficult tradeoff with respect to biological effects (some degree of toxicity with the ingestion of the toxicant versus limited total food intake which has its own obvious biological effects).

The investigator appears to be well trained and productive based on documentation provided. In fact, it is regrettable that the project was not as thoroughly documented (4 pages) as the background, publications and other activities of the principal investigator (11 pages).

For the budget, the high pressure liquid chromatography and equipment and supply categories appear to be very high based on knowledge of cost vs. comparable items in the United States. Subject to discussion from other subcommittee members, it is my recommendation that there be a deferral of this proposal until there is greater elaboration of the research proposal and some discussion of approaches to prevent or minimize the growth of the fungi producing the toxins. There seems to be a linked phase to this project unless extensive research has already been done. In the latter case, comment is essential to give this project proper evaluation. It would also be helpful if we could have some biographical data on the coordinator, Dr. Page of the Bureau of Foods. It would be most reassuring to have some documentation of his qualifications. In view of these facts, I have not provided a quality ranking for the four categories proposed. Certainly, the capacity strengthening and relevance would have a high rating of 1. It is not possible to judge the scientific merit adequately from the limited material provided. Consequently, I am deferring this subject to the discussion in the RAC meeting.

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II. OTHER COMMENTS

Dr. Peterson remarked that he contacted Dr. Glenn Burton, an expert in this field who pointed out to him that some improved varieties of grain may eliminate ergot contamination through crop breeding.

Dr. Skinner asked about the nature of the system through which the project was being judged. He felt that he would give this particular proposal a score of 1 on capacity building but otherwise, it should be deferred. He pointed out that some LDCs would look at capacity building and other features of that type much more than they will at the scientific merit of the proposal.

Dr. Schweigert suggested that without scientific merit there was no point in funding a project for capacity building.

Dr. Greathouse questioned why the project proposal did not refer to the CRSP on sorghum and millet. He pointed out that ICCRISAT had been working on sorghum and millet and this work had not been recognized in the proposal.

Dr. Long suggested that if the project is only to determine the frequency and the magnitude of ergot contamination there might be reason to defer it. The interesting question is how to prevent ergot contamination. It would appear that the researchers are asking \$150,000 to determine if this is the problem.

Dr. Schweigert pointed out that deferral does not mean that the work would be done. He agreed that the principal investigator did not seem to be aware of all the developments in this area when he prepared the proposal.

Dr. Wishik suggested that an approach to ergot prevention might emerge from the epidemiology. He thought the proposal needed much more work.

III. MOTION

A motion was made to defer the project and that motion was approved unanimously.

Dr. Linder asked what was the purpose of the voting if the motions would not go forward to the Administrator.

Dr. Smuckler then explained that when a project is approved, RAC members would be asked to rate that project on various factors on a scale of 1 to 3. He suggested that when a project was deferred the RAC subcommittee chairman should say why it was deferred but deferred projects and disapproved projects would not require the rating of the individual factors.

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Food from Wood: A New Enzymatic System for Degradation of Lignocellulose - University of Idaho

Dr. Ehrenreich, who is on the faculty of University of Idaho, left the room during the discussion of the project.

Dr. Greathouse, chairman of the subcommittee, that also included Drs. Schweigert and Wishik reported as follows:

BACKGROUND

According to the author of this project, the proposed study is to explore a newly discovered lignin biodegradation system and to evaluate its potential for large-scale technical use. The author anticipates that this system will enable man to feed the resulting upgraded fibrous plant materials to livestock.

It has been well established that fibrous plants represent the most abundant renewable raw material on earth, yet they are practically useless to man. Efficient conversion of lignocellulosic (woody plant) fiber to animal products would help relieve world shortages of food and materials while utilizing an abundant natural resource. The principal barrier to such conversion is lignin. It is believed that lignin is totally undigestible to mammals and that increasing lignification directly decreases cellulose digestibility. Before high quality livestock feeds can be generated from liquified plant materials the lignin barrier must be removed and the lignin degradation products changed into non-toxic and perhaps even nutritionally useful molecules.

The proposed project is to investigate a biosystem by which lignin is removed and apparently utilized by an unusual and highly specialized rodent, Aplodontia rufa (mountain beaver). This biosystem, recently discovered by the authors, appears to efficiently degrade and utilize both cellulose and lignin from high fiber diets. The plan is to evaluate the system's technical potential for increasing digestibility of low quality fibrous plant materials for farm livestock.

Mountain beaver is a unique, primitive and highly specialized rodent with separate evolution dating back to the Pleistocene period. It is endemic and well adapted to the mild, rainy areas of the Pacific Northwest. The main feed sources of mountain beavers are coarse, fibrous and highly lignified shrubs, trees and ferns. Given the choice, mountain beavers,

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according to the authors, consistently selected plants with higher fiber and lignin content as their feed. These animals apparently are able to utilize more than half of this lignified fiber which to other animals is practically undigestible. With their history of separate evolution, it appears that mountain beavers have evolved with an efficient and highly specialized lignin degradation system. The authors speculate that since lignin degradation systems appear to be very useful and efficient in supplying mountain beavers with adequate nutrients from other wise poor quality roughage, the system, if properly identified and duplicated, could supply farm animals with highly nutritious feed prepared from millions of tons of waste product.

The general experimental design being proposed involves the evaluation of the lignin degradation biosystem under controlled laboratory conditions in vivo and in vitro. This eighteen month study will involve a series of nutritional studies to evaluate the amount of lignin removed from various fibrous feeds using fistulated beavers.

II. DISCUSSION

Dr. Wishik described the project as consisting of a very exciting idea. He wondered how the principal investigators could be helped along in defining an acceptable research proposal. He raised a question, however, that after four years of working on the project, why has nothing significant been produced from the research? What would the principal investigators do differently in this project proposal than they have been during in the past four years?

III. MOTION

Dr. Schweigert recommended that the proposal not be supported by AID because of its lack of clarity in describing the research that would be done.

This was put in the form of a motion to disapprove the project. The vote was 14 in favor and 2 opposed to disapproving the project.

This project was the second project in the group of 41 to be considered by the RAC. After some discussion of the procedures that would be used to rate the projects the Committee felt that a revote on this particular project was necessary.

Dr. Ehrenreich left the room once again.

Dr. Greathouse then moved that the project be deferred until the issues raised in the comments before the Committee were resolved.

Dr. Farrell explained that one of the reasons for deferral was that the linkage between the enzymatic degradation of lignocellulose and food production was not clear in the proposal. There was little explanation as to how the enzymes responsible for the degradation of lignocellulose would be isolated and studied.

The vote on the proposal was unanimous in favor of deferral.

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Resource Enhancement of Giant Clams (Tridocnidae) in the Republic of Belau (Palau), West Caroline Island - Palau Marine Research Institute

I. SUBCOMMITTEE REPORT

The report of Dr. Idyll, chairman of the subcommittee that also included Drs. Ehrenreich and Farrell follows:

It would be desirable to reestablish and strengthen the populations of giant clams in the areas of the South Pacific where they have been reduced or eliminated, even though it seems doubtful that the real impact of this on the food supply of the inhabitants of the islands would be significant. But it is not clear that this project will go far toward accomplishing this end. The level of technology in the culture of these clams is too low at the moment to proceed with most of the work proposed, and the understanding of the problem by the principal investigators suggests that the chance of accomplishment of the objectives stated is small.

The proposal is soft in its statement of objectives and of procedures. Of the four objectives, only one is really relevant to the project: this is to conduct experiments on propagation, reproduction, larval biology, growth and survival rates of clams. One objective is to establish sanctuaries for the clams, whereas it turns out that these have already been established by action of the legislature. Another objective is to spawn and raise juvenile clams to reseed the reefs. This is stated as though this were an established technique, whereas there are very few if any solidly established examples of the success of such a procedure. The last objective, to reseed reefs at Ponape, is stated in the project to be largely a public relations gesture.

Perhaps, the weakest part of the proposal is that the principal investigator does not make it clear how far the culture technique for these animals has progressed, nor what the next steps will be.

II. MOTION

To disapprove the project.

III. VOTE

Unanimous in favor of the motion.

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Spawning and Developmental Physiology of Tridacnid (Mollusca, Bivalvia) - University of California, at Santa Barbara

I. SUBCOMMITTEE REPORT

The report of Dr. Idyll, chairman of the subcommittee that also included Drs. Ehrenreich and Greathouse follows:

The subcommittee recommends that this proposal be funded. It shares with the other project on giant clams (from the Palau Marine Research Institute) some of the same strengths (e.g. the desirability of reestablishing these stocks in South Pacific Islands) and weakness (e.g. the uncertainty that any significant food supply would be available from these stocks). But this project is stronger. First it establishes by a well prepared background of the biology of the clams that laboratory culture and eventually mass/commercial culture cannot succeed without an understanding of and an ability to manipulate the symbiosis of the clam and certain dinoflagellates.

Because of the complexity of this symbiosis, the time seems rather distant when mass culture hatcheries can be maintained. But since this desirable objective apparently cannot be realized without the fundamental biology proposed here, the subcommittee suggests support for the research.

The level of science is high and the description of the proposed research appears sound.

II. DISCUSSION

Dr. Ehrenreich explained that the approaches in this project and the one preceeding it on giant clams were greatly different. This particular project is proposing basic biological research. The proposal outlines carefully what the researchers plan to do. In the previous proposals such details were lacking.

Dr. Greathouse remarked that the budget caused him some problems. He thought that it was somewhat high.

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Dr. Pimentel noted that the subcommittee thought the science in the project was high but that the capacity strengthening and relevance to LDC problems was low yet. He asked for some clarification.

Dr. Carter remarked that the project carried with it a very low chance of increasing food production. Why shouldn't AID let others fund the project?

III. MOTION

The motion was made to approve the project.

IV. VOTE

Two Committee members opposed the motion; the remainder were in favor. The motion carried.

It was further explained in response to Dr. Pimentel's question that the ranking on the various factors would be done on the forms that were available to each RAC member. The overall project could be approved and yet some factors within the project could be rated low. Since there was some confusion over the evaluation process a discussion on this topic followed.

Dr. Smuckler summarized the approach which was being used. The RAC would be asked to vote for one of three choices; approve, defer or diasapprove. Only for those that were approved would RAC members be asked to vote on the various factors.

Dr. Smuckler suggested that Dr. Minners would rely heavily on the views of the subcommittee, whose members will have spent more time on the projects than the Committee as a whole. He suggested that the subcommittee members put a small s in the upper right hand corner of their rating sheets so that in the event of differences of opinion it would be possible to defer the subcommittee.

Dr. Carter asked how did the Science Advisor receive so many proposals on such short notice?

Dr. Minners responded that once information that a new source of funding is available gets out, it gets around very quickly and there was no difficulty in getting numerous proposals submitted. One should ask if there were any criteria for the distribution of projects by the region or sectors of the country or by subject matter. What was the range of the projects that were before the RAC today? Such a tabulation of the projects had not been done.

Analysis of Southeast Asian Aquaculture Systems for the Enhancement of Production and Improvement of Institutional Research and Teaching Capabilities - University of Michigan

I. SUBCOMMITTEE REPORT

The report of Dr. Idyll, chairman of the subcommittee that also included Drs. Ehrenreich and Farrell follows:

The subcommittee recommends that this project be accepted in principle and that the principal investigator be asked to re-write and refocus it.

The focus of the proposal is somewhat fuzzy, but if its main thrust is that Thai aquaculture could be enhanced and its productivity increased by the application of modern science on top of successful and ancient trial and error methods, then it should be funded.

The need for food in Thailand is high, and the contribution of fish culture is already important. The chance of higher per unit yields is good if some modern practices could be introduced. The principal investigator is competent and he has a long experience in Southeast Asia. The local research and development facility (built within the last few years with Canadian funds) is excellent, and the Thai staff which works on the project is competent.

Reservations by the subcommittee about the project center around the lack of focus of the objectives of the project, and the slowness with which practical results are promised. It appears to the subcommittee that given the advanced state of aquaculture in Thailand and the competency and experience of the principal investigator, it should not take so long (three years) to identify the factors holding back higher production, collect background information, identify gaps, establish priorities--the gist of the objectives stated in the proposal.

It is recommended that the project be deferred until the principal investigator can satisfy the RAC that practical results can be achieved more quickly. As it stands now the proposal appears too much as an academic exercise and too little of an application of modern technology.

II. OTHER COMMENTS

Dr. Farrell thought that this project was headed in the right direction. The project should specify its objectives clearly particularly with regard to the modelling that it proposes. He thought that the project had very good potential but should get down to cases.

Dr. Pimentel remarked that there is a great deal of information about shrimp and fishes. There is a need to assemble the information in some logical fashion and then determine which factors should be studied. The proposal does not do this.

Dr. Ehrenreich thought that one of the strong points was the model. He thought modelling in the biological sciences is on the forefront science in those areas. He thought that using the model it would be possible to look at and analyze related areas. He agreed the project should be tightened up and that the time frame should be expanded to two years.

Dr. Pimentel said he recognized the value of models but that it was impossible to build good models without information. He thought that in this case the old rule "garbage in garbage out" applied very clearly.

Dr. Long, of AID, thought the researcher was suggesting more than could be made use of in AID. He reviews the technology that is now available but has outstripped AID's use of that technology. He thought the researcher should be required to deliver a usable product within a finite period and a finite budget.

Dr. Minners pointed out that deferral does not mean that quality's lacking but additional information is necessary.

Dr. Wishik suggested that on several of the projects before RAC researchers should go back and make improvements before actually starting the research.

III. MOTION

The motion was made to defer the project and the vote was unanimous in favor of the motion.

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Anatomy and Histology of Carp - University of Idaho

I. SUBCOMMITTEE REPORT

The report of Dr. Idyll, chairman of the subcommittee, that also includes Drs. Greathouse and Schweigert follows:

The subcommittee recommends that this project not be approved. This is despite the information that RAC has already in the past approved the project, but was unable to fund it. The recommendation is based on the view that the relevance of this work seems lower than that of other proposals, and that it does not promise significant contributions to the aquaculture efforts of LDCs.

This kind of work should be funded by publishing companies. The principal investigator is reputedly a meticulous worker and an excellent artist, so that the product should be of interest to certain publishers. One member of the subcommittee has made inquiries and found that at least one publishing house might be persuaded to undertake this project.

II. OTHER COMMENTS

Dr. Pimentel remarked that the principal investigator is a very good scientist but that, to his knowledge, he has never worked with carp. Therefore, he personally would question the principal investigator's credibility in this field.

Dr. Schweigert pointed to one statement in the project proposal that he did not agree with. The statement was that in the field of aquaculture, there is enough information available on the disease and histopathology of the carp. He thought this was not true.

Dr. Greathouse was the subcommittee member who had contacted a publishing house. The publishing house appeared to be interested in this project and Dr. Greathouse suggested that this particular type of project could be funded through that source rather than through AID.

A motion was made to disapprove the project, the vote was unanimous in favor.

The Potential for Utilizing Seagrasses to Restore Caribbean Benthic Nearshore Impacted Areas - Florida International University

I. SUBCOMMITTEE REPORT

Dr. Idyll, chairman of subcommittee, that also includes Drs. Ehrenreich and Farrell reported as follows:

The members of the subcommittee have some reservations about the proposal, which is somewhat untidily written, but they agree that it has potential for benefit. These potential returns are long-term.

The principal investigator has good experience and background in the research proposed, and in the Caribbean area where the work would be conducted. The link with Jamaica is good, and facilities at the Jamaican institution are apparently adequate for this work.

The main concern of the subcommittee is whether the work as described can be accomplished in a reasonable time. On top of that, the proposal does not specify how long the research is expected to take. The budget is only for one year, but the research obviously cannot be performed short of several years. It is unclear whether the AID is being asked for multi-year support.

There is a major scientific flaw in the proposal in that the experimental design appears to be unrealistic, with too many variables to be handled properly within the apparent scope of the research project. There are to be three species of seagrasses, three planting techniques, an unspecified but apparently multiple number of different kinds of areas, and an unspecified number of different densities to be compared. The number of replications necessary to get statistically reliable results would seem to be unrealistic. It is suggested that before a final decision is made on the grant, the principal investigator be asked to narrow the scope of the work, and to inform the RAC of the details of an experimental design that can be accomplished in a reasonable (specified) period of time.

II. DISCUSSION

Dr. Peterson made four points. First he pointed out that it was not clear in the proposal whether the researchers intended to plant seagrasses in areas under stress from pollution or not. Second, the type of experimental design that would be used was not indicated. Third, there was no indication of duration the project. Dr. Peterson pointed out that plant sessions take time to develop. And fourth, Dr. Peterson wondered who would pay for the work other than salaries since this was not given in the proposal.

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Dr. Pimentel asked how Jamacia had avoided pollution in these areas? If they were continuing to pollute there is no reason to plant seagrasses. He thought the proposal needed more careful documentation.

Dr. Smuckler was bothered by the indeterminate nature of the proposal.

Dr. Greathouse suggested that deferral would be an inappropriate way to handle this project. He thought the project should be disapproved.

Dr. Farrell disagreed. He thought a middle area was needed or else the Committee would be making decisions on partial information.

Dr. Connell pointed out that procedurally the deferrals were falling into a pattern. There were those projects that could be better structured and those that should be written.

Dr. Smuckler suggested that the boxes on the rating sheet should be checked for all projects and that the reasons for projects being deferred should be put into the motion.

Dr. Long remarked that if a project is deferred it could be interpreted that the Committee favors the project but that there is a specific road block to approval. He suggested that the four criteria on the rating sheet are not equal to each other. He asked whether the lack of scientific merit should not result in the project being dropped or declared irrelevant. He felt that capacity and innovation should be given weights only as only additional value to scientific merit.

Dr. Minners said that he would accept that.

Dr. Long remarked that he attaches a lot of importance to innovation.

Dr. Skinner remarked that he had quite a lot of concern with this particular project on seagrasses. It had good science, but he wondered if it was practical.

Dr. Peterson suggested that an alternative to the particular evaluation system would be to rank the projects using a mental process and criteria for evaluation and then to summarize those ranks over the Committee.

Dr. Wishik suggested that deferral meant it had merit but needs more work. Disapproved meant that it was inappropriate for AID.

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Dr. Rechcigl noted that when a project was disapproved there was no reason that it couldn't be resubmitted in the same way that deferred projects were.

Dr. Wishik then suggested that, on the basis of the discussion, the mountain beaver project should be subjected to a second vote. He thought that the proposal was a good idea with merit but had obstacles which prevented the Committee from approving it.

A motion was then made to defer the project on seagrasses with the suggestions (1) that the principal investigator should specify the design of experiment, (2) that the length of time of the project should be indicated and (3) that it be made clearer as to who would do the field work.

The vote on the motion was unanimous in favor.

Prior to beginning the review of projects on the afternoon of the 21st, the Committee settled on 17th and 18th of November as the tentative dates for the next RAC meeting. A motion was then made to approve the minutes from the March 3 and 4 meeting.

Dr. Wishik indicated that he had found some typos that changed the meaning of some of the minutes and with the understanding that these typos would be corrected he supported approval of the minutes.

The vote was to approve the minutes of March 3 and 4.

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Development of Fishery Research and Management Capabilities in the West Coast Region of North and Central Africa - University of Maryland

I. SUBCOMMITTEE REPORT

Dr. Idyll, chairman of the subcommittee, that also included Drs. Ehrenreich and Swanson reported as follows:

The subcommittee recommends that this proposal be approved in principle. The problem that it proposes to address--shortage of food--is urgent in that area, and the possibility of being able to increase the supply of food by successful completion of the project is reasonable.

The qualifications of the two principal investigators are very high, and their experience in this field of endeavor and their understanding of the problem are both broad.

The reservations of the subcommittee concerning the proposal center around the concern that too much is being suggested in too broad a geographic area in the time proposed. The objectives are broad and ambitious, and the problems too varied and complex for reasonable solutions to be achieved in two years.

The subcommittee recommends that before final decisions are made about a grant, that the principal investigators be asked to provide more details and specific directions for the work proposed, and that they narrow the scope of the activity. The budget seems high for what is likely to emerge in the first two years of effort.

The link with the African countries to be involved in the work may be good, the formal arrangements that must precede successful collaboration appear to be soft so far; two countries have expressed tentative agreements of cooperation, but it may be useful to make these more solid.

II. DISCUSSION

Dr. Idyll remarked that Sea Grant had considered funding this particular project and in its review had made the same suggestions as did the subcommittee. Revisions of the project proposal were already underway as a result of the Sea Grant review. Therefore, a requirement for revisions should not be a heavy burden for the principal investigators.

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Dr. Ehrenreich added that the principal investigator should also try to reduce the budget.

Dr. Pimentel hoped that the principal investigators will document their contacts in West Africa. He thought that it was necessary that there be agreements from both sides before the grant is made.

Dr. Smuckler pointed out that this was not a research proposal. It was really an establishment of collaboration between an African institution and the University of Maryland.

Dr. Peterson agreed that this is not a research project. Whether it is a developmental project was also open to question.

Dr. Minners noted that he had several questions about the project. It was in reality a capacity strengthening project. Therefore there was not much in the way of technical information provided by the researchers.

Dr. Long suggested that one way out would be not to let the talent go to waste. To ask the principal investigators if they could develop a feasible research project.

Dr. Greathouse asked what involvement would the project have with REDSO, the Regional AID African Organization. Also he wondered if there had been enough internal discussion of the project.

Dr. Idyll agreed that it was not a research project. There would be some research involved but it would deal much less with agriculture than ocean fisheries and would be in areas that could not be considered research.

Dr. Farrell suggested that the principal investigators should identify the problems they want solved before requesting a grant.

Dr. Elmendorf pointed out that this is a capacity strengthening proposal and that more of the money was actually being budgeted for the University of Maryland than for African institutions. She wondered which institution would be strengthened.

The motion was made to defer the project and ask the principal investigator to narrow the scope of the research, to sharpen the objectives of the proposal and to reduce the budget.

The vote on the motion was unanimous in favor.

An Inventory of the Plant Resources of Selected Areas on the Eastern Slope of the Andes in Peru - Missouri Botanical Garden

I. SUBCOMMITTEE REPORT

Dr. Farrell, chairman of the subcommittee, that also included Drs. Ehrenreich and Sloger reported as follows:

This proposal is essentially a taxonomic effort in an area of rapidly declining naturalness due to agricultural penetration of virgin vegetation cover. Its scientific worth is ultimately great, but probably long term in its potential utility.

II. DISCUSSION

Dr. Ehrenreich pointed out that this type of study collects basic information before launching into the revegetation program. He suggested that before the project is funded collection sites should be identified.

Dr. Pimentel raised a question about the relevance of the project. He felt that it may not be strong enough for approval. He personally gave more weight to relevance than did some other members of the RAC.

Dr. Skinner wondered whether new or useful plants would be found. The utility of the project would then go beyond that suggested in the proposal. This is virgin territory. There is no other approach that can be used except a basic taxonomic effort. It is a very fundamental step. It is difficult for him to see this project as an AID undertaking except maybe as a long range project.

Dr. Greathouse pointed out that the authors did not summarize what their predecessors had done in this geographic area. He could not believe that nothing had been done there.

Dr. Ehrenreich noted that some other institutions had worked out there. However, no information had been provided on the basic taxonomy of the areas.

Dr. Elmendorf felt the project could place more emphasis on food and medicinal plants. She also pointed out that none of the Latin American bibliography was included in the proposal.

Dr. Pimentel remarked that the project does not deal with preserving the environment. It is simply collecting and classifying plants.

Dr. Farrell responded that before the ecology of the area could be understood, one must understand what plants are there. This is a virgin area. The interdependence of the species there must be determined first before any steps toward revegetation can be taken. This is really a modest first step.

Dr. Long remarked that he personally gets turned off by the search for new medicinal plants. He felt there was a sampling problem involved in the project. Is it the intent of the principal investigators to use the two areas they have identified as a proxy for a larger area? Otherwise you can't move from the information collected to an AID program in a larger area. If the areas are a proxy for a larger area then there must be some intention to do something more than simply collect the basic taxonomic information.

Dr. Farrell pointed out that the researchers had identified two key areas. The characteristics of these two areas could be better defined. However, there is no way the researchers could undertake taxonomic effort for the entire Andes region.

A motion was made to approve the project.

The vote was 8 in favor of the motion and 8 not in favor of the motion. The 8 that voted to disapprove the motion could be broken down as 3 who would vote for deferral and 5 for outright disapproval of the project.

Reference Manual for Forest Biomass Energy Resource Assessment
- The Solar Energy Research Institute

I. SUBCOMMITTEE REPORT

Dr. Farrell, chairman of the subcommittee, that also included Drs. Ehrenreich and Pimentel reported briefly on the project.

Dr. Farrell said that the subcommittee recommended disapproval of this particular proposal because of its low relevance to the AID program. In addition, he pointed out that the objectives had already been met by a Forest Service contract which is well on its way to completion.

Dr. Ehrenreich pointed out that another project funded by AID at \$1.5 million included the objectives of this project. The report from this large project was to be delivered to AID on October 1.

The contractor was contemplating the possibility of renewal of the contract.

Without further discussion the motion was made to disapprove the project.

The vote was unanimous in favor of the motion.

Domestication of the Babassu Palm - New York Botanical Garden

I. SUBCOMMITTEE REPORT

Dr. Ehrenreich, chairman of the subcommittee, that also included Drs. Farrell and Peterson reported as follows:

This project looks interesting and well prepared and the principal investigator certainly seems capable of carrying out the objectives. Although the project is titled "domestication", it is really background work, although necessary, for a domestication project (which may have to be done by other persons). As the author states, "From an agronomic viewpoint, it is impossible to know with this group of palms exactly what organisms are, and how they are related to each other, vital information that must be determined before an expensive domestication program is undertaken. For as much time and effort is spent blindly collecting seeds with no knowledge of their true identity or phenotypic plasticity (here I would add ecological amplitives), the danger is that one might end up with collections of things thought to be different, but under identical conditions of cultivation found to have been only ecotypes or similar forms. The background information is well reviewed and points out the potential economic values from possible domestication (food, fiber, fuel, etc.).

The basic inventory work seems logical and appropriate, although anticipated counter cooperation may be more difficult to obtain than indicated. It would seem that while the inventory is being made that some information could be obtained on *Oenocarpus* and *Jessenia* Palms at no extra cost to this project.

The scientific approach seems logical, sound, and achievable from the taxonomic and ecological points of view. It appears that the principal investigator has excellent experience in the regions to be studied, good LDC contacts, and excellent opportunities for LDC participation and institutional strengthening.

II. DISCUSSION

Dr. Peterson pointed out that the project mentions a Brazilian institution. Has such an institution been identified?

Dr. Greathouse asked if Brazil is the most appropriate country? How secure are the linkages to further development? Where will the funds to continue come from?

Dr. Pimentel remarked that the babassu palm is a valuable tree but it has some problems. The collection of samples and taxonomic work is being proposed. The proposal never suggests how the palm might be domesticated.

Dr. Pimentel also pointed out some inconsistencies in the report concerning comments on high yield and on the average fruit yielded.

Dr. Ehrenreich remarked that the main point of the proposal is that this was a predomestication effort. Domestication would take place in several places with similar habitats.

Dr. Smuckler asked the subcommittee chairman if the germplasm bank would be established and maintained in Brazil? The response was yes.

Dr. Long asked who would provide later funding?

Dr. Ehrenreich responded that the Brazilians should be able to do it without too much trouble. The maintenance of the germplasm bank would not require much of an investment.

Dr. Smuckler suggested that the question of who would provide future funding should be answered if the project is approved.

Dr. Long pointed out that in this project collaboration with some institution in Brazil would be totally necessary.

Dr. Farrell remarked that botanists do know enough about the babassu palm to say that some high yielders are there. However, they must be labeled and characterized prior to any domestication efforts.

Dr. Pimentel pointed out that yields are not measured in a single year. He also thought the project proposal was weak in terms of its attempts to domesticate the babassu palm.

Dr. Farrell responded that scientists have to begin somewhere and in this case the first step was identification of the species available and their characteristics.

Dr. Ehrenreich pointed out that while the author called it domestication it really was predomestication.

Dr. Peterson suggested that the researchers should find the best plants and then look at them next year.

Dr. Smuckler asked if this was a 10 to 15 year plant breeding project to obtain good plants.

Dr. Peterson thought that it was. Only some identification will be accomplished in this project.

Dr. Pimentel pointed out that the researchers were plant taxonomists.

Dr. Peterson suggested that the researchers would need to know whether the palm trees are cross pollenating and other features of this type before attempting to domesticate the palm.

A motion was made that the project be approved.

Dr. Pimentel then commented that if in this project, information on palm trees is collected and then we leave that information in Brazil, the project would be a deadend activity.

Dr. Greathouse suggested that the project be deferred. There are too many missing components of the research proposal.

Mr. Roseborough, of AID staff, pointed out that the MAB project was now finishing its second year and that this particular proposal would be an extension of a project in the MAB program. He knew that there was enthusiasm from Brazilian researchers about this activity. There was already in fact a seed bank for such palm trees in existence.

Mr. Bengé, of AID staff, pointed out that there was no indication in the proposal that the researchers plan to link up with any international group. He felt that the proposal would be strong if there were such linkage.

The vote on the motion was 8 to approve and 8 to disapprove and of those 8 all of them would have preferred to defer the project.

Dr. Pimentel suggested that they defer the project with a condition suggested earlier by Dr. Ehrenreich. Dr. Wishik also thought that modification would be more like AID wants it if the project is deferred.

Dr. Long said that if RAC is advisory, the administration could proceed even if the RAC recommended that the project be deferred. The issue is not so closed. Project broadening is apparently being suggested. He felt this particular project was a launching pad for later events. It involves predomestication of the babassu palm. If all conditions were met, this group should pack up and leave the next step to others. If for \$150,000 this project can get something started, then the proposal is a good idea.

Dr. Peterson says he could go from approval to deferral because different type of people are needed for the next step in the research.

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Agronomic and Domestication Studies of Oenocarpus - Jessenia
Palms - New York Botanical Garden

Dr. Ehrenreich, chairman of the subcommittee, that also included Drs. Peterson and Sloger reported as follows:

This proposal does not have the well defined objectives and approach of the other palm proposal. The approach is not crisp and logical, but rather vague, which leaves one with uncertainty as to exactly how the principal investigator plans to carry it out. This is even more important because the principal investigator is a recently graduated Ph.D. and does not have the depth of experience of the principal investigator on the other palm proposal.

The proposal has a number of subprojects such as somatic hybridization, which seems out of place for this particular proposal given the objective, time frame, and budget. There are also too many unanswered questions for one to feel comfortable that the principal investigator can indeed carry out the objectives such as description of the sampling method (see the Babassu project), the so-called suitable research center for germ plasm banks, the number and place of the "selected populations" of species to visit, the tie with an LDC institution seems vague and uncertain, the "learning" of ethnobotanical uses from aboriginal people is vague. Also, it said that mycorrhizal studies will be done; it is not specific how, where, or when they would be done.

For the above reasons the Committee could disapprove this proposal for funding, but suggest that the principal investigator could work with the principal investigator of the Babassu proposal.

II. MOTION

The motion to disapprove was made by the subcommittee chairman.

III. VOTE

The vote was unanimous in favor of the motion.

Sesbania - University of Hawaii

I. SUBCOMMITTEE REPORT

Dr. Peterson, chairman of the subcommittee, that also included Drs. Sloger and Skinner reported that the Sesbania plant had proven not to be very useful in California where it was tried some years ago. He recommended disapproval of the proposal.

Dr. Wishik remarked that if the Agency was looking for innovative ideas why not consider this legume? The proposal certainly was innovative.

Dr. Peterson replied that it was an old old legume. There had been a lot of work done with it and it had not proven to be useful.

Dr. Elmendorf remarked that she had inquired about sesbania at the National Academy of Sciences where she was told that its a fairly common source of animal feed and was potentially a nutritious human food.

Dr. Greathouse suggested that this proposal could tie into existing efforts on sesbania.

Dr. Pimentel agreed. He pointed out that in India rice plants are sometimes interplanted with sesbania since the sesbania also provide biological nitrogen from the air. He suggested that this type of technology might prove to be useful.

Dr. Long remarked that he too had seen sesbania growing in rice bunds in India and in quite large quantities. He suggested that what was needed is a survey of what has been done with sesbania around the world.

Dr. Sloger remarked that sesbania is a fairly interesting plant that fixes nitrogen in water on its roots and as well as on its stem. The measured rate of nitrogen that is fixed from the air with sesbania is ten or more times as much as that for soybeans. There is some interest in the scientific world in sesbania as a green manure because of its excellent nitrogen fixing characteristics. There is a lot of interest in the collection of the rhizobia from sesbania. It belongs to the cowpea group so that in most cases, the required bacteria is already in the soil.

Dr. Elmendorf suggested that there was an opportunity to tie into the University of Hawaii project Niftal and the other AID research projects in soils. Also there was a potential tie in agriforestry projects done in Hawaii.

Mr. Bengé, from AID, cited a study he had done in 1974 on sesbania. The Office of Food and Natural Resources is very interested in this particular plant. He felt that it was widely used as a crop.

Dr. Peterson then changed his motion to the following:

II. MOTION

RAC moves that this project be deferred for purposes of requesting that a revised project be submitted which will; 1) provide a review of current literature showing where and how sesbania is currently being used in LDCs, 2) show evidence that the project leader has been in communication with other investigators working on sesbania and has taken their ideas into consideration and 3) provide information clarifying project leadership which is not clear since the proposers and investigators are different people. Assurance is requested that adequate leadership is provided.

Dr. Wishik asked Mr. Bengé if there would be any merit to reviewing what is going on with this particular plant.

Dr. Sloger then pointed out that since part of the proposal is to prepare a state of the art paper, the type of review suggested by Dr. Wishik would be done.

Dr. Peterson said he would be concerned if the uses and purposes of the research were not cited. He felt that more documentation should be done on the proposal.

Dr. Long then summarized some of the points that were made. First the proposal should cite the literature on this subject, second, information is needed on who is doing what should be the main subject of this project. Third, there should be a tie-in with other projects.

Dr. Greathouse thought the potential was here for a young researcher to find out what the state of art is in this field.

III. VOTE

The vote on the motion was unanimous in favor.

Selection of Prosopis Pod and Woody Biomass Producing
Characters for Use in LDCs - Texas A&I University

I. SUBCOMMITTEE REPORT

Dr. Moss, chairman of the subcommittee, that also included
Drs. Ehrenreich and Farrell reported as follows:

The four best pod producers of widely divergent backgrounds developed from the California screening trials i.e., a P. pubescens (screwbean), a P. Alba from South America, a P. velutina from Arizona, and a California native P. glandulosa var torreyana selection with 17% protein has been planted in three replicates of 25 trees each at three phosphate levels of 0, 60, and 120 kg/ha PO_5 , respectively. Sixteen - 0.09 ha plots of our best woody biomass producer have been planted to allow harvest of four replicates every year for four years and thus determine biomass accumulation as a function of time. Two small trials of Leucaena leucocephala (Hawaii Giant K-8) have been planted to examine woody biomass production and forage production in the South Texas climate which receives more frost and less rainfall than Hawaii or the Philippines. A 0.8 ha plot has been established to examine selections for fast growing thornless ornamentals. Two hundred trees from a thorny parent with high sugar content and large pods have been planted to examine progeny for segregation of pod and thorn characters. To meet seed requests of developing countries, 100 trees of 8 outstanding selections possessing either high woody biomass production, early pod production, salt tolerance or frost tolerance have been planted at wide spacing (7.5 m or 24.6 ft.) to serve as a seed orchard. A small screening nursery has been planted to examine recently obtained P. tamarugo from Chile, carob from Cyprus, other tree legumes and sympatric non-leguminous species such as jojoba.

All previously mentioned plots have been planted by Felker and one technician. Pod collection, data analyses, cultural practices such as tree pruning and staking, weed control, on this multi-facted 12 ha plantation is a far greater task than can be completed by Felker and one technician, two graduate students, and three part-time undergrads for data collection, data analyses and maintenance of these plots.

With funding from the UNDP and with the cooperation of Mr. Musnad and Mr. Hourri of the Forestry Research Institute at Khartoum, Sudan, Felker has established a screening trial of 30 tree legume species near Khartoum. Felker hand carried seeds and rhizobia to Khartoum in the summer of 1979 and provided

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special planting containers. Some of these trees are now producing pods in only a little more than a year from transplanting. Excellent relationships have been developed with Mr. Hourri, Director of the Institute, and Mr. Musnad, his associate.

A student under Mr. Hourri's guidance is seeking to study applied aspects of tree legume physiology with Felker in Texas. Support is requested from AID to visit the Sudan Prosopis plantings and to renew collaborative efforts with Musnad and Hourri.

Seeds, rhizobia, and technical expertise in both woody biomass production and pod production will be provided to "Operation Double Harvest" in Haiti through a visit by Felker. Mr. Micheal Benge has agreed to assist in providing contacts with operation double harvest.

This proposal is, then, a follow-up of work done in California. It is not a proposal for specific work, but rather an add-on to an ongoing Texas A&I project--essentially help so they can do a little more.

The subcommittee does not know the principal investigator. No vita was attached so we cannot judge his ability. We have no way of knowing how much work has been done since there is not literature review. Dr. Ehrenreich is familiar with much Prosopis done in many southern states going back at least 70 years. Work on animal food value has been done in Arizona, New Mexico, and California. Texas Tech has a major project on control of Prosopis, which is a troublesome "weed" in much of West Texas.

The proposal has some positive shreds, although its lack of completeness makes it hard to judge. The subcommittee recommends rejection of the proposal as written. It may be that the RAC would want to support a specific proposal outlining a clearly defined piece of work on Prosopis which was applicable to LDCs. The subcommittee cannot recommend support of the general, add-on proposal which is before us.

II. DISCUSSION

Dr. Greathouse suggested disapproval of the project as it was laid out.

Mr. Benge, of AID, remarked that this project would not be introducing a plan to Haiti. It was already a good firewood crop there. It is a multi-purpose plant that also is good for forage.

The King Ranch in the United States now looks at it as a promising plant even though at one time they considered it a pest. There is a need to link up with the LDCs in carrying out the work. A graduate student should be able to carry out this project.

Dr. Ehrenreich suggested that one of the problems of the proposal is its scientific merit.

Dr. Schweigert asked how Texas planned to use this plant in the future. He was very sensitive to the priorities that had been given to eradicating the plant in the past.

Dr. Greathouse remarked that he preferred AID to work through major institutions in Texas and elsewhere in the West if such research on this plant is to be done.

Dr. Peterson asked Mr. Bengé why the King Ranch now likes the plant?

Mr. Bengé responded that the state of Texas was looking at it for biomass for energy. They were particularly interested in the thornless variety as a potential forage for cattle.

MOTION

The motion was made to disapprove the proposal.

VOTE

The vote was unanimous in favor.

Feasibility and Planning Studies for Low-Cost Television Systems Adapted to Less Developed Country Use - Battelle Pacific Northwest Laboratories

I. SUBCOMMITTEE REPORT

Dr. Anderson, chairman of the subcommittee, that also included Drs. Hrones and Elmendorf reported as follows:

The subcommittee felt that if the production of these sets could be calculated as in the proposal it would seem that this particular venture is now ready to turn over to a manufacturer and there would be no reason for AID to become involved in it. He felt that a manufacturing company could carry out the remaining engineering productions design much better than could Battelle.

Dr. Hrones while not present at the RAC meeting had suggested in his letter to Dr. Anderson that the estimate of the cost for the television tubes was considerably underestimated by at least a factor of four.

Dr. Elmendorf suggested that the work should also go to a firm if production of these television sets was as far along as indicated.

The subcommittee was not sure what Battelle was agreeing to deliver to AID beyond the analysis of production possibilities that were already stated in the proposal.

Finally, the subcommittee asked what provisions or patents would be made if the research was carried out?

II. DISCUSSION

Dr. Shenkan, from the Office of Education, remarked that this was an unsolicited proposal. He felt that Dr. Anderson's questions were legitimate. However, the proposal, while looking at receivers, also looked at the entire television system. He pointed out that television technology has gotten more complicated rather than less so. The costs probably were not underestimated, however one must go through this project in order to determine if the cost can be reduced. Senegal now has 1,000 such receivers. If sets could be produced locally then some time could be commanded for education by television.

Dr. Weller suggested that the project seemed to be illogical and out of phase.

Dr. Wishik said he could react positively to the proposal under some circumstances. However, why wouldn't Battelle invest \$100,000 dollars to carry out the project.

Dr. Sanderson had problems with the proposal. He said that it was merely a proposal to strip some of the components from the standard television set and to degrade video quality for the purpose of reducing costs, one channel would be left. In fact, the television would be stripped down so that it is viewable only in a dim room. You would need a receiver in each village. If you look at the overall cost for a system that would not be satisfactory, the proposal was not one that should be supported.

Dr. Peterson asked why hasn't private industry done this?

Mr. Shenkkan from DS/ED, said that at this time there was no market. He found the comments about costs of the television to be curious and pointed out the transistor radios in developing countries sell quickly for \$20.00 to \$30.00. This television set would cost less than that. He felt that it would be useful as an educational device.

Dr. Skinner pointed out that there are certain types of technology that are already available. Therefore, why aren't these television sets already produced by commercial firms?

Mr. Shenkkan from DS/ED, remarked that he thought that there would be a market. It was a matter of introducing the idea into developing countries.

Dr. Anderson pointed out both Dr. Hrones and Dr. Sanderson thought the development of a high quality but cheap television receiver was probably not feasible.

Dr. Elmendorf thought the Committee should not overlook the fact that this project is innovative and it is relevant; but apparently it is not feasible.

III. MOTION

A motion was made to disapprove the project.

IV. VOTE

The vote was in favor of the motion. However, there was one abstention from the voting and several RAC members voted against the motion.

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Policy Studies on Information Technology and Development
Academy for Educational Development

I. SUBCOMMITTEE REPORT

Dr. Skinner, chairman of the subcommittee that also included Drs. Hrones and Anderson reported as follows:

The object of this proposal is to study the relationship of various kinds of communication technologies on development. The communication devices would include radio, telephony, television (both full - motion and "slow scan") audio and video teleconferencing, data storage and data communications, cassette and disc storage of video and audio, and message storage and retrieval technologies, especially by low-cost micro-computers. These technologies were often used in combination. Certain of them are more important for major LDC uses than others.

We are urged to imagine the following scenario: "A Kentucky farmer sits down at a video terminal called a Green Thumb Bus at his local agricultural cooperative to find out the latest price for corn and hogs. An Alaskan village school teacher sends a message over the electronic mail system to find out what teaching materials are available on tundra technology... In the South Pacific an agricultural extension worker goes to the local university of the South Pacific extension center to find out what can be done with citrus by products. His question is typed into a microcomputer terminal and sent via satellite to the College of Agriculture in Western Samoa. In an Egyptian village, a vegetable wholesaler phones Alexandria to find out how much produce will be needed in the next twenty-four hours."

What our investigators would like to find out are:

- 1) What socio-economic effects these techniques have on development?
- 2) What criteria should be used to determine what technologies necessary for what kind of projects?
- 3) What technologies AID should consider in its strategies for development? What information is available along these lines? What can AID do to get government agencies to transfer these technologies?
- 4) How can information technologies supplement or partially substitute for other forms of technical assistance?

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The hope is that:

- a) a set of recommendations on policy evaluation could be provided for AID in helping LDCs.
- b) recommendations for U.S. industry to provide technologies for LDC markets
- c) exchange data among U.S. government bureaus, representatives of LDCs
- d) increase understanding by AID of other governmental and non-governmental use of technologies
- e) write a book on information technologies and development.

The cost of this would be a modest \$500,000.

The Committee's views are that the proposal is too diffuse and general. There was not enough thought given to conditions in the LDCs to facilitate the use of the proposed technologies, nor were data provided to show what is now being done in the LDCs with the technologies at hand. In fact there is no evidence that one of the principal participants in this proposal ever was in the Ivory Coast even though this fact was cited.

There was a feeling on the part of one Committee member that there was a need for the kind of data and analysis suggested, but this proposal does not suggest the methodology for such a project. Another Committee member raised a number of questions dealing with the lack of attention in the proposal of dealing with the "level of development" as linked to technology. Moreover, there was the suspicion here that A.E.D. has not clearly thought out all the implications of this topic and others such as whether AID should turn over its "Policy Analysis" to others.

In summary, then, the unanimous opinion of the Committee was to reject the proposal.

Dr. Anderson felt that the institute at least on the surface was not competent to carry out the project. There wasn't complete information on this however.

Dr. Linder pointed out that he had similar questions about the qualifications of the principal investigator. The principal investigator has had eight jobs in nine years.

Dr. Smuckler commented that his views tended to reinforce the negative comments of Dr. Linder.

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Dr. Wishik thought the idea was good, this very simplistic project talks about seminars and writing a book.

Dr. Block, from the Office of Education, pointed out that the idea of doing the study came from discussions between Deputy Asministrator Wheeler and COMSAT and others.

Dr. Block then described the qualifications of the Academy and pointed out that generally they were considered to be qualified for this type of work. In any event, the Academy would not function alone.

Dr. Sanderson was concerned about the amount of money involved. There is enough information already available in this area to indicate that its a very fast moving technology. He was concerned that the information obtained might be obsolete in three to four years. The study was a very limited study and the information could be obtained a lot cheaper.

Dr. Skinner questioned whether or not the technology that was proposed was appropriate.

II. MOTION

A motion was made to disapprove the proposal.

III. VOTE

The vote was in favor of the motion with no abstentions from the vote.

Investigation of the Feasibility of Using Microcomputers in
Less Developed Countries Statistical Processing Applications
U.S. Bureau of the Census

The report of Dr. Linder, chairman of the subcommittee that included Drs. Falcon and Swanson follows:

I. SUBCOMMITTEE REPORT

The project proposal makes the case that many statistical operations in the LDCs fail at the data tabulation stage. This is undoubtedly true. Bilateral and multilateral technical assistance agencies have contributed literally millions of dollars to assist the taking of censuses and large scale surveys in LDCs and yet all too often the results never appear because of tabulation failures. The WFS, for example, has spent something like \$40 million in assisting some 40 countries to take fertility surveys yet in many cases no results have appeared due to tabulation problems and in many cases the data had to be brought to London for tabulation.

Successful tabulation of a census or a large survey require the computer input of a very large number of items, a relatively small amount of computer manipulation of those data, and then a large output of detailed tables. This now requires a large scale mainframe computer needing a special environment, and a specialized staff.

At the present stage of development, microcomputers are not feasible for these large input-output applications. Input is difficult, and data-editing is limited. Microcomputers are especially useful in managing census operations or the tabulation of very small surveys, but present microprocessors are really not suitable for large-scale tabulation because of their limited memories, limited auxilliary equipment and the non-existence of appropriate software.

The Census Bureau proposal believes that a potential solution to these problems may be at hand with the rapid development of large-scale capacity on microcomputers and the development of appropriate software.

The proposal is for the Census Bureau to study:

1. the state-of-the-art of microcomputer hardware, including capacity, availability and maintenance problems in LDCs.
2. the state-of-the-art of existing software as it is available for large-scale data processing.

3. the feasibility of software redesign by adapting existing Census software packages (Cents, Concor, etc.) to microcomputer use.
4. to design one demonstration software module.
5. to make recommendations as to the feasibility of turnkey microcomputer systems for LDC use.

In the short range, there is some doubt that this project could produce an immediately useful evaluation report. But the stakes are high and the prospective results would be valuable in scientifically advancing the successful tabulation capability of the LDCs.

In the longer run, although there is still some gamble, the potential payoff would be very high.

The project draft which was distributed is somewhat deficient in that no detailed budget is given, no overall time table is given (except for man-months for each project element) and no indication is given of the professional level of the Census staff which would undertake the work. All subcommittee members remarked on this point as well as Dr. Ehrenreich. A revised draft satisfies these points.

Someone raised the question of the capacity of the Census Bureau to undertake this type of work. The outstandingly creative history of the Census Bureau in the data processing field is preeminent. The Census Bureau designed and built electrical punch card equipment before IBM existed; it designed and built mechanical punch card equipment before the power equipment was commercial; the first data processing computer (UNIVAC 1) was built under the direction of, and with Census Bureau funds; Census Bureau has taken worldwide leadership in developing data processing software (CENTS, CONCOR, COCENTS); the Census Bureau has taken worldwide leadership in introducing computer methods in LDCs and is training LDC nationals in the use of various hardware and software applications as well as general statistical and data collection methods.

If the Census sees, which it obviously does, the evolution of microcomputers to have an important role in computer data processing, this project deserves high priority.

The subcommittee recommends approval of this project as presented with an understanding that the focus stated in the RAC discussions be clarified.

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OTHER COMMENTS

Dr. Swanson felt the focus on the data processing might be misleading in a number of countries where resources are needed to improve coverage and quality of data. He could be more supportive if the proposal were divided into two parts, one part to look at the state of the art and the second part, depending on the information obtained in part I, to implement the project. There is a tendency in the proposal to be presumptive about the state of the art. The project would proceed without first accumulating information on where we stand now with respect to microcomputers.

Dr. Wishik thought the project proposal could be a very important document. It is concerned with a very important problem in developing countries. Also, the Bureau of the Census is the best institution to carry out this work. However, he thought the proposal should be better defined. One part of the proposal is concerned with exploration. A second part moves the Census Bureau directly into implementation.

Dr. Wishik thought the study should focus on a single type of microcomputer. It appears that the researchers want to do a complete overview of the field. On a second point, Dr. Wishik pointed out that the microcomputer is useful for many types of data; the researchers will apparently look at them all. It would be better to focus on just a few types of data.

Dr. Wishik felt the proposal needed considerable modification and should be deferred. As a final point he suggested that it would be premature to have in mind "a clearinghouse" for the shipment of microcomputer software overseas, for software development, and hardware maintenance.

Dr. Long, of A.I.D., hoped that the Agency could go forward with this proposal. It is an innovative idea in an area that needs some work. He felt this proposal required some follow-up but was a candidate for outright approval rather than deferral.

Dr. Skinner remarked that in his view, the problem in studies involving surveys were in collecting good data and not in data processing.

Dr. Pimentel thought the project could make a contribution to the developing world. He thought the value of the project was high.

Dr. Wishik suggested that the work be confined to a single country.

III. MOTION: That the project be approved with an understanding that the focus of the project as stated in the RAC discussions, be clarified.

IV. VOTE: The motion was unanimously approved.

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International Awards for Population Research: Program on the Determinants of Fertility in Developing Countries - The Population Council

I. SUBCOMMITTEE REPORT

Dr. Linder, chairman of the subcommittee, that also included Drs. Connell, Swanson and Wishik reported as follows:

This project was approved by RAC in June 1980 by a close vote and with RAC expressing a number of concerns. The project considered last year was for a 5-year contract with the Population Council for some \$7.3 million, under which the Population Council would establish a program of subcontracts for research relating to determinants of fertility having a bearing on national population policy decisions.

The need and desirability for this type of research was accepted by RAC in accord with the statement made by Dr. Speidel that "...the project was designed to redress an imbalance (a view held by some) between socioeconomic and medical research in the population program." RAC's concerns were not so much with the relevance of the proposal as with its probable effectiveness and the policies and mechanics of its operation.

Because of these concerns, RAC requested a review after the first year of operation. This review is the subject of the agenda item now before RAC.

RAC will recall that two years ago, a contract was approved for the NAS to set up a committee to identify areas which appeared to be promising for future research in factors determining fertility rates in LDCs which would seem to be related to policy decision-making. This Committee was not to undertake new data collecting or analytical research but to formulate an "agenda" for future research which would seem to be promising.

This NAS Committee has been established and has had a number of meetings. It has commissioned some 39 short papers on various topics and 27 of these are now on hand. These will be incorporated into 8 critical essays. It is not a RAC task at this time to review the work of this NAS Committee, but it will be recalled that the "agenda" to be developed by this Committee is considered a major base for the research granting program of the Population Council. Early input to the Population Council program and a general coordination of these two activities is achieved by an overlapping of three members of the NAS group and the Program Committee for the Population Council work.

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A word should be said about the steps taken in the past year by the Population Council to implement its program.

1. A Program Committee has been appointed and has had several meetings.
2. The first action of the Program Committee was to formulate a statement about the purpose, policies and procedures for requesting a research subcontract. Over 14,000 of these announcements were distributed worldwide in December 1980 and January 1981.
3. In response, the Council has received 96 short preliminary research grant proposals.
4. These 96 short proposals were reviewed by the Program Committee and, of these 24 applicants were invited to submit full proposals. Sixteen of these full proposals have now been received.
5. These 16 proposals are now being reviewed by peer groups which will assign scores or rankings to them.
6. Later this month (July 1981), the Program Committee will meet again and make awards to some of the 16 proposals based on the scores and on the funds available.

In its original consideration of this project in June of last year, RAC expressed a number of concerns. Primarily these were: a) the composition of the Population Council Program Committee, b) the coordination with the NAS "agenda" committee, c) the priority to be given to LDC institutions as research sites, d) the open-ended nature of the statement of program priorities, e) whether new ideas could be produced, f) possible conflict of interest in the granting process, and g) the balance between overhead costs and grant costs.

The RAC subcommittee believes most of these concerns have been satisfied:

1. The Program Committee consists of 8 persons, all of whom have extensive LDC experience and 2 of whom originate from LDC universities.
2. Coordination with the NAS "agenda" working group is achieved by an overlapping of three of the Program Committee members.

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3. LDC institutions as sites for the proposed research is shown by the fact that of the 16 institutions invited to submit detailed proposals 6 were from LDC institutions, 8 were from American, English or Australian institutions, and only 2 were from developed countries institutions (Harvard, Edinburgh) without specification of LDC participation.
4. The open-ended character of the program has been narrowed down by the preparation of a detailed "Program Priorities Statement." Presumably, this first statement will be further revised and narrowed as the input from the NAS "agenda" working group nears completion.
5. Detailed research proposals for the 16 projects now under consideration show a variety of innovative projects. While the detailed research proposals have not been submitted to RAC, the research topics are available and show relevance to policy decision making. The subcommittee believes these topics indicate a progressive program.
6. The Population Council has prepared a formal statement on conflict of interest which should satisfy this possible criticism of the program (Tab 7). This statement seems to follow the NIH policy on this point.
7. No information is given in this report relating to overhead costs.

It is the subcommittee view that this project is progressing well and it is unanimous in recommending continuation of this project.

RAC may wish to consider if it wishes to have an annual review of the project, or, as suggested earlier, a more detailed review at the end of the third year (i.e., 2 years from now) when the form and content of the program will be fully developed.

II. DISCUSSION

Dr. Connell added that she felt the Population Council had done everything that they had been asked to do after the last review by the RAC. They have an excellent program committee. She raised a question of the budget split of 60% for projects and 40% for management, and thought this particular issue might require some attention.

Dr. Wishik remarked that he fully subscribed to Dr. Linder's remarks. He felt the chief accomplishment of the year was the preparation of the statement on research. The statement needed to be paraphrased and broken into discrete pieces and then translated into practical jobs. However, the overall statement was of high quality. He suggested that the RAC go on record as urging the Population Council to move into the field and aggressively search for projects. He agrees that another review in one year is necessary.

Dr. Wishik asked the question; how can AID closely oversee the project? One approach might be to have a RAC member invited to be present at the program meeting. Generally, he felt the project thus far had been well done and should be continued.

Dr. Anderson thought the document on priorities was lacking in coherence and in novelty. He asked whether if the docket of projects supported through this program should be new and different. If so, he did not feel encouraged by that particular paper.

Dr. Schweigert found the report extremely interesting. He thought the mechanism that had been established was very effective and the committee appeared to be highly qualified.

Dr. Speidel, of AID, remarked that the Population Office had been pleased with the progress of the program. The Population Council had tried to ascertain AID needs. He felt the program statement prepared by the Population Council was of high quality and the quality of consultants which were available to the Population Council was also very high. He pointed out that they had achieved a balance between overhead and project cost. That in itself was quite an accomplishment. Working with developing countries requires frequent use of staff, therefore heavy input from the Population Council and this shows up as overhead in the budget.

Dr. Jansen, of AID, described the interchange between the Population Office and the Population Council. He felt the program that had been developed was excellent. The Population Council had actually sent staff members down to Washington to discuss the program and to make sure that it was addressing the needs of AID. They culled information from the Agency and that information was showing up in their work.

Dr. Wishik asked what will the program committee do next year? He felt the committee should be giving the program an overview.

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Dr. Wishik thought there was a mosaic of ideas that needed investigating and the committee should be looking at the gaps. He thought the cost should be broken down into two subsections. One would be overhead and other would be implementation.

Dr. Clark, from AID, said that when the research agenda is in full form, Dr. Wishik's suggestion will be conveyed to the Population Council.

Dr. Anderson pointed out that the priorities do not say how the linkage will be made between socio-economic factors in fertility. He felt this was an important omission in the program prepared by the Population Council.

Dr. Linder, pointing out that the RAC should express its opinion after reviewing the project, made the following motion.

III. MOTION

That the continuation of the project for the 5-year term be approved. Because of the size of the project, RAC requests a regular annual report of progress and the issues considered.

Dr. Long asked what would the review be about?

Dr. Linder responded that some of the issues raised here today would be gone over each year.

Dr. Speidel added that if the program is going well and is trouble free, then the regular annual review by AID staff should be appropriate. If problems are found then RAC can request a full review.

Dr. Peterson then asked if there was not an issue that this type of program is taking the research program out of AID and outside the review of the RAC.

Dr. Linder pointed out that he initially had been troubled by this but these projects were very small and the review mechanism set up by the Population Council is excellent. He felt in this case "wholesaling" was necessary.

Dr. Smuckler pointed out that the removal of projects from RAC review is something that the annual review of project by the RAC would include.

Immunization Against Trypanosomiasis - University of California, San Diego

II. SUBCOMMITTEE REPORT

Dr. Weller, chairman of the subcommittee, that also included Drs. Carter, Greathouse and Schweigert reported as follows:

This application, prepared in September, requested \$79,940 from the U.S. Army Research and Development Command for support of research on immunization against Trypanosoma brucei rhodesiense for the year January 1 to December 31, 1981. Lack of money for new projects in the Army R&D budget prevented funding, and the request is now sent to AID. This time delay poses problems per se. The project, which is extensive and ambitious, lists three scientists and one technician: the scientific component consists of Dr. Davis at 10% time, Dr. Braude at 5% time, and an infectious disease fellow, Dr. Ballon-Landa at 50% time. However, Dr. Ballon-Landa's funding expired as of July 1, 1981. Further, Dr. Davis is currently a Fogarty Fellow working on trypanosomiasis in Nairobi, Kenya at the International Laboratory for Research on Animal Diseases (ILRAD); he will return to San Diego in September 1981.

The research proposal is highly technical and documented by an extensive review of pertinent literature. It is now well known that typanosomes in the invertebrate host escape host immune defense mechanism by a sequential process of antigenic change which involves variation in the nature of the glycoprotein surface coat. The shifting antigenic composition of the coat precludes development of effective humoral and cellular immune responses. Additionally, there is some evidence that the coat glycoproteins are involved in immunosuppressive phenomena which are a feature of trypanosomal infections.

In view of these considerations the investigators propose to prepare other surface components and trypanosomal products free of glycoprotein and to test their immunizing capacity in rats. Additionally, whole procyclic organisms that lack a glycoprotein coat will be examined. Briefly, they plan to study:

- 1) A toxic substance produced by trypanosomes, tryptophol; available as indole-3-ethanol.
- 2) A platelet aggregating factor, probably a protein, previously isolated by the investigators in the course of studies on thrombocytopenia in trypanosomiasis.

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- 3) A lipo-polysaccharide substance, known as complement activating factor (CAF-T).
- 4) Cytoplasmic membranes.
- 5) Flagellae.
- 6) Glycoprotein coat degraded with trypsin.

The facilities for the proposed work are adequate and well equipped. The budget, as prepared for one year, i.e., 1981, is reasonable in terms of supplies and equipment but not relevant with respect to personnel in view of changes since preparation. The proposed research is sound and excellently described. The applicants indicate that the several objectives outlined can not all be accomplished in one year. This statement bears on the single area of concern revealed on review of the proposal, namely, the relatively small proportion of time that the scientific staff plan to devote to the project. Dr. Davis indicates 10% effort and Dr. Braude 5% effort. The research Fellow, Dr. Ballon-Landa is listed at 50% time. There is only one technician who listed for 100% effort. It appears doubtful that a team of this size will in fact be able to prepare and test the 7 different antigens that are the justification for the purchase of 490 rats, and to additionally utilize 200 mice and 50 rabbits as budgeted.

In summary; this is a sound proposal that rates a "1" on the basis of scientific merit, relevance, and innovative character. It has no capacity strengthening component. However, the budget needs updating and the limited staffing deserves consideration. If AID desires to support a new initiative of this type, then it should be visualized as a multi-year project and the group at San Diego should be prepared to invest an augmented and commensurate proportion of their scientific effort.

II. DISCUSSION

Dr. Smuckler asked if the Army was running out of money for research purposes.

Dr. Weller responded that while the Army wasn't running out of money, research funding at the Department of the Army was indeed becoming scarce.

Dr. Schweigert pointed out that the budget was very modest for the amount of work that would be done. Neither he nor Dr. Weller had requested a budget reduction.

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Dr. Wishik described his visit to the research facility at the University of California in San Diego and where he found a great variety of research being done on this topic. The trypanosomes keep changing and the researchers have worked out a way of bypassing the changes. The idea for doing this had occurred quite accidentally when a San Diego man who had contracted trypanosomiasis had extensive bleeding. Through caring for this man an antigen was identified that is the common factor for all trypanosomes. There are some problems yet to be worked out in this activity, however, and that's the purpose of the present proposal.

The co-investigator is to return from Nairobi and will bring an African strain of trypanosomiasis with which future research will be done.

Dr. Smuckler asked if this was one of the diseases that is included in the WHO research program?

Dr. Weller said yes. There was a tremendous amount of research going on not only in WHO but by some commercial companies also. However, this particular project has unique aspects.

Dr. Long asked if this would have merit at \$800,000? Would it be an inadequate framework to examine whether AID should get into this vaccine work?

Dr. Weller replied that with a reasonable extension it may stand on its own.

Dr. Wishik agrees that the field will pick up the research if this particular project were successful. He recommended increasing the budget and extending the project to 2 years.

Mr. Ed Smith, of AID, pointed out that this proposal does not compete for money with malaria proposals. The continuation of the project would depend on the interest of the Africa Bureau. However, he felt that we should not think of this as strictly an AID funded project. The trend in this area is very much in the direction of multi-donor projects.

III. MOTION

The motion was made to approve the project with the suggestion that the staff explore a new higher budget for a two-year period to provide for of higher supervisory inputs into the project.

The motion was accepted unanimously.

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Effects of Insect Pathogens on the Ability of Mosquitoes to Transmit Malaria - University of Florida

I. SUBCOMMITTEE REPORT

Dr. Pimentel, chairman of the subcommittee, that also included Drs. Weller and Wishik reported as follows:

The incidence of malaria in the world population is growing rapidly due to insecticide resistance in mosquitoes, drug resistance in the pathogen, and other factors related to malaria control. One of the worst situations, for example, is in India where the incidence of malaria that had been reduced to about 100,000 cases per year in 1961 has now (1978) been estimated at 50 million cases. Clearly, malaria remains one of the major diseases of mankind.

The objective of this research project is to determine if induced sublethal infections in mosquitoes by some insect pathogens will reduce the ability of the Anopheles quadrimaculatus mosquitoes to transmit malaria protozoan organisms (Plasmodium sp.). The research has merit if it can be documented that mosquito pathogens kill mosquitoes and/or reduce the ability of the mosquitoes to transmit the human malaria parasite when mosquitoes themselves are infected with a pathogen. Thus, some mosquito pathogens may offer two methods for human malaria control.

The sublethal effects of some pathogen types on mosquitoes warrant investigation as proposed by Dr. Martin Young. Previous investigations have demonstrated that Nosema algerae (protozoan) reduced malarial oocyst development of P. falciparum in the mosquito, A. gambiae, by more than 90%. Additional studies have further confirmed that if Anopheles mosquitoes are infected with the protozoan Nosema sp., then the development of protozoan Plasmodium sp. is suppressed. Thus, the investigator's selection of Nosema algerae and Vavrai culicis as two protozoans with potential to reduce infections in Anopheles quadrimaculata of Plasmodium berghei is sound. The sublethal development of either of the two mosquito protozoan pathogens appears to have the possibility of interfering with the development of the malarian protozoan in the mosquito. Indeed, the potential benefits of this research are significant.

The inclusion of Bacillus thuringiensis (BT) and E. sphaericus (BS) in this investigation is questionable. Both BT and BS live and multiply extracellularly, therefore, the chance of

either Bacillus interfering with Plasmodium infections in mosquitoes is probably zero. In contrast, Nosema and Vavraia are both microsporidians that live and multiply intracellularly within the mosquito, in a manner similar to that of Plasmodium sp. This is probably the biological reason why these protozoans interfere with Plasmodium development within mosquitoes.

Some recent data were sent that indicated that the longevity of adults of A. abimanus that survived an exposure to BT (H-14) had a 70% reduced longevity when 73% of the larvae died. Eclosion was somewhat affected at this high larval mortality level.

These findings are not surprising with BT that includes a toxicant. Sublethal dosages of any toxicant (including insecticides) may give similar results to BT on mosquitoes. If normal feeding, growth, the development are affected by a toxicant (BT or insecticide), then effects may be noted in the pupal and adult stages. The impact may be increased pupal mortality, reduced longevity in adults, and reduced egg production.

The possible advantage of the protozoans over the bacterial organisms is that they might be self-sustaining infections in some mosquito populations. If this were so, they have several advantages over the BT and BS because these organisms have to be cultured and sprayed like insecticides for mosquito control.

Once some data are obtained on the effectiveness of the protozoans in suppressing malarial parasite development in the mosquito both by killing the mosquito and suppressing development of the malarial parasite, it would be beneficial to make a theoretical assessment of the potential of this control system for malaria under natural conditions.

Details on how the experiments are done are not included. No time scale for the research over the three-year period is given for the study.

Ratings of this proposal are as follows:

- (1) Scientific ability of the Principal Investigator (Outstanding)
- (2) Scientific merit (Medium)
- (3) Transferability (Low)
- (4) Relevance (High)
- (5) Innovation (Protozoan = high - Bacteria = low)

Three items in the budget deserve examination:

The salary for a graduate student at the full-time equivalent of almost \$16,000 a year seems excessive. Travel costs to meetings are excessive by NIH standards. The item, "supplies - animal work" is not justified. On the other hand, a figure of \$200 a year in years 2 and 3 for publication costs seems inadequate. A dissecting microscope is also listed in the budget with no justification.

No discussion was included concerning the environmental impact of employing pathogens for mosquito control. Insect pathogens may affect beneficial non-target species. Although this project is only a preliminary laboratory study, it would be of value for the investigators to be cognizant of the potential environmental impact of employing insect pathogens for mosquito control.

II. DISCUSSION

Dr. Rechcigl asked if there would be environmental problems with this project and if so should they be part of the investigation.

Dr. Wishik remarked that these two projects would add two more approaches to the malaria network. He suggested that this work be limited to the lab to avoid any environmental problems.

Mr. Smith, from AID, pointed out that he was a little confused by the RAC procedures. This project had been approved by the RAC earlier and now a motion was being made to defer it. He thought that the researchers should be given a free hand. He pointed out that they were top investigators, the research was innovative and he did not think the researchers hands should be tied.

Dr. Pimentel remarked that the project had been approved by the RAC with provisos at an earlier meeting. Now it was being submitted for consideration for the Science Advisors program. In the context of special review for the Science Advisor's program he would recommend deferral with the same conditions as in the RAC meeting. He finds that the study of bacillus is still in the project, whereas the RAC at the earlier review, suggested that it be removed.

Mr. Smith said that he could not provide a great of the details on the bacillus research.

Dr. Pimentel thought that the researchers should at least provide some information such as which strains, etc.

Dr. Farrell asked if these protozoans were hazardous to human health if they were to escape in the laboratory?

Dr. Pimentel thought that based on general evidence that these protozoans were quite specific to the insect and the effect on humans should be minimal or zero.

III. MOTION

That this project be deferred with the hope that the investigators would focus their research on the potential of protozoan insect pathogens interfering with malarial development and transmission in Anopheles mosquitoes. The research plan should include details on lab procedures to be emphasized. Justifications are needed for the following budget items: travel, microscope, animal work, and publications.

IV. VOTE

There was one vote opposed to the motion. The motion carried.

The Use of the Annual Fish for Nothobranchius guentheri Mosquito Control in Sri Lanka - Oerenreich Foundation for the Advancement of Science

I. SUBCOMMITTEE REPORT

Dr. Pimentel did not vote on this project since the investigator is a member of the faculty at Cornell.

The report of Dr. Weller, chairman of the subcommittee, that also included Drs. Pimentel and Wishik reported as follows:

Annual fish inhabit bodies of water that are dry seasonally and have the potential for mosquito control in arid areas of alternating rainy and dry seasons. They survive the dry season in the form of embryos encased in the dry substrate. The embryos enter a diapause, or developmental arrest, similar to that observed in species of insects. Thus, the East African annual fish, Nothobranchius guentheri has been proposed as a unique means of mosquito control in temporary waters.

In this study, embryos and fish are proposed to be introduced in Sri Lanka into specific habitats that are known for their large mosquito populations and that undergo periodic drying. Both the fish and mosquito populations will be followed to study the effectiveness of the fish as a larvivore in nature.

The use of N. guentheri (Ng) for mosquito control in temporary ponds and pools has merit and should be investigated. However, this should not be done without knowing a great deal more about these fish. Introductions of new species into country always offer a potential threat and problem. This has been documented many times in the past.

In East Africa in the native habitat of the fish Ng, the investigator should obtain information concerning the following essential questions before the fish is introduced into Sri Lanka:

- (1) How effective is Ng in feeding on mosquitoes and how many fish per mosquitoes are required to provide control under natural conditions?
- (2) What is the minimum time required for Ng fish population to develop from the few surviving embryos and to reach a density suitable for mosquito control? (Mosquitoes have a generation time of about 1 month and produce hundreds of eggs).

- (3) Ng feed on aquatic and terrestrial organisms in addition to mosquito larvae (Bailey, 1972), but do they favor mosquitoes? (The investigator said that the environmental impact of Ng will be "non-existent." This is not the case if, as Bailey reports, Ng fish prey on aquatic organisms in temporary ponds and pools. Beneficial frogs, toads, salamanders, and insects also breed in these ponds. If Ng prey on these beneficial organisms, their introduction into Sri Lanka may result in a worse mosquito situation than at present).
- (4) What is the minimum time that temporary ponds and pools must contain water annually for Ng to maintain its population?
- (5) How do Ng disperse in their native habitat?
- (6) What about considering other species of annual fish that might be more effective in preying on mosquito larvae?

II. DISCUSSION

Dr. Wishik agreed with Dr. Weller on the points he made. However, he found that this particular project and the previous one on the mountain beaver to be the most stimulating of projects submitted to the RAC for review. There were lots of questions about the project but he felt that it was much too innovative an idea to discard lightly. The principal investigator is very enthusiastic. He felt that breeding of the fish was premature. The first phase should be in the laboratory and then from there see where the project would go.

Dr. Ehrenreich said that he had talked with an agriculturalist at the Department of Agriculture, who mentioned that the USDA was funding a large program in Arkansas in this area. He wondered why the investigator, who was using a species of fish from Africa did not want to carry out his work in Africa.

Dr. Idyll was concerned about the introduction of the fish species to Asia. He felt that the project should be deferred or disapproved because of the potential hazards of this activity.

Dr. Skinner said the principal investigator did point out that the fish would later be used in Zambia.

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Dr. Peterson asked why couldn't other mosquito fish be used? They were commonly known and in fact mosquito fish were used in mosquito control in other areas. Why this particular species that would have to be introduced in a new area of the world?

Dr. Weller pointed out that the investigators were planning to control vectors when the rains are high. In Sri Lanka the transmission of malaria is low in high rains. It goes up when the monsoon fails. The controlling of mosquitoes in gem pits may not be enough to reduce the rate of malaria.

Mr. Smith, from AID, pointed out that the gem pits in Sri Lanka were responsible for a tremendous upsurge in malaria in the country. The principle investigator had discussed the problems of introducing the fish into Asia but had assured Mr. Smith that this fish could not live in an open environment, that he must live in a very closed environment such as the gem pits. This information had not appeared in the proposal.

Dr. Long asked if there were any problems in Burma with the gem pits causing an upsurge in malaria? Mr. Smith responded that the pits had been filled in Burma.

III. MOTION

The motion was made to disapprove the project. One member of RAC abstained from voting and there was one negative vote which would have preferred deferring the project rather to disapproving it. The motion to disapprove carried.

The Role of VA Mycorrhizae in the Phosphorus Nutrition of Legume Seed Crops in Thailand - Department of Agriculture, Bangkok, Thailand and University of Hawaii

I. SUBCOMMITTEE REPORT

The report of Dr. Sloger, chairman of the subcommittee, that also included Drs. Moss and Peterson is as follows:

The subcommittee recommends disapproval of this project as it is written. There is little chance that the investigators will accomplish objective B because during the past 40 years all attempts to get in vitro growth of mycorrhizal fungi have been unsuccessful. The proposal fails to state the nature of the scientific interaction between the principal investigator and the cooperating scientists. In addition, the plan of research lacks sufficient detail which, to me, indicates a lack of knowledge by the principal investigator in this area of research. The proposal does not cite any published or preliminary reports suggesting that grain legumes grown in paddy rice fields have limited or no mycorrhizae. Yet, this phenomenon has been observed in rice fields of California. However, plants, inoculated with mycorrhizal fungi, show the best yield responses in fumigated soils containing low levels of phosphate.

The project does have scientific merit in regard to determining the survival of mycorrhizal fungi that associate with grain legumes after growing rice. Also, the agronomic experiments involving applications of appropriate inoculum to ensure mycorrhizae and rock phosphate should give meaningful results.

The proposed research to utilize the fluorescent antibody technique to identify mycorrhizal fungi is interesting but this is a project by itself.

The scientific skills of the cooperating scientists are excellent. But how do they interact with the principal investigator and where will their research be done?

II. DISCUSSION

Dr. Peterson remarked that the best information so far indicates that non flooded rice performs poorly when inoculated with the mycorrhizal fungi. Phosphorus is actually bound up so that it cannot be released. He found the proposal interesting

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but doubts that it would work. He thought that the amount of additional phosphorus from this type of inoculation would be insufficient.

Dr. Moss remarked that there was no way of knowing if the principal investigator had capabilities in this field. It's a very difficult project to do even with the most highly qualified investigators. There was one investigator who appeared to be qualified but the proposal did not say what his role would be.

III. MOTION

A motion was made to disapprove the project and the vote was unanimous in favor of the motion.

Solar Pond Applications in Developing Countries - The Solar Energy Research Institute

I. SUBCOMMITTEE REPORT

Dr. Sanderson, chairman of the subcommittee, that also included Drs. Ehrenreich and Hrones reported that this project and the three following are from the Solar Energy Research Institute or SERI. SERI is an institute that devote its efforts entirely to energy research. He described what the investigators proposed to do. They would like to develop a technique to store energy in a pond by creating a salt gradient. There are two parts to the project. In the first case, \$35,000 would be spent to develop solar ponds as a source of heat for cooking. He felt that there was a very severe problem with the project, not in the development of the solar pond itself, but in the acceptability of a diet based on slow cooking and acceptability of this method of cooking in LDCs. He thought this particular part of the project would be more worthwhile if done jointly with an Asian institute than an LDC.

The second part of the study involved a site specific study and he felt that this part should be rejected.

II. DISCUSSION

Dr. Ehrenreich thought that this particular proposal was the best of the five in the series of proposals.

Dr. Sanderson asked if the motion could be to disapprove without prejudice.

Dr. Pimentel supported instead deferral of the project. He said 90% of energy in LDCs is for cooking. The solar pond was a substitute for the use of biomass as a fuel and it could be used anytime.

Dr. Schweigert suggested that a food scientist should help the principal investigators avoid temperatures where microbes thrive which would perhaps introduce a serious health hazards to people using this particular method of cooking.

Dr. Long asked if there is any techniques to accumulate heat and to which Dr. Sanderson responded that all such methods consume more energy than is generated. He gave as an example the heat pump.

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Dr. Long asked how do you harness the concept of the solar pond in a LDC? And Dr. Sanderson responded this only peripherally what the project is about. There are a number of constraints to harnessing the concept of the solar pond that have been looked at before by people who have worked outside the United States on this problem. This particular group of researchers have never worked outside the United States.

III. MOTION

The motion was made to defer part A but to disapprove part B of the proposal. There one no vote by a RAC member who would have preferred to disapprove the entire project.

Dr. Pimentel added the remark with regard to the use of cow chips as a fuel that he felt that it was better to use cow chips on the land as a source of fertilizer for food production than as a fuel. The solar pond would be a source of heat for cooking that would not be competing with other uses.

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Developing and Testing of Prototype Small-Scale Biomass Gasifier to Power Rural Irrigation Systems and Generator Sets - The Solar Energy Research Institute

I. SUBCOMMITTEE REPORT

Dr. Sanderson reported that this particular proposal looks back to some techniques that were in Germany during World War II to burn biomass to produce gas. He felt that the proposal does not demonstrate competitive advantage over using direct terminal energy. The burning of biomass produces a very high percentage of carbon monoxide. In addition, the proposal lacked detail in its discussion of the research plan.

Dr. Pimentel added that simplified units for this particular type of activity existed elsewhere.

II. MOTION

The motion was made to disapprove the project and that motion was unanimously accepted.

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Solar Energy - Education: Opportunities for Study in the United States - The Solar Energy Research Institute

I. DISCUSSION

Dr. Sanderson, chairman of the subcommittee, that also included Drs. Hrones and Pimentel pointed out that this particular project would prepare a simplified listing of available solar energy institutes where educational services are available. He was concerned that the proposal would list sources where foreign students could study.

Dr. Sanderson would prefer site specific adaptation of solar energy applications.

Dr. Smuckler suggested that this was non research.

Dr. Pimentel remarked that it was not good just to get a long list of courses without checking the quality of the institution.

Dr. Ehrenreich supported the views of the subcommittee members.

II. MOTION

The motion was made to disapprove the project and that motion was unanimously accepted.

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The Development of a High Efficiency, High-Lift Photovoltaic
- Powered Water Pumping System - The Solar Energy Research In-
stitute

I. SUBCOMMITTEE REPORT

Dr. Ehrenreich, chairman of the subcommittee, that also included Drs. Hrones and Sanderson reported that the proposal did not appear relevant for application in LDCs at this particular time because of the stage of development of the technology and cost factors. He pointed out that even if a pumping system is developed the rest of the technology would be too expensive for the U.S. to be relevant and it certainly would be too costly for use in LDCs. This appears to be an excellent project whose time has not come for application in the developing countries.

Dr. Sanderson agreed that this particular proposal was a technology in search of a use and that it may in time become useful. The project assumes that photovoltaic exist at a moderately higher cost than traditionally sources of electricity and he did not believe this could be the case. He explained briefly how the engineering would work in this particular application. He felt that in field applications such systems have not been continued to an optimum performance therefore, the efficiency of the system was questionable. He felt the proposal would not address this particular issue. Photovoltaic cells were far too expensive to be used in LDCs.

II. DISCUSSION

Dr. Sanderson suggested that there were alternatives such as the windmill which is simple and mechanical.

Dr. Bosken, from AID, disagreed that photovoltaic was not useable in some LDCs. He gave examples where the cost of producing electricity was extremely high in remote areas. He felt that in such areas where the cost of electricity was high that the photovoltaic cell was a reliable source and was competitive costwise with traditional sources of electricity. He did not think, however, that pumping water was a particularly good use for the photovoltaic cells.

III. MOTION

The motion was made to disapprove the project and the vote was unanimous in favor of the motion.

Continued Development and Testing of Prototype Low-Cost Solar Collectors for Developing Country Applications - The Solar Energy Research Institute

I. SUBCOMMITTEE REPORT

Dr. Sanderson, chairman of the subcommittee, that also included Drs. Hrones and Pimentel reported that in general there was a need for testing of solar collectors. There was, however, a number of other organizations already testing them. It was hard to accept that this type of technology should be exported to developing countries for research. There is a substantial industry in the U.S. and the application in LDCs is not a unique problem for solar collectors.

II. MOTION

A motion was made to disapprove the project and that motion was unanimously supported.

174.

Tissue Culture of Banana and Plantain (Musa spp.) for Improving Yield Potential - CATIE, Costa Rica

I. SUBCOMMITTEE REPORT

Dr. Sloger, chairman of the subcommittee, that also included Drs. Moss and Peterson reported as follows:

The subcommittee recommends approval with an average ranking. Bananas are important cash crops and plantains are an important component of the diet of people in rural areas of the tropics. The lack of genetic diversity in banana and plantains has permitted epidemic diseases to damage the banana industry. The banana breeding programs are complicated, difficult and very time-consuming, and only a few commercial cultivars have resulted. This limitation could possibly be helped by tissue culture techniques.

Micropropagation of banana from meristem cultures is a method to produce large numbers of individual plants free of disease. These plants could easily be sent to other parts of the world. Since micropropagation of other plants has been successful, this objective of the proposal has merit and should be successful for both banana and plantain. However, there is one concern. According to the proposal, Vissey and Rivera (in press) in Turrialba, Costa Rica, are working on rapid micropropagation of banana. What is their relationship to CATIE? Why is Dr. Muller proposing this research project when others are already working on it?

The objective for increasing genetic variability by cloning individual cells and regeneration of plants has considerable scientific merit. This has never been done in banana or plantain. However, it is uncertain whether one would take six months or twenty years to successfully regenerate banana or plantain plants from singel cells.

The proposed project wants only one research assistant to work on both objectives. This is unrealistic. It is also strongly suggested that the budget for international and consultation fees be reduced to \$6,000 and \$8,000, respectively.

II. DISCUSSION

Dr. Peterson supported the comments by Dr. Sloger.

Dr. Smuckler asked if the duration of this project was such that it could be accomplished with \$150,000 or are we talking about a 8-10 year plant breeding project?

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Dr. Moss responded that he thought that most of the goals could be reached short of regeneration of the plant. He would encourage AID monitoring of the project to avoid protoplasmic hybridization.

Dr. Peterson thought that in a three year period it should be possible to accomplish a more efficient way of propagating bananas. He felt that it was high risk to continue propagating homogeneous varieties of bananas. It is necessary to change genetics somewhat.

Dr. Smuckler said that this reminded him of the problem with the Colorado State project on tissue culture. He thought the subject of tissue culture should be explored as a potential technique for use in developing countries.

Dr. Carter asked if AID would be in the position of working on bananas for cash crops? The response by Dr. Sloger was, yes.

Dr. Ehrenreich suggested that if the proposal is approved, AID should encourage finding out what role the large companies would play. He thought that CATIE was a good institute and a good place for the research to be located. However, he thought the project managers should look carefully at the curriculum vitae of the principal investigator.

III. MOTION

The motion was made to approve the project. The vote was unanimous except for one abstention.

Application of Plant Tissue Culture Technology to Pyrethrum Improvement in Kenya - Plant Resources Institute

I. SUBCOMMITTEE REPORT

Dr. Peterson, chairman of the subcommittee, that also included Drs. Moss and Sloger reported as follows:

The need to use this technology in Kenya to accomplish the project objectives is not obvious to members of the subcommittee. This is a perennial plant belonging to the family Compositae. The plant is described as forming clumps 6-12 inches in diameter at the base from which numerous stems arise. Presently, it is propagated from seeds. Just 9 ounces of seed planted on 300 square feet of bed space are capable of producing 15 to 30 thousand plants. When about 3 months old, they are transplanted at the rate of about 10,000 plants per acre.

There are two simpler procedures that appear possible to propagate selected superior plants. First, cuttings would be made from superior plants using techniques well known to any practical gardener. Secondly, selected superior plants could be propagated in an isolated polycross nursery, allowed to intercross and the seed harvested and sown for propagation purposes.

Further improvement would be possible by several cycles of reselection. This is a widely used system for improving cross pollinated crops. If pyrethrum plants are self pollinated (this is unlikely), a simple selection process could be used. As a final comment, synthetic pyrethrums are now available and the natural pyrethrum industry may eventually be doomed anyway.

This project appears to be unnecessary and impractical to accomplish the stated goals.

II. DISCUSSION

Dr. Moss remarked that he shared Dr. Peterson concern about the project. He felt that the synthetic pyrethrums will eventually run the natural pyrethrums off the market because of their competitive advantages. He wondered why this should be prevented. He did think, however, that the Plant Resources Institute was a very competent organization.

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Dr. Schweigert asked if there was any known situation where tissue culture would have a role to produce seeds.

Dr. Peterson responded that to his knowledge no varieties had been developed from the tissue culture method.

Dr. Sloger described some tissue culture work in the laboratory experiment station in Beltsville on rice and wheat, where an attempt is being made to develop new varieties.

Dr. Long described the project on tissue culture from Colorado State University that had come before the RAC earlier as research on one plant where the researchers would proceed to sexual reproduction. He asked if this had been proven.

Dr. Sloger suggested perhaps it had with tobacco.

Dr. Peterson remarked that he would recommend that we pull the experts together to get an update on the Colorado State experiment.

III. MOTION

The motion was made to disapprove the project. One RAC member opposed and one abstained. The member who opposed the motion would have approved the project.

Pilot Program for Use of Traditional Agricultural Technologies to Improve Present Agricultural Practices - ESPOL

I. SUBCOMMITTEE REPORT

Dr. Peterson, chairman of the subcommittee, that also included Drs. Falcon and Swanson reported as follows:

The more detailed description of this project which arrived after the subcommittee first reviewed it, puts the project in a somewhat more favorable light - but does not alter the basic conclusion that this is a high cost - low potential project although certainly an interesting one. The subcommittee's feeling is that there are other areas with more potential for use of AID's money.

If the archeological studies were not included and a small pilot - demonstration area could be done for about one-third the cost, it would have more appeal - but still questionable.

II. DISCUSSION

Dr. Swanson added that the proposal appeared to him to be interesting. However, he wondered what the increment to our knowledge would be at the end of the project. To him the project did not appear to be cost effective.

Dr. Skinner felt the principal investigator was not knowledgeable about scales. To him the project did not make sense.

Dr. Wishik found the project proposal to be stimulating. However, he felt that it required extreme revision before it would be suitable for AID funding.

Dr. Moss thought the project was an attempt to show that an area once used had been productive. He didn't see how it could have an impact on agricultural production.

Dr. Long thought that if the area could go back to a more productive system the project could be supported.

Dr. Skinner pointed out that restocking the previous species can't be done. You can't recreate the total system that was in place originally.

III. MOTION

Disapproval

Vote: Unanimous in favor of the motion.

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Crop Diversification Potential in Jamaica - USDA

I. SUBCOMMITTEE REPORT

Dr. Peterson, chairman of the subcommittee, that also included Drs. Moss and Pimentel reported as follows:

This is an interesting pre-proposal worthy of developing into a finished one for consideration at the next RAC meeting. As the proposer (Dr. Duke) indicated, it has been put together hastily without the opportunity of consultation with his counterpart.

The idea that native plants are indicators of potential agricultural productivity is a very old idea at one time advocated by Breggs and Shoutz. However, plants are not distributed on the basis of climate alone but by soil and other factors as well. There would be a serious oversight if soil, slope, direction and sun were not noted as well.

It is not clear who is going to fund and what these nature plants are good for and how it is to be done. A lot of explaining is needed to make this a project that could be funded.

II. DISCUSSION

Dr. Farrell remarked that some of the information in the proposal would be useful but the objectives were very broad and sweeping and there was no path for reaching those objectives.

Dr. Pimentel thought that it would be useful to have the taxonomy in the area. He would like to see it done but he can't see giving it very high priority. He wondered if this information would really reach rural farmers.

Dr. Skinner asked if Dr. Duke had ever been to Jamaica? The main original plant cover is still there. Also, the island is too small for climatic conditions except for elevation.

Dr. Ehrenreich pointed out that this was not an ecosystematic study. It was a basic taxonomic study.

Dr. Weller raised one point. He asked Dr. Skinner about there not being much climatic variation in Jamaica. Puerto Rico, which is near by has much variation.

III. MOTION

The motion was made to disapprove the project.

Dr. Bosken, from AID, asked if there was a consensus on the RAC not to do biological surveys? Dr. Smuckler responded that there was no such consensus.

The vote was unanimous in favor of the motion.

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Report of a Regional Conference on Seismic Risk Mitigation in Southeastern Asia - Inter-Union Commission on the Lithosphere, ICSU

I. SUBCOMMITTEE REPORT

Dr. Finger, chairman of the subcommittee, that also included Drs. Farrell and Hrones reported as follows:

Background

The ICL (Inter-Union Commission on the Lithosphere) is a newly formed organization within the International Council of Scientific Unions. The ICL was conceived to deal with a new program called "Dynamics and Evolution of the Lithosphere: The Framework for Earth Resources and Reduction of Hazards," which is concerned with the current state, origin, evolution and dynamics of the lithosphere.

This proposal requests funding to help support an ICL conference on the application of modern technology to the mitigation of seismic risk in Southeastern Asia. The proposed conference would bring together geologists, geophysicists, communications engineers, and disaster relief specialists of LDCs in order to increase the communication among these groups, and to make them aware of the potentialities of modern technology in mitigating seismic risk. The technology to be discussed includes satellite data communication, microcomputer applications, and other applications of space technology.

In evaluating the proposal, comments from other experts were solicited and those of Dr. Roger Stewart of the Office of Earthquakes, U.S.G.S. were primarily used for the subcommittee's assessments.

Discussion

There is without doubt severe risk in many Southeast Asian countries from earthquakes and volcanoes, and associated sea waves. Modern instrumentation and technical skills are scarce, so it is very likely that government officials and possibly scientists in the affected countries are not fully aware of recent advances in technology. It should be noted that AID currently is sponsoring a three-year study of seismic risk in ASEAN countries ("A Planning Study for Earthquake Hazard Mitigation in Southeast Asia" submitted by the S.E. Asia

Association of Seismology and Earthquake Engineering). This study, funded by OFDA (Office of Foreign Disaster Assistance [AID]), will address many of the topics which are being considered for this proposed conference, and is in fact more comprehensive. It would be a good idea to insure close coordination between the proposed conference and the ongoing study.

Members of the subcommittee were in agreement that the technical proposal lacked the detail necessary for proper evaluation. It is realized that the Conference Steering Committee has yet to be formed, but a more detailed account of the agenda should be available as well as information on how the conference results may be useful to the Southeastern Asian LDCs.

The selection of invited speakers will be of utmost importance. Most of the conference output may well hinge on their presentations. Contributed papers, on the other hand, may not add very much useful information. This has been the experience of the Committee in Space Research (COSPAR), which has had more success with workshops.

Subcommittee's recommendations

1. The proposal contains a great deal of information on the operational structure of the ICL, but there seems to be minimum discussion on the details of the conference. Although the conference appears to be quite worthwhile, final approval of AID funding should be contingent on the additional conference information. This other information should include:

- a) A list of possible invited speakers.
- b) Other key participants.
- c) The possible interaction between participants.
- d) The knowledge that might possibly be gained by the LDCs.
- e) The methods for conference preparation and advertising.

2. Additional funding should be made available for the inclusion of topics from the proposed Support of a Regional Conference on Possible Earthquake Premonitory Behavior in Biological Systems.*

3. There should be close coordination between the proposed conference and the S.E. Asia Association of Seismology and Earthquake Engineering.

*See Subcommittee's report.

II. DISCUSSION

Dr. Marrell added to Dr. Finger's report that he thought the proposal should define what would be done at the conference.

Mr. Bosken, of AID, remarked that this was a long term project that actually came from the mission in Indonesia. There was in fact a volcano project in Indonesia sponsored by AID.

Dr. Pimentel was disturbed to see that the people involved in the proposal did not include LDC scientists.

Dr. Sanderson pointed that there was potential for benefit from the conference. Japan and the United States are quite advanced in their techniques. There was, however, no indication that this particular research crew had built on the expertise in the National Scientific Foundation. Likewise, there was no indication of knowledge of the work done by the U.S. Coast and Geodetic Survey. It appeared to him that the project proposed to discover a well worn wheel. The project would profitably involve developing countries, however.

Dr. Long asked if the conference was really international or just for many countries?

Dr. Finger felt that the project would be truly international. However, he would prefer to have a workshop with knowledgeable people even though from fewer countries.

Dr. Sanderson suggested that the conferences could combine into one conference three to four days rather than the eight to ten days that were suggested.

III. MOTION

The motion was proposed to defer the first conference until information could be obtained from which the proposal could be evaluated.

The vote on the motion was unanimous in favor.

Dr. Finger then reported on the second conference proposed by the ICSU. His report follows:

Background

This proposal requests AID to help support an international conference on earthquake premonitory phenomena in biological systems. The requesting organization is the Inter-Union Commission on the Lithosphere/ICSU.

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Studies on anomalous animal behavior prior to earthquakes are recorded in historical records in Asia and have been studied intensively in Japan and China and more recently in the United States. The sensitivity of fish, birds, and mammals to geophysical phenomena are to be reviewed at the conference. Plans for future research and coordination of research projects are to be discussed also.

In evaluating the proposal, comments were solicited from Dr. Roger Stewart of the Office of Earthquakes, U.S.G.S.

Discussion

Possible premonitory behavior of animals has long been reported. Recently, photographic and other kinds of evidence have been presented to document these reports. It is well established that various animals may be extremely sensitive to changes in environment, for example, magnetic or electric fields, or atmospheric gas concentration. It is indeed the case that more attention has been paid to animal behavior in Asian countries than elsewhere. It is by no means generally accepted that "anomalous behavior in biological systems" is one of the "most promising" phenomena for practical earthquake prediction systems." The difficulties of using premonitory animal behavior are well known from experience in countries (chiefly China) where such methods have been used, and are in any case obvious. The difficulties include the fact that: 1) animals respond to many stimuli other than earthquake precursors, 2) animal behavior must be reported by untrained observers with other cares, 3) results of observations are spotty and give little information about the size of any impending earthquake, and 4) false alarms and missed earthquakes are common.

The record for animal behavior as a premonitory phenomenon is no better than that of many physical measurements. In countries where animal behavior has been extensively used, notably China, standard geophysical measurements are also employed and instrumental data are given more credence than animal behavior reports. In fact, the goal of research into premonitory animal behavior is to discover what, if anything, animals are responding to so that a machine can be designed to sense the same thing. The author implicitly points in this direction when he points out that change in water level affects electrical fields. This is true enough, but why not measure well-levels or electrical fields? It seems that biological systems are being oversold, and it would be a disservice to do this when discussing solutions to earthquake prediction in a developing country.

The positive aspect of animal behavior studies, as the author has noted, is that they are relatively inexpensive and well-suited to rural environments where people and animals are on more intimate terms. It may well be the case that such studies would be useful in Southeast Asia. However, not every country is organized like China, and not every country can or will place as much emphasis on mass participation as did the Chinese during the cultural revolution. It is a defect of the proposal not to mention the social aspects of prediction using animal behavior.

There certainly is a positive aspect in passing as much information as possible to officials in rural emerging countries. However, it would be much better to present information on all methods of prediction, rather than to sell any particular method as a solution. This is probably even more pertinent since no country in the world has found a single prediction method satisfactory. This conference may best be included as a subject in the other conference proposed by the author (see report on Seismic Risk Mitigation in SE Asia).

Subcommittee Recommendations

The subcommittee, reinforced by the experts comments, feels that there is no present need for the proposed conference. Yet some discussion of the subject is warranted. It is recommended that the general subject be included in the Agenda of the Regional Conference on Seismic Risk Mitigation in Southeastern Asia. As stated in the subcommittee's report, the level of funding for that conference should be increased in order to incorporate the biological subjects.

Dr. Finger hoped that the subject of this conference could be included in the previous conference.

IV. MOTION

The motion was to disapprove the proposal on the basis that the substance of the conference should be incorporated into the previous conference.

Dr. Long pointed out that these conferences must be well thought through. The critical issue in deferral is to set in motion some action. In this case the big question is how to get the ground work started.

The vote on the motion to disapprove the project was unanimous.

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Strengthening Environmental Microbiology and Pollution Monitoring Capabilities at the High Institute of Public Health, Alexandria, Egypt - University of Maryland

I. SUBCOMMITTEE REPORT

Dr. Weller, chairman of the subcommittee, that also included Drs. Connell and Farrell reported as follows:

This proposal requests \$135,000 to extend for two years a project currently active under the Maryland Sea Grants program that is based at Johns Hopkins and at the High Institute of Public Health, Alexandria, Egypt. The primary objective is to develop capabilities in environmental microbiology and pollution monitoring at Alexandria. The ongoing project involves exchange of senior professional staff, the holding of workshops and seminars, and the development in Alexandria of the capacity to utilize a tracer virus technique to monitor waste water outfalls near heavily utilized bathing beaches. The tracer technique involves growing a bacteriophage, "f2" in the laboratory; high titers exceeding 1×10^{12} PFU/ml can be obtained; the concentrated virus is then introduced into the wastewater system and can be detected after extreme dilution has occurred.

In the two year renewal various marine sewage outfalls will be examined for the die-off of selected pathogens.

Critique:

The investigators are highly competent and there is every reason to assume that the project will be carried out as planned. The development of a cadre of Egyptian scientists competent in the area of pollution monitoring is a desirable objective, and the budget is realistic. However, there are concerns as to whether the social-political situation in Alexandria has developed sufficiently to justify such training. Simple techniques of monitoring could be used on a continuing basis. Mention is made of interruption of electrical and water services at the Institute in Alexandria. A new sewage disposal plant is said to be "often in a partial state of operation." Waste water pumping stations operate only during the day-time. In such an environment, it is doubtful if the local authorities will continue pollution monitoring after the outside support from AID terminates. Thus, while the project rates high, i.e. a "1" in terms of scientific merit, in potential for capacity strengthening, and with respect to relevance and innovative nature, this reviewer questions whether there actually will be long-term benefits.

II. DISCUSSION

Dr. Schweigert asked if there was an existing monitoring system? To which Dr. Weller responded that one must assume there is none.

Dr. Wishik suggested that the Committee keep in mind that this is the major seashore in all of Egypt and there may be more motivation by the Egyptians to carry out this project successfully than there would be for the ordinary project.

Dr. Connell remarked that the Egyptians have an ongoing program in this area, why should the researchers come to AID? The technology is easily transferred. It's a good proposal. The technical problems identified in the proposal should be resolved in the future.

Dr. Carter pointed out that the particular site for the research has great potential as a tourist area. He was unclear, however, whether the principal contractor would be Johns Hopkins or University of Maryland.

Dr. Idyll pointed out that this was part of the Maryland sea grant program and that even though some of the investigators may be from Johns Hopkins, it is a University of Maryland project.

Mr. Roseborough, of AID, informed the RAC that there is a multi-million dollar project on this topic in Alexandria. He would be surprised if this particular research activity is not included in that \$765 million dollar project.

Dr. Farrell said that he was not aware of an environmental study being done in the area in Egypt proposed for this research. The project would provide recommendations toward monitoring pollution along the beach.

Dr. Weller proposed that the project be approved.

Dr. Long remarked that this was an opportunity for RAC to voice its concerns with the Agency's program through the Science Advisors Office. The mission and the government can take into consideration whatever it wants to from the RAC's remarks to build into the program.

Dr. Peterson asked if the projects had been looked at by the mission? Dr. Minners responded that some comments were coming back now.

Dr. Long suggested that this is a case where the Committee, through it's comments and recommendations, could begin to use some leverage on country or mission programs to affect the quality of those programs.

III. MOTION

The vote for the motion was unanimous in favor.

IV. OTHER COMMENTS

Dr. Wishik then pointed out that the Agency has been asking what is the role of research in AID's program? He suggested that in addition to the research study that was already done where two topics in health and education had looked at that AID could pick a country and ask what research is in the Egypt program.

Dr. Peterson thought that it would not be particularly useful unless you get total research not only that by AID.

Dr. Long suggested that it would be useful to make it known that research is being utilized in Egypt.

Bringing to Bear Scientific and Technological Resources on
Problems of Development in the Western Hemisphere - AAAS

I. SUBCOMMITTEE REPORT

Dr. Weller, chairman of the subcommittee, that also included Drs. Falcon and Moss is reported follows:

This application from the AAAS is for continued support for three years in the amount of \$439,820 to stimulate scientific and technical resources in the Americas that bear on problems of development. The goals are to identify development-related problems, to help develop the capability at the national level to deal with these problems by convening multinational, multi-disciplinary scientific meetings, and to subsequently disseminate the findings.

These goals are currently being met under the auspices of a hemispheric Intersciencia Association that has eight affiliates subsidized in part by AID support. The problems to be addressed are selected by consensus, an operation in which the AAAS secretariat plays a major role. Three symposia are planned over the next three year period. Planning is now underway for four such meetings which offer representation. Two are on fuels from biomass; one scheduled for October, 1981 in Brazil and a second in January, 1982 at the AAAS annual meeting in Washington. A third on "Creativity and the Effective Teaching of Science" scheduled for Costa Rica in March, 1982, was selected because "the teaching of science in some countries has been said to stifle creativity." The fourth, entitled "Biotechnology in the Americas; perspectives for developing countries" is scheduled for Guatemala in October, 1982.

Concurrently, attempts are made to strengthen or to bring into being national AAAS - like organizations. Reference is made to strong associations in Brazil and Venezuela; parenthetically, it would appear that science is well organized in these two countries and little outside stimulus is needed. At the other extreme, discussions are underway to form associations in the Dominican Republic and in Trinidad; one might question whether the U.S. pattern of a national scientific group is appropriate or needed in small countries that lack a critical scientific mass.

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The Interciencia Association is headquartered at the AAAS; of the U.S. funds amounting to \$606,057 expended for this activity between 1974 and 1980, \$210,748 was contributed by AID. Currently, funds are being sought from other sources; a grant of \$100,000 was obtained in June from the Inter-American Bank.

Information re. conclusions of symposia is disseminated in a 64 page bimonthly journal "Interciencia" which has been published since 1976 with AID support. The current circulation of this journal is not mentioned; nor is the number of paid subscriptions. Currently discussions are underway with the Pergamon Press to assume publishing responsibilities; this move will certainly augment page costs and reprint costs.

The tangible benefits of this project, however, meritorious in concept, can not be assessed. Such programs inherently develop a self perpetuating bureaucratic secretariat that by nature tends to proliferate. Of the \$439,000 requested \$174,000 is for the salaries and operations of the AAAS secretariat; to this added \$41,000 overhead. Separately, each budgeted symposium includes an additional \$6,000 for overhead, and \$3,200 for added AAAS secretarial services. Without including administrative travel and per diem costs of the AAAS secretariat, it thus appears that at least \$242,600 or more than half of the grant will be required to maintain the AAAS secretariat.

This does not appear to be cost-effective. Disapproval is recommended.

II. DISCUSSION

Dr. Moss pointed out that AID support to the AAAS would increase under this proposal from \$200,000 to \$439,000.

Dr. Pimentel suggested that this was not a good investment for science.

Dr. Schweigert also thought that this proposal, if funded, would be a high cost activity per unit of achievement. It was not cost effective.

Dr. Farrell agreed that, despite the prestigious nature of the AAAS, \$439,000 would be spent to define problems and then to find solutions.

Dr. Anderson suggested that this particular proposal be turned over to the OAS.

Dr. Skinner pointed out that the proposal was to encourage LDC projects. The AAAS would try to create institutions. AID is involved in the larger process of trying to link up with LDC institutions.

Dr. Smuckler pointed out that the development of the science community was to be a part of the Science Advisors program.

A representative from the LAC Bureau said that one of the problems was the fact that this project was for institution building rather than research. He suggested that the past performances of the AAAS should be evaluated before further funding by A.I.D. is considered.

III. MOTION

Dr. Weller made the motion to disapprove the proposal. The motion carried with one abstention.

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Research and Capability Strengthening Project on Pest Management
- The International Centre of Insect Physiology and Ecology

I. SUBCOMMITTEE REPORT

The report of Dr. Pimentel, chairman of the subcommittee, that also included Drs. Ehrenreich and Weller follows:

This proposed project focused on the following four main features to strengthen the research capabilities of ICIPE:

(1) Insect Pathology - To investigate insect pathogens of several target insect pests, and examine the possibilities and means for pest and vector control through the use of insect pathogens.

(2) Visceral Leishmaniasis - The concentrated exploratory work already accomplished by ICIPE's pioneer research programme at the start of a major outbreak of kala-azar in Machakos district of Kenya, has placed the ICIPE in a pivotal position to make rapid advances on this tropical disease. The project support will enable the ICIPE to bring in a number of disciplinary approaches - immunology, biochemistry, etc. - to help to accelerate developments in this problem area.

(3) Research Training - The ICIPE, in collaboration with a number of university institutions in Africa, which cut across language barriers in this continent, have agreed to launch an intensive International Postgraduate Studies Programme in Insect Science with specializations in agricultural pests, veterinary pests, and vectors of tropical diseases, later this year. The final planning conference will be held at the Rockefeller Foundation Conference Centre at Bellagio, near Lake Como, in Italy in September, 1981, at which final decisions on implementation and mechanisms will be agreed upon by the ICIPE and participating universities.

(4) Scientific Editorial Services - A semi-autonomous editorial unit will be formed under the chief editorship of Professor Thomas R. Odhiambo, to encourage scientific publications in the area of tropical pests and vector management.

ICIPE has made an outstanding start in research and training in insect science and pest control. If the several objectives listed above were funded, this would help further strengthen ICIPE's research in training in several areas.

This proposal and the specific projects have strengths and weaknesses and these are evaluated. In Insect Pathology it would be nice to conduct a survey of the occurrence, distribution, and diversity of the pathogens of insects in Kenya. However, the principal investigators must have a knowledge of one insect pest where the use of a pathogen offers real potential for insect pest control. This potential should be investigated by focusing the research effort. Then some progress can be made in insect pest management of a serious pest in Kenya.

A specific research plan for this pest and pathogen should be developed, detailed, and submitted for consideration.

In the Visceral Leishmaniasis portion of this proposal, identification of the various Leishmania strains is desirable but the research plan is poorly developed.

The research on the defense mechanisms of sandflies to the Leishmania organisms is interesting, but the relevance of this in limiting the disease was not identified. If such information would benefit control, this relationship should be specified.

The Research Training aspect of this proposal has attractive features. It would appear that to make the best use of funds for both research and training, postdoctoral students would be associated with the research project. These postdoctoral students could supply the technical manpower.

For example, the insect pathology research on a specific pest and pathogen could be assisted employing postdoctoral student trainees. The same is true of the Leishmania study.

Development of Scientific Editorial Services would obviously benefit ICIPE but it is doubted that this would help strengthen the research and investigations of insect pest control. The responsibility of this need can best be handled by the individual scientific staff of ICIPE.

The proposed budget for aspects of ICIPE proposal appears to be excessive for scientific and technical staffs and material services and expendables. The research in particular on insect pathology and Leishmania appear to have too many staff and material services and supplies for what is proposed. The budget items need to be justified relative to each part of the study.

II. DISCUSSION

Dr. Ehrenreich added that the proposal in general was seeking support for ICIPE from AID. This is the fourth time that the RAC has reviewed a proposal from ICIPE they always have been asked to come back with a specific proposal.

Dr. Ehrenreich suggested that something should be done either to get general support or to get an appropriate research proposal out of the organization.

Dr. Schweigert pointed out that one piece of the last research proposal was funded by AID.

Dr. Pimentel remarked that the progress on their work on ticks, which was only partially supported by AID, was much greater than ICIPE had reported. He noted that the staff at ICIPE was very good.

Dr. Weller commented as follows:

ICIPE proposes to supplement its extensive ongoing program by augmenting work in insect pathology, visceral leishmaniasis, by expanding research training, and by further developing an office for editorial reviews. The proposals are stretched with a broad brush, and with a long shopping list of equipment. "CV's" are appended for ten individuals; these are inadequate for no publications are listed and there is no way to judge past research productivity or accomplishment. Some have recently received a Ph.D. in Nairobi. Two referred to as "Doctor" apparently have master's level qualifications.

The insect pathogen study is diffuse in outline, refers to a spectrum of agents from viruses to nematodes, and focuses on a range of insect vectors or pests. This is to be accomplished by three professional workers; one of whom will be paid from other funds. There is no indication that the staff is qualified to handle the spectrum of agents. The budget is given in general terms without justification. For example, 9 microscopes, 4 refrigerators, and 2 autoclaves at \$24,000 each, are budgeted over a four year period. By now ICIPE must have enumerable microscopes, etc.

The proposed work on leishmaniasis is also poorly outlined. Three more microscopes and five centrifuges appear in budget. A separate budget for cryopreservation, adds two more microscopes, another centrifuge, and an unjustified Counter

counter. Parenthetically the staff and budget demands are unrealistic - 20% time of one technician would be adequate. Further, the preservation methodology is outdated and has a element of hazard.

The training proposed is also troublesome. Early on there will be a conference in the grand style at Bellagio, Italy. Justification for expansion of the currently outlined training effort is not presented.

Finally, a request is made to expand ICIPE editorial services.

ICIPE is financed by a multiplicity of grants from different organizations. The application does not indicate multiple sources of funding, etc. It would be of interest to have a detailed on-site audit of those projects that have been supported by AID.

There is no reference to the concurrent WHO special program covering both research and institution building in the subject areas.

This proposal is scientifically and administratively inadequate. It should be disapproved, for AID should not provide the equivalent of a blank check.

Dr. Skinner noted that the researchers at ICIPE were young and that this would be an opportunity for the transfer of technology. The young staff did not have a record of publication, but he felt that they should be supported and looked at carefully. He felt the institution needed support. It was seeking funds from various places. To turn down this proposal might be discouraging to these young LDC researchers.

Dr. Wishik thought the application was inadequate. He pointed out that there was a broader question. What would be an appropriate mechanism for the RAC to review this application in the manner that it is written? AID needs a policy for assisting institutions such as ICIPE. He was concerned about the quality of work produced at ICIPE. The first tick proposal by ICIPE was the second worst proposal encountered in the RAC. He felt that it would be appropriate for broader assistance to build up the institution to bring it up to a level of higher scientific quality.

Dr. Minners pointed out that ICIPE applied to enter the CGIAR, but it was turned down. AID tried to get help for it through CGIAR. The Agency is beginning to take steps towards some kind of overall support for ICIPE. ICIPE has a wide swings in funding and hopefully this will be controlled over time. Dr. Minners had visited ICIPE and the Director of ICIPE had visited him here in Washington, so he got to know the institution well.

Dr. Pimentel said that he would like to encourage the young scientists in this LDC institution to draft a new proposal and to get input from well known experts in the field. They need this help, they do have senior staff who know how to write a proposal but they were not involved in writing this particular one.

III. MOTION

The motion was made to disapprove the proposal but to encourage ICIPE to develop a proposal with more research details.

Dr. Pimentel pointed out that the work on ticks by ICIPE had good links with the Ministry of Agriculture in Kenya. There is, therefore, a mechanism through which research results can be put into effect.

Dr. Wishik suggested that the caveat attached to the motion be deleted and Dr. Pimentel agreed.

The vote on the motion without the caveat was unanimous.

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Cooperative Coastal Issues Advisory and Training Assistance Program with Universite du Benin, Togo - South Carolina Sea Grant Consortium

I. SUBCOMMITTEE REPORT

Dr. Idyll, chairman of the subcommittee, that also included Drs. Ehrenreich and Farrell reported as follows:

The subcommittee recommends the funding of this project. It addresses a real and urgent need for assistance in complex engineering problems whose solution would be of immediate practical benefit.

The proposal is well conceived and presented, offering convincing evidence that the principal investigator understands the local situation in Togo and the technical aspects of the proposed approach. The linkage with Togo is obviously very strong, and the collaboration and cooperation from local government and technical organizations seems assured.

There is apparently a strong chance of quick return to Togo in terms of improved beach management, and if this success is indeed achieved, excellent opportunity to extend the influence of this project to other West African countries. The South Carolina Sea Grant Consortium has a good record of delivery of results.

It does appear that there are some rapid changes occurring in the engineering concepts being used in beach erosion control; it seems probable that the South Carolina group is aware of these, but the subcommittee suggests that the principal investigator should talk to engineers in the U.S. National Park Service who have been active recently in this area of endeavor.

There is some question in the minds of the subcommittee that the budget for this project may be too high and the principal investigator might be asked to reexamine this.

II. DISCUSSION

Dr. Ehrenreich thought the budget was a bit high particularly in the international travel area. The budget in general could be tightened considerably.

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Dr. Farrell, while not opposed to it, thought the proposal showed a high degree of ambivalence. The objectives were worthy enough. However, the proposed solutions were loaded heavily with engineering approaches. Dr. Farrell then described a Park Service experience the peer engineering solutions that didn't work because other aspects of the project were not taken into account. He didn't see any recognition beyond straight engineering in land use planning. He felt that it was a worthwhile effort but something was missing that needed direction. The researchers should be encouraged to look at broader experiences in this field before beginning the project.

Dr. Wishik pointed out that the people from South Carolina were planning 11 or 12 trips. He wondered what the travel was for, but to him it appeared to be a serious effort to provide technical assistance to the LDCs. AID has always encouraged such technical assistance and this particular feature of the proposal might be viewed favorably.

MOTION

The motion was to approve the project. The vote was unanimous in favor.

MR

Assessment and Development of Living Marine Resources in Kenya
- University of California, Davis

I. SUBCOMMITTEE REPORT

The chairman requested that it be recorded that Drs. Peterson and Schweigert and did not participate in the discussion of this project.

Dr. Idyll, chairman of the subcommittee, that also included Drs. Ehrenreich and Farrell reported as follows:

The subcommittee recommends that this project not be funded. It is a fisheries development project without a fisheries expert on the staff. This deficiency is reflected in some naive suggestions for procedures. The proposal gives little promise of providing impetus to fisheries development in Kenya.

The project includes the proposal to measure the sizes of exploitable invertebrate fishery stocks without an suggestion of techniques to be used. It is doubtful that any of the experts listed could accomplish this complicated procedure.

A major weakness is the proposed measuring of the nutritional needs of the populace, the nutritional value of potential invertebrate stocks, and the matching of these.

The proposal seems to be the research desires of a mixed group of specialists who want to work in Kenya.

The budget is very large, especially compared to probable results.

II. DISCUSSION

Dr. Ehrenreich doubted that the project would provide much in the way of institution strengthening because of the low probability that the researchers will accomplish what they propose. The researchers would hope to match protein requirements of natives to marine resources and then solve the nutritional problems of Kenya. In some cases they indicate that health care clinics would be involved in direct studies but don't make provisions for such studies in the proposal. The researchers also propose to compare the existing and projected socio-cultural framework of setting policy priorities related to food production and consumption with various other means of setting

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policy such as considering export related investments and associated earnings. But they don't say how the required information will be obtained and analyzed. The researchers also say the biological research under both natural and controlled environments will determine supply constraints on and/or opportunities but don't say how the biological research will be done to meet this goal. Dr. Ehrenreich could not see those approaches providing a solid basis for decision making.

Dr. Farrell thought the objectives of the project were too numerous and were too vaguely related to each other to be operational in any studies sense. The cost estimates were also highly unexplainable. The nutritional relationships were overblown. The need for biological consultants to a team of biological experts was unclear. He felt the project director should be a marine biologist rather than a geologist. He also thought the study structure did not provide for specific planning sufficient to assure that the assessment of yields would be obtained as claimed.

III. MOTION

The motion was made to disapprove the project and carried, although two RAC members abstained from voting.

Technical Training Center for Aquaculture in Costa Rica - CODESA

I. SUBCOMMITTEE REPORT

The report of Dr. Idyll, chairman of the subcommittee, that also included Drs. Ehrenreich and Farrell follows:

The subcommittee recommends that this proposal not be funded.

The objective of training practical prawn culture operators is good as a long-term objective, since the technology has just about reached the paying stage if efficient management is exerted. But the proposal does not make it clear who would be the instructors, nor whether there are jobs yet for the graduates.

It is stated in the beginning to be for the funding of a laboratory/training facility, but the budget asks also for operating costs for one year. AID does not ordinarily fund facilities. And since no indication is given that funds would be available from other sources for operating the facility after the first year, the training center is apparently expected to be supported indefinitely by AID.

II. DISCUSSION

Dr. Ehrenreich thought the creation of the proposed training center was a meritorious proposal. He thought AID would do well to invest in such an undertaking, especially if the project has regional implications. The budget was modest, in fact too modest for what they hoped to accomplish.

Dr. Ehrenreich was concerned that there would be a continuing annual request to extend this project. He doubted whether the researcher listed in the proposal had the capability of establishing and carrying out in a qualified manner the correct curriculum as presented. He thought they should seek expert consultants from proven aquaculture programs in the U.S.

Dr. Farrell also thought the proposal had significant merit but was inadequately explained. He would have suggested deferral until some of the questions are cleared up.

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Dr. Smuckler thought the proposal appeared to fit within a much broader framework than just research.

Dr. Long was concerned that the field missions and LDCs would not be responsive to aquaculture opportunities. He wouldn't want to start something and have it wither away. If the proposal is technically sound and is very good, it would make sense to work with the mission to assure follow-on; otherwise it probably should not be supported. The history of aquaculture is that the LDCs has not picked up when projects have terminated.

Dr. Schweigert agreed that there was a considerable span between promise and reality. He was pleased to see that in this proposal expectations might not be out of line with reality.

III. MOTION

A proposal was made to defer the project until inquiries could be made with the mission and to determine whether perhaps the project might be mission funded.

The vote was unanimous in favor.

Program for the Stimulation of Azolla Use in Tropical LDCs -
University of Hawaii

I. SUBCOMMITTEE REPORT

Dr. Moss, chairman of the subcommittee, that included Drs. Peterson and Sloger reported as follows:

Next to water, nitrogen fertilizer is probably the most important factor limiting food production. Unfortunately the availability of nitrogen fertilizer to peasant farmers is deteriorating. The past three years has seen a doubling of both the price of nitrogen fertilizer and the interest rate on the borrowed money needed to buy it. Under the present economic situation, even peasant farmers who were beginning to adopt higher technology, are being forced back to subsistence agriculture. To help relieve their plight, peasant farmers in LDCs must be presented with practical alternative to chemical nitrogen fertilizer. Certain nitrogen fixing plants are a practical alternative.

Nitrogen-fixing legumes have the widest potential application and a considerable amount of effort is going into promoting their use.

For peasants who cultivate flooded crops such as rice, a nitrogen-fixing aquatic fern-alga symbiosis, Azolla-Anabaena, offers the best hope. Azolla has the potential of fixing three or more kg N/ha/day, and is thus capable of fixing the entire nitrogen requirement for a high yielding crop of rice within three weeks.

Azolla is a labor intensive green manure crop appropriate for many rice regions in tropical LDCs, where the supply of water is relatively stable for a few weeks required for its field cultivation.

Hawaii's Azolla program began in 1976 and has emerged as one of the leading information centers on the subject. As of spring 1981, four professors were partially involved, and four graduate students were fully involved in research on Azolla, primarily for application in LDCs. Hawaii has the largest library of Azolla reference material, including over 400 Chinese publications, with all citations filed in computer memory for each subject retrieval.

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The proposed approach is to utilize previously established contacts with international and LDC agriculture institutes for support in conducting a series of three 2-week workshops on the practical use of Azolla. The first workshop would be held during February 1983 in Chiclayo, Peru, in cooperation with the Peruvian National Rice Experiment Station and CIAT. The second workshop would be held during July 1983 near Dakar, Senegal, in cooperation with ORSTOM and WARDA. The third and final workshop would be held in February 1984 in Bangkok, Thailand, in cooperation with the Thai Ministry of Agriculture and IRRI.

The workshops will consist of a series of field demonstrations and seminars that will cover all aspects of Azolla management in the tropics including oversummering, nursery multiplication, field cultivation, soil incorporation and pest management. The trainees will be required to actively participate in all field demonstrations. The final days of each workshop will be spent in drafting a handbook on Azolla cultivation in the local language of each trainee. Preparation of the drafts will draw on reference material provided by the principal investigator, the trainee's knowledge of local rice cultivation conditions, and experience gained at the workshop. The drafts will be edited and published with support from this grant and other institutions cooperating with the program.

In support of the workshops, a graduate student will arrive from China for the purpose of preparing training materials and writing a review based on over 400 Chinese Azolla publications recently brought from China.

The one question which is not addressed in the proposal is the state-of-the-art of Azolla technology as a means of supplying nitrogen. Assuming that technology is available, the next logical step is as proposed herein.

This project is an outreach training program to get the present Azolla technology into use in LDCs. It is not research.

The University of Hawaii has a good track record in research and training in programs for tropical agriculture. They appear to have good capability to conduct this program.

II. DISCUSSION

Dr. Pimentel thought the principal investigator showed a lot of enthusiasm and that the workshops would be very effective at disseminating information.

Dr. Sloger thought the proposal was attractive and well presented. It involved technology transfer and would produce a definite end product.

The Azolla plant is a water fern with two large cavities in which blue-green algae form. In Dr. Sloger's opinion, where there was nothing else in the way of nitrogen fixing plants, Azolla was important.

Dr. Peterson was opposed to the proposal based on work that was going on at the University of California. He did not think the development of the plant had reached the point where it could be passed to LDCs. There is a need to feed the plant phosphorus to obtain nitrogen fixation. This has proven to be very expensive.

It cannot be grown in association with rice. The only possibility is to grow it ahead of rice and then it is difficult to transfer. He had not seen it used in China during his visit there but had seen pictures of it. He thought some of the statements in the proposal were questionable.

Dr. Schweigert suggested that this was once again a case of high expectation versus reality.

Dr. Long thought there was a need to sort out exactly what was in the proposal. One part of it deals with dissemination and other parts with other aspects.

III. MOTION

Dr. Moss moved that the project be approved with a careful analysis of staff time in the budget and that groups with expertise in this area be contacted before beginning the program

Dr. Pimentel proposed a friendly amendment that the project be one of assessment for potential and information gathering.

Dr. Moss thought the workshop was to be for dissemination of information.

VOTE: Six in favor; eight opposed. A second motion to defer the project was made until a proposal could be prepared that concentrated on a workshop on assembling information on Azolla.

Dr. Wishik suggested the friendly amendment - deferral with attention to points raised in RAC's discussion.

VOTE: Unanimous in favor.

Comparative Study on Vector Competence of Simulium damnosum Complex in the Rain Forest of Liberia and the Savanna Region of Sierra Leone - Johns Hopkins University

I. SUBCOMMITTEE REPORT

Dr. Pimentel, chairman of the subcommittee, that also included Drs. Weller and Wishik reported as follows:

A study of the ecology of transmission of human onchocerciasis and vector competence of different man-biting species of black flies will be undertaken in Liberia and Sierra Leone. In Liberia the study will be done in three rain forest biotopes: (a) man-arranged biotope of the Firestone Rubber Plantation at Harbel; (b) along a large river -- St. Paul River; (c) along a medium size river -- Cestos River; (d) the fourth study site will be chosen in the northern savanna region of Sierra Leone. This research work will provide information on the role of individual man-biting species of the Simulium damnosum complex in transmission of human onchocerciasis ("River Blindness"). Vectorial capacity of different species will be assessed by study of anthropophily/zoophily of individual species. It will also provide information on dynamics of transmission of Onchocerca volvulus in different climatic zones. Beside direct evidence of attraction of individual species of blackflies to man in nature detected by means of landing-biting collections on man, additional information on anthropophily will be obtained by laboratory tests using an olfactometer. Special effort will be made to assess potential means of control of vectors in different foci. Attempts will be made to colonize anthropophilic blackflies. Existing collaboration between the Johns Hopkins University and the Liberian Institute for Biomedical Research will be strengthened and a collaboration between the Johns Hopkins University and Sierra Leone Ministry of Health will be developed. Two young Africans will be trained as medical entomologists with funds coming from other sources.

The primary objective of the study is to identify the comparative vector competence of several species of blackflies in the S. damnosum complex in regions of Africa where it has not been done. This is needed and will be an asset in determining the potential threat for "river blindness" for these regions.

In addition, the principal investigator will: (1) investigate the dispersion and longevity of the important vector species; (2) attempt to colonize some man-biting species; and (3) develop possible methods of insecticide control.

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To examine the population ecology and host-feeding behavior of several species of S. damnosum in four locations in Africa as described appears excessive. The principal investigator also mentions examining the effectiveness of parasites, predators, and pathogens in attacking the larvae and adults of the vector blackflies.

The first objective as described in the title will probably be accomplished. The remaining numerous objectives would, if thoroughly researched, require a similar investment in time and money. Realistically, the principal investigator plans to include these other aspects probably as preliminary type investigations. The information will be of help but recognized as only preliminary investigations.

Dr. Milan Trpis is an outstanding and highly productive scientist. The principal investigator should accomplish the major objective of the study.

Two items in the budget are questioned. Why can't the principal investigator supply some of the needed microscopes from his laboratory?

Why can't the principal investigator rent a vehicle? Where are the comparative costs of renting versus purchase? What will happen to the vehicle at the end of the third year?

II. DISCUSSION

Dr. Weller thought the principal investigator should focus on one geographic area and limit the scope of the techniques employed.

Dr. Wishik was in favor of approval for the proposal but thought the experiments involving humans and the colonization of flies should be looked at carefully.

Dr. Long asked what the end product would be.

Dr. Pimentel responded that the most important vectors for river blindness would be identified. He thought at least three geographic areas were necessary.

Dr. Pimentel thought this type of study would be more profitable if the species complex for the flies is worked out first, but broad specifications for this aspect of the research are also acceptable.

III. MOTION

To approve proposal with the suggestion that the research should be focused in one geographic area and that the various items in the budget be carefully examined.

VOTE: Unanimous in favor of the motion.

Control of Barley Diseases for Lesser Developed Countries of the World - Montana State University

I. SUBCOMMITTEE REPORT

Dr. Peterson, chairman of the subcommittee, that also included Drs. Moss, Schweigert and Moss reported as follows:

The basic goal of this project is to improve the control of barley diseases for the lesser developed countries (LDCs). This will be done by accumulation of resistance genes into acceptable barley stocks. After the various different sources of resistance have been determined, crosses will be made to incorporate these resistance genes into a base of widely adapted but diverse barley germplasm including both 2-row and 6-row types. Barley male steriles will be utilized to facilitate various crossings for pyramiding genes for resistance and to establish recurrent selection populations (RSPs) for various diseases. Evaluations will be made on both seedlings and mature plants (field) exposed to a wide range of virulences. Selections of the most resistant and agronomically acceptable barley types will be repeated in several cycles of recombination. When resistance is adequately established for specific diseases, combinations will be made to combine resistances to two or more diseases.

This project is intended to augment and assist the efforts of CIMMYT and ICARDA as well as LDCs' regional and country programs. Emphasis will be on improving the control of barley diseases for the semi-arid areas of the world but the findings should be applicable to all barley growing areas. Additional cooperative nurseries may be established in Asia as desired and future potential areas for inclusion would be the countries of Peru and Brazil in South America.

This project has been developed through working level coordination with CIMMYT and ICARDA and various scientists stationed in target LDCs. The diseases listed for study are important throughout most of the barley growing areas of the world. The approach of producing resistant stocks rather than varieties is geared to enable CIMMYT and ICARDA to utilize the superior material in their barley breeding programs. The methodical assembling and pyramiding of major and minor genes for resistance cannot be done easily at CIMMYT or ICARDA but fits well into the developed expertise of the barley program at Montana State University thus resulting in a very desirable and worthwhile relationship.

During the past two years, considerable progress has been made in development of barley stocks with broad-based resistance to specific diseases. Emphasis has been placed on barley scald and net blotch. Resistant stocks are also being developed for leaf rust, bacterial streak, and barley yellow dwarf virus (BYDV). Leaf rust has received major input during the past year. Basic preliminary work has begun on root rots and powdery mildew. The number of applicants to the training program has exceeded financial and space limitations.

The major diseases of North Africa and the Middle East have been identified and specific nursery sites have been located where a high probability for natural infections exists. Many isolates of barley stripe mosaic virus have been collected from the target areas and these have served to give information on the virulence pool and at the same time have been used for determining different sources of resistance. Since barley yellow dwarf virus (BYDV) does not lend itself readily to such types of isolation, there is little known about its virulence pool. Diagnostic antisera is required for the determination of virulence pools of this virus.

The basic breeding method used was recurrent selection facilitated by male sterility. A base population of barley was established using barley lines of diverse origin but of wide adaptation. In case of the six row barley population for scald resistance, for example, 14 barley cultivars were crossed on to a male sterile line and equal amounts of seed for each cross constituted the base population (Table 5). As new and different resistant sources were identified these were crossed into the population. Each cycle consisted of selection in disease nurseries for resistant types, followed by a recombination nursery where seed was harvested only from the male sterile plants thus assuring recombination and pyramiding of disease resistance genes. Two generations were obtained each year by growing the selection nurseries at a number of locations and the recombination nurseries in Arizona.

Diseases are often the most limiting factor in barley production; though quantitative information on losses caused by specific diseases of barley is largely lacking for the LDCs, extrapolation can be made from findings for other areas. Net Blotch, caused by *H. teres*, for example may result in losses as high as 30% as found by Piëning and Kaufman (1969) in Western Canada. The greatest losses were also noted under low fertility and other stress conditions. In the virus diseases, barley stripe mosaic virus (BSMV) and barley yellow dwarf virus (BYDV) infection levels of 10% e.g. may result in 3-5% loss in yield and losses as high as 50% may occur. BYDV, depending on strain, barley cultivar, stage of plant development at time of

infection, may cause severe losses (50% yield red.). In diseases such as the smuts and Helminthosporium stripe there is generally a one-for-one relationship between percent infection and percent yield loss. The amount of loss for many diseases may be influenced by many factors such as initial onset. In the rusts early infection may result in devastating losses but a high severity of infection first developing after the soft dough stage may be of minor consequence.

In the past few years, investigators Sharp, Carroll, Eslick, Sands, and Scharen, as well as SEA/USDA cooperator Hockett have had the opportunity to visit the Middle East area and survey some of the major barley growing areas. They found that barley diseases were widely prevalent and that they received little, if any attention by the local barley breeders. A better appraisal of the problems with barley in the Middle East has been made through disease surveys and collections, contacts with people working with barley during the workshop and tours held in Jordan and Syria in April, 1977, and especially in annual evaluations of nurseries in the Middle East. This has resulted in a much better idea where barley disease nurseries for specific diseases should be located to achieve suitable natural infection. This is leading to a better sampling of the virulence pool for the specific diseases and in turn should lead to development of broad based resistances in the barley stock populations.

Virus diseases such as barley stripe mosaic and barley yellow dwarf (BYDV) have received very little attention in the Middle East to date. Visits in the area revealed that these diseases, particularly BYDV, often limited barley production to a significant degree. Moreover, recent observations by Dubin (CIMMYT-Personal Communication) in South America strongly suggest that barley yellow dwarf virus is a very serious disease on that continent and a special CIMMYT sponsored workshop on this disease was conducted in Mexico this past year.

It has become quite clear that Montana pathologists involved in the AID program for improving barley will be expected to take an active role in the disease ratings and selections within the various nurseries in the LDCs. This aspect is reflected in the travel portion of the budget.

There has been much interest and several inquiries from foreign students regarding the possibility of receiving training with barley diseases at MSU. This category of agricultural scientist is in short supply and participants can fill definite niches in barley improvement programs in the developing countries. A continuing budget for training is thus proposed.

The following report was given by August 1979 site visit team:

Training

The training sponsored by this project is very good. The Graduate Program has five Ph.D. candidates, three foreigners and two from the U.S. The Masters Program has three M. S. candidates. The six-month trainee program is well developed and currently has six members all from developing nations. Graduate students and trainees funded under this project have been selected with full knowledge of the missions in their respective countries.

In addition to a general orientation on major research being conducted on barley, and the tailoring of work of special interest to individual trainees, the training program allows both staff and students to work on important barley problems not strictly part of the RSP program per se., i.e., work on root rots and soil-borne pathogens. This type of training and work related programs should continue.

Montana provides a very good setting for "short course" and training work during a barley production cycle. The evaluation team suggested that the project staff evaluate the long-term needs for establishing a short course as a regular feature of the university program.

Student morale appeared to be high and typically informal western, but highly professional, relationship existed between staff and students. Institutional backstopping and facilities were likewise excellent. Montana State capabilities are well recognized by CIMMYT and ICARDA and it is expected that the supply of trainees and degree candidates will soon tax the considerable capability of the plant pathology and agronomy departments.

This project was an outgrowth of an earlier, much more general barley improvement program which had received critical review by RAC and also by an on-site review team, which included RAC member Dr. Clanton Black, during a one-year extension of that earlier project. The recommendation of that team was to terminate the earlier project and invite a new project proposal on barley diseases.

Perhaps because of this earlier experience the current project has had careful monitoring. It has been reviewed by a site visit team which included RAC member Dr. Maurice Peterson and the LDC involvement was reviewed by a prominent, experienced plant breeder from Missouri, Dr. J. M. Poehlman, who visited

sites in Morocco, Tunisia, and Egypt. Both of these reviews gave the project highly positive evaluations and gave strong recommendations to AID to continue to fund the project.

The budget contains significant travel and training funds; however, these do not appear to be excessive and have been justified adequately in the opinion of the subcommittee. All members of the subcommittee have expressed support for the project.

II. DISCUSSION

Dr. Yohe of A.I.D., remembered that this was the first project review before the RAC. He was grateful for RAC assistance over the years.

Dr. Long asked if any other universities were participating in this work.

Dr. Moss responded that at the University of Arizona one plant breeder was very active in his participation in this work.

Dr. Yohe pointed out that Montana State University distributes new varieties to nurseries throughout the U.S. for trial and review.

III. MOTION

Approval

VOTE: Unanimous in favor of the proposal

Dr. Peterson said that during the site visit review of the project that it had been suggested that the project should be transferred to ICARDA. The reviewers strongly objected. Subsequent events have shown these objections to have been well founded.

Discussion on the Review Process

Dr. Smuckler summarized his views of how this special RAC meeting went as follows:

1. The four categories on which the projects were judged did not apply to non-research.
2. The term innovativeness should imply that the project was at the forefront of science - not that it was an unusual project.
3. The procedures worked alright after the first three or four projects.
4. It worked well to permit the subcommittee chairman to speak for the subcommittee.
5. The idea of supplementing RAC with ad hoc reviewers worked very well.
6. The format of the project descriptions could be more uniform from project to project.
7. The process should have a sense of competitiveness from the beginning.

Dr. Moss thought the workload for RAC members required more lead time. Dr. Peterson added that the volume was too great for the available time.

Dr. Schweigert thought broader participation from RAC would be useful. Most discussion on any one project was by 6 or 7 people.

Dr. Long summarized his views as follows:

1. The value of the RAC output was much greater than he had thought it would be in this type of review.
2. The importance of inter-disciplinary review was obvious.
3. The ad hoc reviewers were essential to the success of the meeting. This feature should be institutionalized.
4. Input from A.I.D. staff resulted in discussion rather than defense of projects.

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5. Events during the reviews forced discussion on what A.I.D. should do. This was good.
6. From 40-50 people will be necessary to have this type of review. There must be an upper limit, yet the required expertise will be necessary for good research review.
7. The Agency now needs advice on how best to obtain advice on its research programs.
8. The Science Advisor may not be in full position to realize how helpful RAC has been.

Dr. Wishik remarked that he had been very comfortable in knowing that these projects were not going to the Administrator.

Dr. Minners summarized his views as follows:

1. He was satisfied with the "yield" from the review. He had needed to make a strong effort to get some projects going.
2. In the future, his projects can fit into the regular RAC review.
3. He wanted the RAC meeting to be unencumbered without advocates for the projects.
4. He wondered if \$150,000 per project or per year per project would be most appropriate.
5. He recognized that breakthroughs in science were not common, that incremental gains in knowledge were more often the case. However, innovativeness was one of the features that the program was supposed to have. He would approach this positively.
6. He appreciated the RAC practice of "calling it like it is."
7. In the future he would advertise and remain reasonably small.

Dr. Schweigert thought the ad hoc members of RAC performed very well and this practice should continue. He felt the limit on size should be variable, \$150,000 is too small for some types of projects. Very large projects should require unusual merit.

Dr. Smuckler agreed that projects over \$150,000 should require unusual merit. Other requirements should be a time of 1-3 years and a usable or observable end product.

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Dr. Rechcigl thanked the ad hoc members for their contribution to the meeting and noted that all of them were government scientists.

Dr. Long remarked that employees of the Federal Government had in the past served on RAC and probably would in the future.

Dr. Peterson questioned the 1-3 year time limit. The better research project seen by RAC were much longer term. He would be in favor of extensions.

RESEARCH ADVISORY COMMITTEE MEETING
July 21-23, 1981
ATTENDANCE

RAC Members

Dr. Ralph H. Smuckler
Dr. C. Arnold Anderson
Dr. James P. Carter
Dr. Elizabeth B. Connell
Dr. John H. Ehrenreich
Dr. Mary L. Elmendorf
Dr. Terrence R. Greathouse
Dr. Forrest E. Linder
Dr. Dale N. Moss
Dr. Maurice L. Peterson
Dr. David Pimentel
Dr. B. S. Schweigert
Dr. Elliott P. Skinner
Dr. Earl R. Swanson
Dr. Thomas H. Weller
Dr. Samuel Wishik

Ad Hoc Reviewers

Dr. John H. Farrell, DOI
Mr. Frederick G. Finger, NOAA
Dr. Clarence P. Idyll, NACOA
Dr. Jack Sanderson, NSF
Dr. Charles Sloger, USDA/ARS

Other

David Dellson, NATURE
Peter E. Hoekstra, FS/USDA
Chris Jozie, New Science Mag.
J. Wortman, Calf.State Univ.
& College, DC

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PPC/PDPR, J. Silverstone
PPC/PDPR, J. Atherton
PPC/EMS, K. Pouney
AFR/DR, J. Stockard
LAC/DR, J. Smith
LAC/DR, J. F. Swift

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AID CENTRALLY FUNDED RESEARCH PROGRAM
SUMMARY OF FUNDING BY PROJECT AND FUNCTIONAL AREAS FOR
PERIOD JANUARY 1962 - SEPTEMBER 30, 1980

FUNCTIONAL AREA	TOTAL PROGRAM		ACTIVE		COMPLETED	
	No. of Projects	Amount Funded	No. of Projects	Amount Funded	No. of Projects	Amount Funded
DSB						
Food/Nutrition	101	122,020,554	42	75,620,412	59	46,400,142
Rural Development	2	563,782	2	563,782	-	-
Population	36	91,323,115	11	67,363,638	25	23,959,479
Health	35	22,647,371	14	8,459,896	21	14,187,475
Education and Human Resources	16	14,945,187	5	5,645,159	11	9,300,028
Selected Development Problems						
Science & Technology	8	1,400,000	2	1,400,000	6	2,624,307
Industrial Urban Development	8	1,286,373	1	150,000	7	1,136,373
Small Research Projects	85	1,925,898	19	520,581	66	1,405,317
PPC						
	46	24,736,877	6	4,381,020	40	20,355,867
	1	375,055	1	375,055	-	-
TOTALS	338	284,565,519	103	165,196,543	235	119,368,988

DS/PO/RES:6/81

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FOOD AND NUTRITIONActive ProjectsContractAccummulativeContractorDurationObligationAgriculture OfficeCrop Production Technology

1. Improved Varieties of Soybean - Project 931-0560	University of Illinois AID/CM/ta-C-73-19, ta/c-1294	4/20/73- 11/30/80	3,706,000 (11/30/80)
2. Improvement of Tropical Production of Beans and Cowpeas Through Disease and Insect Control - Project 931-0562	University of Puerto Rico AID/CM/ta-C-73-26	5/14/73- 11/30/80	1,860,460 (11/30/80)
3. Development of Improved High Yielding Sorghum Cultivars - Project 575	University of Nebraska AID/ta-C-1068, ta-c-1382	2/12/74- 2/11/80	683,000 (2/11/80)
4. Control of Barley Diseases #931-1318	University of Montana	5/31/74 5/31/81	895,300 (5/31/81)
5. Improvement of Winter Wheat for LDC Based Hybridization of Spring X-Winter Form, Project 931-0621	Oregon State University AID/ta-C-1-68 ta-C-1382	9/30/76- 9/14/82	1,500,740 (9/14/81)
6. Aflatoxin Reduction in Maize Project 931-1181	University of Missouri ta-C-1451	9/30/77 9/30/82	149,000
7. Effects of Mechanization on Small Farms - Project 931-1026	I.R.R.I. AID/ta-c-146	9/20/78 9/18/80	214,960 (9/20/79)
8. Ag. Mechanization #931-1026	ADC	9/30/77 10/1/81	115,000 (10/1/81)
9. Tissue Culture Project 936-4055	Colorado State ta-c-1535	8/15/80 9/30/83	500,000 (9/30/83)

FOOD AND NUTRITION

Active Projects

Contract

Accummulative

Contractor

Duration

Obligation

Livestock Production Technology

1. Tsetse Fly Control #931-0030	USDA PASSA - RA-1-00 AG - TAB - D30-1-00	6/1/62 12/30/80	4,992,000 (12/30/80)
2. Mineral Deficiencies and Toxicities of Grazing Ruminants in Latin America Project #600	University of Florida AID/ta-C-1153	11/1/74- 11/30/81	1,150,000 (11/30/81)
3. Physiology & Ecology of Ticks Project #1038	International Center for Insect Physiology and Ecology (ICIPE)	9/30/78- 11/30/81	364,000 (11/30/81)

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FOOD AND NUTRITION

	<u>Active Projects</u>	<u>Contract</u>	<u>Accummulative</u>
	<u>Contractor</u>	<u>Duration</u>	<u>Obligation</u>
<u>Pest Management</u>			
1. Control of Weeds #0463	Univ. of Oregon	6/30/66 5/31/82	3,091,000 (5/31/82)
2. Control of Vertebrate Pests #931-0473	RSSA - Dept. of Interior D.W.R.C. (J. Walker)	9/30/67 9/30/81	6,761,000 (9/30/81)
3. Root-Knot Nematode #0614	Univ. of Missouri	6/26/75 9/15/83	1,254,00 (9/15/83)

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FOOD AND NUTRITION

	<u>Active Projects</u>	<u>Contract</u>	<u>Accummulative</u>
	<u>Contractor</u>	<u>Duration</u>	<u>Obligation</u>
<u>Water and Soil</u>			
1. Water Management Research in Arid and Sub-Humid Lands of the LDCs - Project #489	Colorado St. University (csd-2162 ta-C-1100 ta-C-1411	6/28/68- 12/30/80	5,682,037a/ (12/30/80)
2. Crop Production and Land Capabilities of a Network of Tropical Soil Families #582	University of Hawaii AID/ta-C-1108	5/31/74- 5/31/83	5,064,368 (8/31/81)
3. Crop Production and Land Potential of Benchmark Soils of Latin American	University of Puerto Rico AID/ta-C-1158	1/1/75- 12/31/80	2,110,037 (12/31/80)
4. N-Fixation - Symbiotic #0613	USDA	6/1/75 6/30/81	,930,000
5. N-Fixation - Associative #1004	Univ. of Florida	1/1/77 4/30/82	928,000
6. Determinants of Irrigation	Cornell University	9/30/77 6/30/81	342,000
7. Nitrogen Fixation Non-Symbiotic Associates - Project #931-1005	University of Florida ta-C-1376	1/1/78- 4/30/82	927,411 4/30/82).

a/ A.I.D. project monitoring and funding transferred to Asia Bureau in 1980.

Active Projects

Contract

Accummulative

Contractor

Duration

Obligation

Rural Development

1. Development of Potential Agr. Settlement - New Lands #936-5303

Institute for Development
Anthropology

4/15/79
8/1/81

147,459
(8/1/81)

250

Urban Development

Population Migration Fertility
in U.D.

Active Projects

Contractor

Contract

Duration

Accummulative

Obligation

Research Triangle
Institute

150,000

231

FOOD AND NUTRITIONActive ProjectsContractAccummulativeContractorDurationObligationNutrition

1. Fortification of Sugar with Iron	INCAP-PAHO	6/30/76 1/31/81	1,110,000 (1/31/81)
2. Effect of Protein Calorie Intervention on Human Growth #931-0625	INCAP AID/ta-c-1342	6/30/76- 9/30/80	2,716,916
3. Consumption Effects of Economic Policy	Michigan State Univ.	6/1/78 10/3/81	485,000
4. Control of Iron Deficiency #931-0227	Kansas St. University Medical Center	9/30/78 1/31/81	1,148,862 (1/31/81)
5. Consumption Effects of Agricultural Policy #931-1274	SIECA/ECID	6/30/79- 5/31/82	1,085,000 (5/31/81)
6. Vitamin A Deficiency #931-0045	Cornell University (ta-1375)	9/30/79 9/30/82	400,000 (5/31/81)

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<u>Population</u>	<u>Active Projects</u> <u>Contractor</u>	<u>Contract</u> <u>Duration</u>	<u>Accummulative</u> <u>Obligation</u>
1. International Fertility Research Program (IFRP) Project #537	University of North Carolina (csd-1172)	6/30/71- 7/31/81	24,146,000 (7/31/81)
2. Program for Applied Research on Fertility Regulation (PARFR) #932-0546	Northwestern University (csd-3608) DSPE 0035	5/30/72- 6/30/81	8,868,000 (6/30/81)
3. World Fertility Survey Project #931-0547	International Statistical Institute (csd-3606)	6/30/72- 9/30/81	20,102,000 (9/30/81)
4. Simplified Techniques of Fertility Control Project #932-0548	Johns Hopkins University (csd-3627)	6/30/72- 9/27/81	5,471,000 (9/27/82)
5. Study of Determinants of Fertility Change	National Academy of Science	7/30/79- 9/29/82	1,322,563 (9/29/82)
6. International Awards for Population Research	Population Council	9/30/80- 9/30/83	807,000 (9/30/82)

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Health

Environmental Health

	<u>Active Projects</u>	<u>Contract</u>	<u>Accummulative</u>
	<u>Contractor</u>	<u>Duration</u>	<u>Obligation</u>
1. In-Vitro Cultivation and Production of Malaria Parasites #931-0453.5	University of Hawaii TA-C-1227	6/30/75- 6/30/81	701,189 (6/30/81)
2. Evaluation of Blood Stage Antigens in the Immunoprophylaxis of Malaria Project #931-0453.1	Univ. of New Mexico AID/csd-3689 AID-C-1259 DSPE-0036	5/1/76- 4/30/82	(4/30/82)
3. Cultivation of Human Malaria Project #931-0453	Rockefeller Univ., N.Y.C. AID/ta-C-1373 Rockefeller-DSPE-0030	1/1/77- 1/1/82	1,707,482 (1/1/82)
4. In-Vitro Production Methods of Human Malaria Parasites #931-0453	N.Y.U. Medical Center AID/ta-c-1486	1/15/78- 1/15/81	285,000 (12/15/80)
5. Immunization & Vaccination Project #931-0453	Gorgas Memorial Institute (0034)	12/1/78- 3/15/81	76,556
6. Ultrastructural & Cytochemical Analysis of Malarial Antigens	Uniformed Services Univ. of the Health Sciences DC-DSB-DZ-0453-4-80	8/31/80- 8/31/83	238,318 (2/28/81)
7. Merozoite Antigen Vaccine	University of Missouri DSPE-0078	5/3/81- 9/29/83	110,000 (5/31/81)

EDUCATION AND HUMAN RESOURCES

Active Projects

Contract

Accummulative

Contractor

Duration

Obligation

Education and Human Resources

1. Education for Preliterate Adults
Project #931-1020

World Education, Inc.
N.Y.C. AID/ta-C-1455

9/14/77-
12/31/80

350,000
(12/31/80)

235

SELECTED DEVELOPMENT PROBLEMS

	<u>Active Projects</u>	<u>Contract</u>	<u>Accummulative</u>
	<u>Contractor</u>	<u>Duration</u>	<u>Obligation</u>
<u>Social/Economic Research and Development (PPC)</u>			
1. Income Distribution and Public Policy - Project #930-0643	Princeton University AID/otr-C-1492	6/30/76- 3/31/81	1,203,000 (3/31/80)
2. The Family in Economic Growth: Promot- ing Growth Through People	Rand Corp	6/25/80- 6/27/83	1,522,000 (6/25/81)
3. Fertility Determinants and Women's Role Project #930-0066	University of Penn. AID/otr-C-1571	8/1/77- 3/30/81	400,768 (3/80)

2/2/80

SELECTED DEVELOPMENT PROBLEMS

Active Projects

Contract

Accummulative

Contractor

Duration

Obligation

Small Projects (DSB)

1. Anti-Genie Model

Michigan State
DSPE-0067

3/31/81-
3/31/82

34,840
(3/31/82)

2. Biomedical Research In-Vitro,
Exoerythryocyte

Biomedical Research
Institute - DSPE-0079

5/1/80-
5/31/83

33,000
(5/31/81)

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ACTION MEMORANDUM FOR DEPUTY ADMINISTRATOR

THRU : EXSEC
FROM : DS/AA, Sander M. Levin
SUBJECT : Proposed Collaborative Research Support Grant in Grain Sorghum and Pearl Millet (CRSP-GS/PM)

Background: The two crops selected for this CRSP, grain sorghum and pearl millet, are extremely important food crops for a very large part of the developing world. More than 40 million hectares of sorghum and 34 million of millet are grown in the LDCs. The production from more than 80 per cent of this area is used for food. Most of the producers of these two grains are primarily subsistence families constituting some of the poorest of the rural poor who live in areas of low rainfall and poor soils. Research aimed at alleviating one or more of the principal constraints to production, marketing, and utilization of sorghum and millet of the subsistence farmers of the LDCs can have a significant impact on the well-being of this A.I.D. target group (See Tab C).

In the planning of this program, the project manager and other technical members of DS/AGR worked very closely with the planning contractor, regional bureaus, and JRC at all stages of the planning process. As a result of this close monitoring and coordination, each group involved had adequate opportunity for technical input. The contractor consulted in great detail with the world's leading sorghum and millet scientists. The JRC approved the program in August 1978. The Chairman commented that there had been more detailed scientific effort put into the planning of this program than any research program with which he had ever been associated. The BIFAD approved the JRC's recommendation that the University of Missouri (UMC) Program Proposal on GS/PM be accepted and that A.I.D. be asked to fund the Collaborative Research Program in fiscal year 1978.

To date, A.I.D. has made two policy decisions related to CRSPs. On August 22, 1978, you approved the processes followed by A.I.D. in selecting the participating U. S. universities and the Management Entity for the small ruminant program (Tab A). This decision applies to the Sorghum/Millet CRSP also. On September 28, 1978, the DA/AID approved a grant schedule and standard provisions to be used for implementing the small ruminants collaborative research support grants under Title XII (Tab B). Although this approval was only related to that Grant, the DA/AID is being requested to approve an identical, or slightly modified version to cover this Grain Sorghum/Pearl Millet CRSP and all subsequent CRSPs. This approval must be consummated before the execution of this grant.

Program Goal: The goal is to increase production of GS/PM among A.I.D.'s client people. This is to be done by developing and testing improved technologies and by enhancing the abilities of developing country scientists to solve problems related to GS/PM production and use. U. S. developing country institutions and international centers will be involved in the research and training. A substantial portion of the research will be done in the developing countries.

Proposed Program: The proposed program is composed of a set of integrated, long-term research projects with related training and technical service capabilities. Its first purpose is to organize and mobilize financial and human resources necessary for mounting a major, multi-institutional U.S.-LDC collaborative effort which in turn is expected to provide the knowledge base necessary to achieve significant advances in alleviating the principal constraints to improved production, marketing, and utilization of GS/PM in LDCs. A second purpose is to improve the capabilities of appropriate LDC institutions to generate, adapt, and apply improved knowledge to local conditions.

Research activities cover a wide range of subject matter categories, all of which are responsive to the most urgent technical needs identified in the planning process as the principal constraints to improved GS/PM production and utilization in LDCs. Constraints and research needs were identified by extensive LDC surveys and analyzed by a panel of internationally recognized sorghum/millet experts. These constraints are:

1. Low fertility of soils;
2. Water-related concerns;
3. Difficulty of stand establishment;
4. Pest problems including weeds, diseases, and insects;
5. Risk-related issues such as yield stability and new technology;
6. Deficiency of genetic, cytological and physiological studies, and germplasm collection and preservation;
7. Lack of seed multiplication and distribution plans;
8. High tannin and reduced nutritional value;
9. Poor communication systems between/among scientists, extension agents, and farmers;
10. Lack of farmers' ability to take risks;
11. Lack of acceptability - considered low status food;
12. Inadequate price and utilization policies;
13. Inadequate market organization and performance;
14. Lack of trained manpower.

A.I.D.'s regional bureaus identified an additional constraint not included in the Program Proposal prepared by UMC. This involves the lack

of technical information on cultivation of sorghum/millet in inter-cropping systems. The Management Entity has agreed to include research on this constraint in its detailed program plan.

This program will build on the existing experience and ongoing research and training activities in participating universities. More specifically, A.I.D. has financed research on sorghum and millet at Texas A&M University, Kansas State University, University of Nebraska, and Purdue. The research programs at these four universities will be phased into the CRSP as they terminate. All of this, combined with the development of effective LDC institution linkages, will contribute to cost-effectiveness, social soundness, and technical feasibility of the program.

Twelve universities will participate initially in the GS/PM CRSP. Four of these, mentioned above, have conducted major GS/PM research programs for several years and have made the major contributions to recent advances in production technology such as protein quality and quantity, and in the development of elite breeding populations carrying high levels of multiple disease and insect resistance. Mississippi State University has carried out a major seed technology program for many years. These five institutions have extensive research experience both in the U.S. and LDCs. In addition, they have provided considerable technical assistance to LDCs in cereal grain improvement over the past several years. Approximately 90 per cent of the CRSP budget for training and research will be utilized by or through these five universities.

Even though their research involvements will not be great, the other seven universities (University of Arizona, University of Florida, Texas Tech University, Colorado State University, University of Georgia, University of Kentucky, and Florida A&M University) will carry out activities that address important constraints not adequately addressed by the five major participating universities. All of the participating universities have a long history of training foreign students. All have staff members who have had experience working in LDCs, in many cases on a long-term basis.

The University of Nebraska has been recommended by the JRC, reflecting also the expressed preference of the participating universities, to act as the Management Entity for this program. The Management Entity has submitted a sorghum/millet proposal which we find acceptable. The program proposed has been derived from the planning reports of UMC (Tab C) as developed through iterative discussions with the Joint Research Committee and A.I.D., and as modified by the Management Entity in response to A.I.D. review and analysis of an earlier proposal by the Management Entity. To supplement the proposal presented by the Management Entity, A.I.D. staff have prepared a program framework and strategy (Tab D), a social soundness analysis (Tab E), an economic analysis (Tab F), and an environmental threshold determination (Tab G).

The Grant provides that in the first stages of the program the Management Entity will work with the Regional Bureaus, A.I.D. Missions, and host country institutions to develop the portions of the program to be done in the developing countries. The Grant also provides that the Management Entity will develop a program plan that displays specific objectives, budget, schedule of expected inputs, outputs, and progress verification indicators of each project (both in the U. S. and with specific institutions in developing countries), and the critical and supporting relationships among projects. The program plan will be submitted to A.I.D. for A.I.D. Project Manager's approval and will be used to assess the progress of the program and its component projects. The Management Entity will also obtain A.I.D. Project Manager's approval of each project prior to disbursement of funds. Project Manager's approval will be based on evidence of completion to technically, fiscally, and operationally sound project plans.

One aspect of the program strategy which is of critical importance to CRSP success is the development of collaborative research relationships between universities participating in the CRSP GS/PM, appropriate LDC institutions, closely associated international centers, and organizations such as STRC in Africa and CATIE in Latin America. It is important that these relationships be established and developed on a coordinated basis in order that there be no undue overlap, conflict, or duplication of effort among the universities, missions, and regional bureaus. This will be done as an integral part of the detailed research project and program planning be undertaken under direction of the Management Entity.

Program Outputs: One of the direct results to be expected from the CRSP is the development of an ongoing, coordinated, comprehensive multi-disciplinary and multi-institutional research program. Further, this program will have made significant contributions to the knowledge base required to alleviate many of the principal constraints to improved GS/PM production, marketing, and utilization in LDCs.

Training activities will result in improved capabilities of LDC personnel to carry out appropriate in-country research, training and extension work directly related to GS/PM production, marketing, and utilization. These improved capabilities of LDC personnel will result in improved LDC institutional capabilities.

The technical services concept is conceived as providing to requesting countries, bureaus, and missions limited technical services for improving sorghum and millet research, production, and utilization. This assistance would be provided under the grant by participating universities through the Management Entity. Where more extensive field services by the participating universities would be required, the requesting institutions would fund these from their own resources.

Proposed Budget: The estimated cost of the program during the first five years is \$21.0 million. Of this, the participating U. S. universities will contribute 5.1 million, or as a minimum, 25 per cent of the total. A.I.D. plans to make an initial grant of \$5,000,000 to cover the A.I.D. portion of the costs during the first two years. It is our intention to roll forward both the planning and funding of the program annually.

GRAIN SORGHUM AND PEARL MILLET CRSP COSTS (\$000)

<u>Year</u>	<u>Total Program</u>	<u>Non-Federal Contribution</u>	<u>Management Entity Cost</u>	<u>A.I.D. Contribution</u>
1	2,903	703	200	2,000
2	3,588	878	210	2,500
3	4,274	1,054	220	3,000
4	4,965	1,230	235	3,500
5	4,980	1,230	250	3,500
	<u>20,710</u>	<u>5,095</u>	<u>1,115</u>	<u>14,500.</u>

Recommendation: Considering the very expert opinions that have gone into the development of this program, A.I.D.'s priorities, and the ability of A.I.D. to affect the direction and magnitude of the program in future years, I recommend that you approve \$14,500,000 to finance a five-year CRSP on GS/PM by signing the attached PAF Part II.

Attachments:

A thru G a/s

Clearances:

DS/AGR/FCP, KMByergo	_____	Dated: _____
DS/AGR, DFPeterson	_____	Dated: _____
DS/XII, ELong	_____	Dated: _____
DS/PO, RSimpson	_____	Dated: _____
DAA/DS/FN, TBabb	_____	Dated: _____
AA/ASIA, JHSullivan	_____	Dated: _____
AA/NE, JCWheeler	_____	Dated: _____
AA/AFR, GTButcher	_____	Dated: _____
AA/LA, ALValdez	_____	Dated: _____
GC, MBall	_____	Dated: _____
GC, RRichstein	_____	Dated: _____
PPC/PDPR, EHogan	_____	Dated: _____

DS/AGR/FCP, RIJackson/cl
1/3/79

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Annual Report
on
Inheritance
and
Improvement
of Protein
Quality and
Content
in
Sorghum

APRIL 1, 1977 — SEPTEMBER 30, 1979
PURDUE UNIVERSITY SORGHUM PROJECT
REPORT NO. 14
CONTRACT AID/ta-C-1212
UNITED STATES AGENCY FOR
INTERNATIONAL DEVELOPMENT
DEPARTMENT OF STATE
WASHINGTON, D.C.

ANNUAL REPORT
ON
"INHERITANCE AND IMPROVEMENT OF PROTEIN
QUALITY AND CONTENT IN SORGHUM BICOLOR (L.) MOENCH"

April 1, 1977 - September 30, 1979

REPORT NO. 14

prepared by:

Departments of Agronomy, Biochemistry,
Animal Science, and International Programs in Agriculture

Contract AID/ta-C-1212

Purdue University

Submitted to:

United States Agency for International Development
Department of State
Washington, D.C.

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REPORT SUMMARY

A. 1. Project Title and Contract Number: "Inheritance and Improvement of Protein Quality and Content in Sorghum bicolor (L.) Moench."
No. PRF-AID/ta-1212.

2. Principal Investigator: John D. Axtell, Department of Agronomy.
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3. Contract Period: April 1, 1975 to September 30, 1979

4. Period covered by report: April 1, 1977 to September 30, 1979

5. Total AID funding of contract to date: \$693,849.97

B. The Ethiopian high-lysine gene is associated with an 80% increase in the lysine concentration of whole sorghum grain. It is generally accepted that the gene reduces yield in all genetic backgrounds. We believe that the Ethiopian high-lysine gene, in its native background genotypes, has greatest applicability as a special purpose grain for people requiring a high-protein food source, i.e. pregnant and lactating women and weaned children, as an alternative to externally supplied supplements. The chemically induced P-721 opaque gene is associated with a 60% increase in the lysine concentration of whole grain in isogenic comparison with the sib

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line. The lower rate of dry matter accumulation during development on a per seed basis confirms that this gene also is associated with reduced seed weight. However, selection experiments indicate that yield component compensation, particularly in seed number, allows high yielding P-721 opaque lines to be developed. The increment in lysine concentration contributed by the gene in high-yielding genetic backgrounds may not be as great as in the isogenic line.

When seven varieties of sorghum grain varying in tannin content were fed to chicks, a high negative correlation ($r = -0.92$) was observed between tannic acid equivalents (TAE) of the grain and growth rate. An identical correlation (0.92) was noted between TAE and feed efficiency (grain/feed). Supplementing a high tannin sorghum (HTS) diet with ferric chloride (0.5 or 1.0%) to enhance tannin oxidation failed to overcome the detrimental effects of tannin. Similarly, the addition of diethyldithiocarbamate (0.1 or 0.2%) to a HTS diet to inhibit polyphenol oxidase, and thus reduce tannin oxidation, did not affect the tannin toxicity. Chicks fed HTS diets produced significantly more excreta while consuming less feed than chicks fed low tannin sorghum (LTS) diets. Hence, dry matter utilization of chicks fed HTS was significantly poorer than chicks fed LTS. The growth depression produced by feeding HTS in a sorghum-soybean meal diet could be completely overcome by methionine supplementation. However, the addition of methionine had no effect on dry matter utilization. These results suggest that the growth depression associated with feeding HTS cannot be explained on the basis of reduced digestibility. Since the reduced feed efficiency observed with HTS is not completely corrected by methionine supplementation, lower digestibility appears to be the cause of the poorer feed efficiency.

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Polyphenolic materials such as tannins constitute a natural defense mechanism which in grain sorghum provides protection against weathering, pre-harvest germination, and bird depredation. In some areas these stresses are so severe that low tannin types are difficult to produce. The agronomic advantages of high tannin sorghums are somewhat offset by the nutritional disadvantages associated with these types, which give relatively low weight gains and feed efficiencies in feeding trials with rats and chicks. A major incentive for our work is the resolution of this conflict between sorghum producers and feeders concerning the presence and effects of tannin in sorghum.

When we began to investigate the occurrence and effects of tannins in sorghum we found that the available techniques for quantitating the tannins were not satisfactory. We have therefore developed, or modified from previous techniques, a series of tannin assays which are more suitable. These assays are somewhat independent of each other because they measure different chemical properties of the tannins. Collectively, therefore, they serve not only to determine the amount of tannin present, but also to characterize it to some extent.

The vanillin assay has been widely used for sorghum tannin, at least partly because of its specificity for the flavanol type units of the condensed tannin polymers such as are present in sorghum. We have made several modifications of this assay which increase its accuracy, reproducibility and convenience. Perhaps the most significant change is the inclusion of a reagent-free blank which corrects for background absorption. We have shortened the extraction time from 24 hours to 20 minutes, and altered the reaction conditions. The reaction is quite temperature sensitive so the temperature must be controlled. Catechin,

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although often used as a standard in this assay, gives non-linear standard curves and much less absorption than equivalent weights of tannin. Purified tannin should be used as standard whenever possible.

We developed a rapid and convenient technique, the Prussian Blue assay, which measures total extractable phenolics. This assay can be adapted for rough estimates of tannin content in the field or at the elevator, or for precise measurements in the laboratory. Non-tannin phenolics in sorghum cause little interference in high tannin varieties. Even this low level of color due to non-tannin phenolics can be largely accounted for and subtracted out by comparing values obtained by extracting with water and with a salt solution. The salt seems to prevent tannin from extracting without interfering with the extraction of other phenolics, so that the difference in values obtained with water and salt solution extraction are due to tannin.

Perhaps the most characteristic property of tannins is their propensity for interacting with proteins; this may largely account for their biological properties. We have devised assays which measure, under standard conditions, the protein precipitating power of tannins. The amount of tannin in the precipitate is determined spectrophotometrically after redissolving the pellet. The amount of protein precipitated is measured using ^{125}I -labelled protein such as serum albumin. These assays are particularly useful for studying tannin-protein interactions. Depending on the conditions, purified sorghum tannin can precipitate up to six times its own weight of serum albumin. The protein precipitating power of high tannin sorghum can be assessed in the ground grain without any extraction or other treatment which might modify or fractionate the tannins, by mixing constant amounts of sample with increasing

concentrations of ^{125}I -albumin. After centrifugation, measurement of unbound ^{125}I in the supernatant gives a titration curve which shows the protein precipitating capacity of the grain at saturation.

These assays, when applied to mature sorghum grain, all give good correlations with nutritional quality as assessed by rat and chick feeding trials measuring weight gain and feed efficiency. Preliminary studies with immature grain show a much poorer correlation between the results of chemical assays and feeding trials. The reasons for this apparent discrepancy are under investigation.

A. GENERAL BACKGROUND

Sorghum is the fourth most important cereal crop grown for human consumption in the world, being surpassed only by rice, wheat, and corn in world-wide importance. In Africa and much of Asia it is the principal source of energy for many millions of people and provides a major source of protein in the human diet. Though it is considered relatively high in carbohydrates and a fair source of total protein compared to most cereal crops, the protein quality is the poorest of the major cereals with regard to amino acid balance and total digestibility.

The purpose of this project is to develop and release for utilization in the LDCs superior varieties or lines of sorghum with genetically controlled higher protein content, improved amino acid balance and total digestibility. Thousands of lines from the world sorghum collection and the various breeding programs are being evaluated for levels of total protein and for quality by chemical and biological means. The more promising strains are recombined in various ways to further improve the amount and quality of protein in breeding populations. The parameters of inheritance of the high nutritional quality factors are in the process of being determined. Lines which do not produce seed at Purdue are sent to Puerto Rico for multiplication. There, these lines are screened for improved yield for the tropics and seed is returned to Purdue for screening of nutritional characteristics. Selected germplasm and supporting cultural practice information are provided to established programs and interested cooperators to test, screen, and to assist in the exchange of breeding materials. Graduate degree and non-degree training programs at Purdue are developed and regularly conducted to increase the research capacity of

developing nations with the desire to improve the nutritional quality of their sorghum.

B. STATEMENT OF PROJECT OBJECTIVES AS STATED IN THE CONTRACT

Objective 1. Identify and evaluate sorghum lines or mutants with improved protein quality and quantity utilizing both chemical and biological methods.

Objective 2. Identify the chemical nature and composition of the protein fractions of selected high lysine sorghum genotypes and correlate the grain fraction composition with nutritional quality.

Objective 3. Ascertain the role of various chemical components of sorghum grain such as polyphenols (tannins), protein quality, and carbohydrate availability to monogastric animal nutrition.

Objective 4. Determine the mechanism of inheritance of high nutritional quality and develop breeding methods and materials including populations with improved biological quality for potential utilization in developing countries.

C. CONTINUED RELEVANCE OF OBJECTIVES

The objectives are currently being pursued under the Title XII Sorghum and Millet CRSP.

D. RESEARCH ACCOMPLISHMENTS

Summary reports of the major research accomplishments are included in the following sections. (See list in Table of Contents.)

CURRENT STATUS OF PROTEIN QUALITY
IMPROVEMENT IN GRAIN SORGHUM

J. D. Axtell, S. W. VanScoyoc, P. J. Christensen and G. Ejeta
Department of Agronomy

Introduction

Sorghum is an important crop species for the production of grain for human consumption in Africa and Asia. The grain provides an important source of carbohydrate and is similar to other major cereals as a source of total protein. The protein quality, however, is relatively poor in comparison with that from other major cereal grains. Mertz *et al.* [1] estimated that the biological value of sorghum grain is poorer than that of any other major cereal, primarily because of the low lysine content of the sorghum endosperm storage proteins. Two genetic mutants, one naturally occurring and one induced, have been identified that increase the lysine content of the sorghum endosperm and improve the protein quality of the grain. The purpose of this paper is to briefly review the origin of these mutants and describe the recent results of experiments on the relationship between improved protein quality and total grain production in sorghum.

Ethiopian High-Lysine Gene

Singh and Axtell [2] screened about 10,000 entries in the World Sorghum Collection and identified two floury endosperm varieties from Ethiopia that contained a gene that significantly increased the level of protein and also increased the lysine concentration of the endosperm.

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proteins. The screening process involved cross-sectioning seeds from each entry to identify those with floury endosperm phenotypes and then evaluating grain samples from those selected entries for protein and lysine concentration. Sixty-two floury endosperm lines were identified, of which two (IS-11167 and IS-11758) had a significantly higher lysine content than normal sorghum. These lines contain approximately 15 - 17% protein in comparison with normal checks averaging about 12% protein. The lysine content of the Ethiopian high-lysine selections is approximately 3.1% (expressed as per cent of protein) and 0.50% (expressed as per cent of sample) in comparison with normal sorghum values of 2.0 and 0.26%, respectively [3]. The biological value of the Ethiopian high-lysine grain is also significantly higher than normal sorghums in isonitrogenous rat feeding experiments [2]. It has been established that the concentration of alcohol-soluble proteins is significantly reduced in high-lysine endosperm, relative to values present in normal sorghum endosperm [4,5].

Utilization of High-Lysine Sorghum in Ethiopia

A collection trip was made in 1973 to determine whether the high-lysine varieties identified in the World Collection were being cultivated by farmers in Ethiopia. The lines originally identified from the World Germplasm Collection were obtained in Wollo Province in the central highlands of Ethiopia. Farmers continue to grow these varieties in mixed plantings of sorghum varieties in this area of Ethiopia. A large number of varieties similar to the original high-lysine variety were collected in addition to an equivalent number of normal varieties for comparative purposes. Ejeta [6] has evaluated the protein and lysine content of grain from high-lysine and normal varieties grown under actual

field conditions in Ethiopia. Figure 1 illustrates the lysine and protein concentration in this series of high-lysine and normal sorghum varieties. The mean lysine concentration, expressed as percent of protein, was 2.88 for the high-lysine entries and 2.17 for the normal sorghum varieties grown in the same environment. Protein values were 15.7 and 11.4%, respectively. It seems likely that the high-lysine gene has been present in Ethiopia for a long period of time, since there is great diversity in panicle morphology, maturity and plant height among the high-lysine genotypes collected. The farmers roast the heads of the high-lysine varieties in the late dough stage and eat the grain in mixtures with grain from normal sorghum varieties prepared in a similar way. There is general recognition by the farmers that the yield of high-lysine varieties is significantly less than normal varieties. The reason given to us for growing these varieties is that the high-lysine grain has superior flavour qualities and improved palatability.

There is a good opportunity to utilize these high-lysine varieties in African countries as high-protein, special-purpose sorghum varieties. The protein concentration is increased by about 30%, along with the significant increase in protein quality. The grain from these varieties is recognizably different for marketing purposes because of the somewhat dented kernel phenotype of the mature grain. The flavour characteristics also appear to make these varieties quite acceptable for human consumption. We propose that these Ethiopian high-lysine varieties should be utilized in rural populations as special-purpose sorghums for people who have a high protein requirement. It should be possible for farmers in rural areas to produce an adequate quantity of high-lysine sorghum grain for use as a weaning food and a supplement for pregnant women and nursing mothers on a

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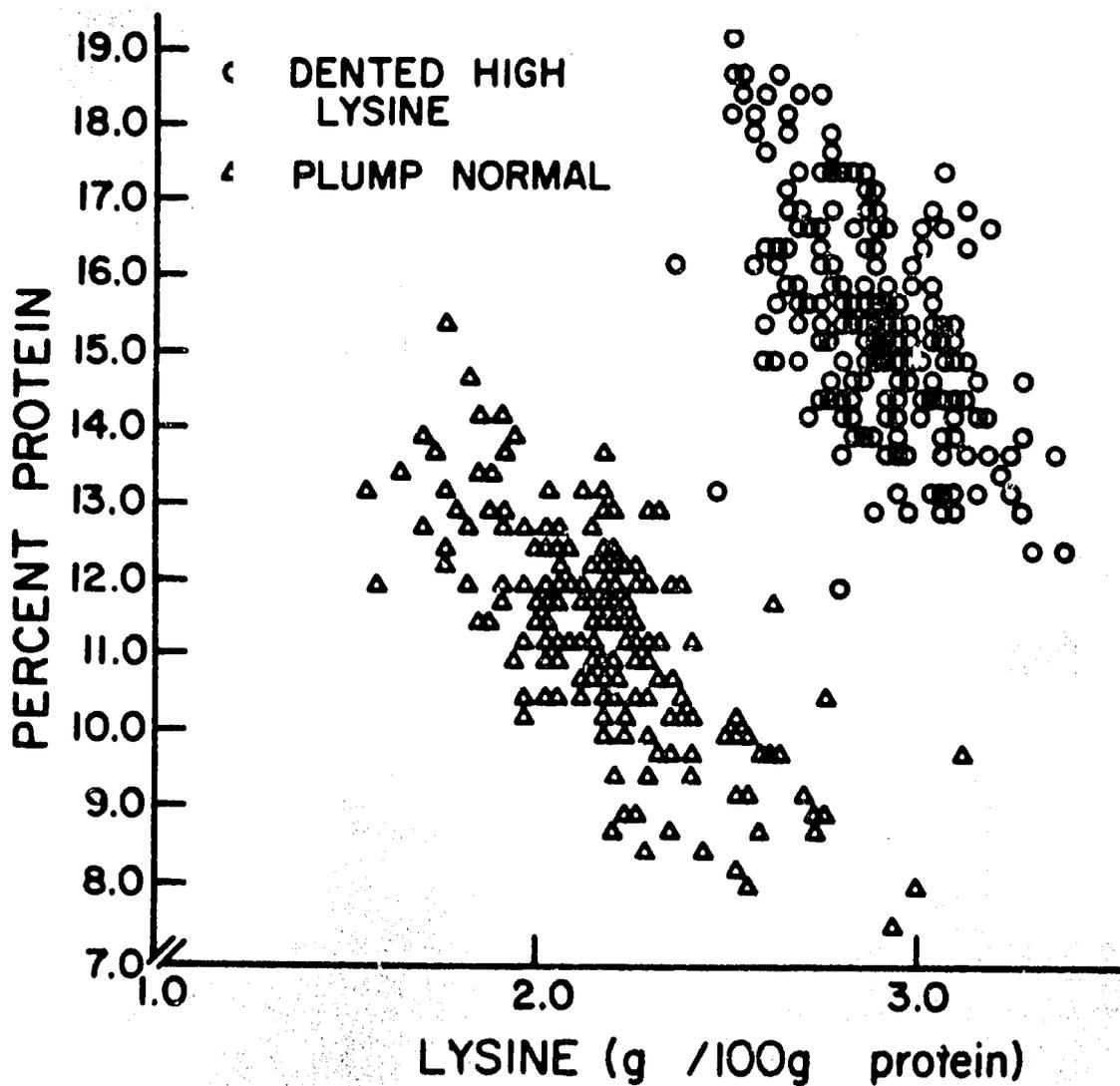


Figure 1 Relationship of protein and lysine in normal and high lysine sorghum lines collected from the same environment in Ethiopia. (From Axtell et al, 1978)

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small section of their farm. It may also be possible to develop a marketing system whereby these grains can receive a market premium when sold in the cities. [Caution - Very recent studies by Graham at Johns Hopkins (personal communication to E. T. Mertz) suggest that sorghum flour is quite indigestible by infants and small children. Since this is contradictory to results with laboratory animals, more information is needed before high-lysine sorghums are utilized as weaning foods.]

Chemically Induced High-Lysine Mutant

Mohan [7] utilized chemical mutagenesis to induce a second high-lysine gene mutation in sorghum. The parent line used for the mutagen treatments was a photoperiod insensitive, three-dwarf sorghum line with relatively broad agronomic adaptability. The parent line also had a colourless pericarp and translucent (vitreous) endosperm so that progeny from the mutagen treatments could be screened for opaque mutant kernels over a light box. Selfed seed was treated with diethyl sulphate by soaking in a solution containing 1 ml DES per 1000 ml of distilled water for three hours. The M_1 plants were grown in Lafayette, Indiana, during 1972 and each head was bagged to ensure self-fertilization. M_2 plants were then grown in Puerto Rico during the winter of 1972-73 and each M_2 head was again bagged to ensure self-fertilization. Approximately 23,000 bagged M_2 heads bearing M_3 seeds were harvested in the spring of 1973 in Puerto Rico and shipped to Lafayette for evaluation.

Seed from each head was threshed and examined for opaque kernel segregates over a light box. A total of 445 putative opaque mutants were identified and seed from each segregating head was separated into vitreous and opaque classes. Both classes of seed from each putative mutant head

were then analysed for protein and lysine concentration. Of the 445 mutants, only 33 were identified that had an increase in lysine concentration greater than 50%. Plants from each of these 33 opaque and normal sib seed lots were grown in paired rows to evaluate them for any morphological changes associated with the change in endosperm phenotype. Most of the opaque mutants were found to drastically affect either plant or seed development. Only one of these 33 (P-721) was found to produce normally appearing plants and seeds. The P-721 opaque mutant produced an increase of about 60% in lysine concentration. It is a single gene that is simply inherited as a partially dominant factor. The biological value of P-721 grain is significantly higher in monogastric feeding experiments than normal sib counterpart grain.

VanScoyoc has examined dry matter accumulation during grain development to determine what effect the P-721 mutant has on grain yield potential. Figure 2 presents the mean seed weight per head of P-721 opaque and normal sib heads at periods ranging from 10-59 days after pollination in a space-planted population. It is evident from these data that there is no difference in dry matter accumulation until approximately 31 days after pollination. After 31 days dry matter accumulation in the P-721 opaque line levels off, whereas dry matter in the normal sib line continues to accumulate for an additional week, plateauing at 38 days after pollination. VanScoyoc has also examined 1000-seed weight during grain development and his data are presented in Table 1. Seed weights of the normal and opaque lines are similar at 31 days after pollination, but diverge at 38 days after pollination. At maturity kernel weight for the opaque line is reduced 11-14% relative to its normal counterpart. No reduction in seed number was observed, so the difference between the lines can largely be

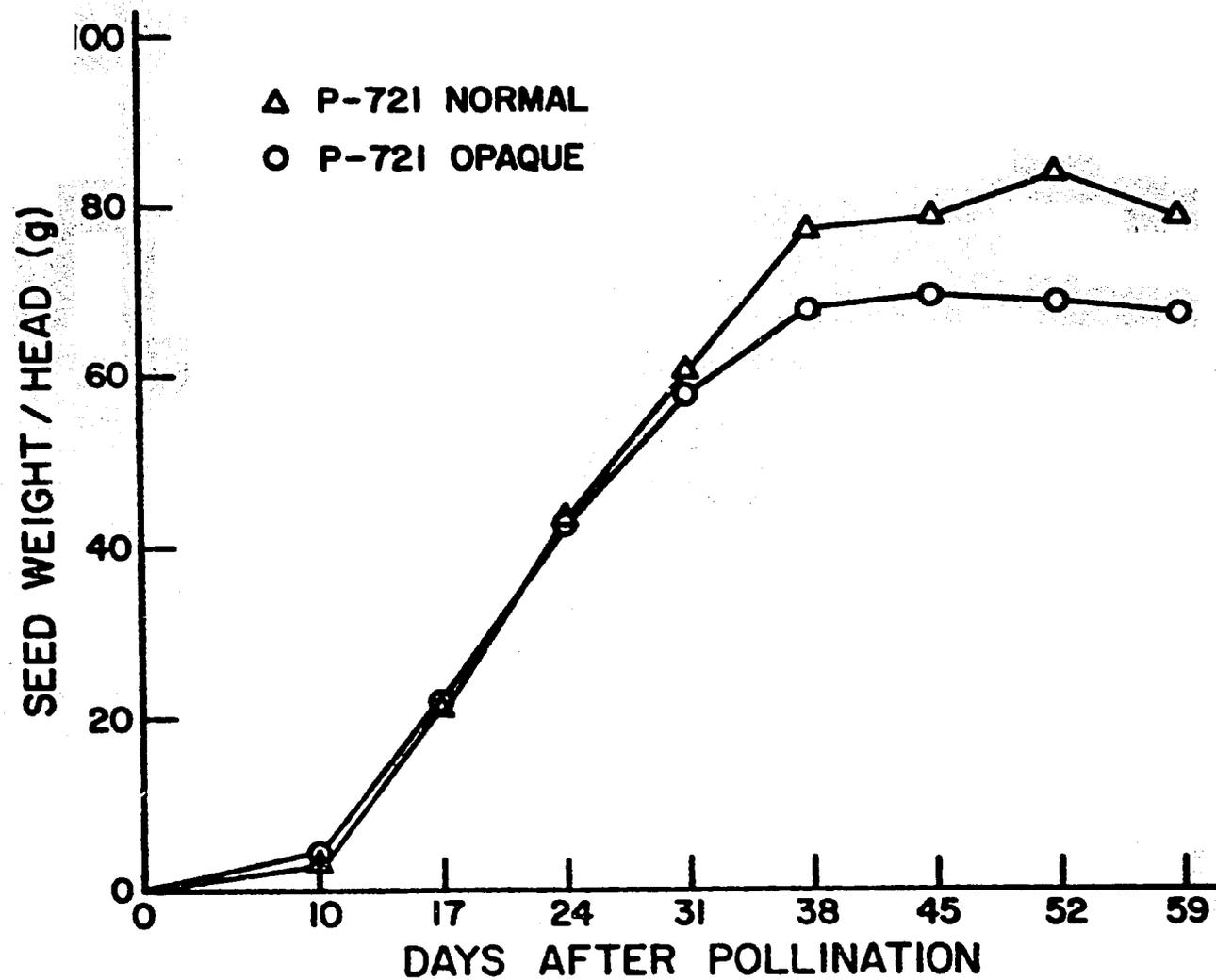


Figure 2. Mean increase in seed weight/head of P-721 opaque and P-721 normal sorghum (*Sorghum bicolor* (L.) Moench) during grain development in a space planted population.

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TABLE 1. MEAN 1000-SEED WEIGHTS OF P-721 OPAQUE AND ITS NORMAL SIB LINE DURING GRAIN DEVELOPMENT IN A SPACE PLANTED POPULATION

Days after pollination	P-721 normal	1000-seed dry weight ^a P-721 opaque	% P-721 opaque of normal
17	7.625	7.875	103.3
24	14.664	14.294	97.5
31	18.562	18.998	97.7
38	23.164	21.652	93.5
45	25.455	21.608	84.9
52	24.099	21.451	89.0
59	24.861	21.358	85.9

^a Mean of three replicates.

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accounted for by reduced kernel density. The reduction in kernel weight is in relative agreement with preliminary data from a 1977 four-replicate yield trial at 147 664 plants/ha showing a 9.4% total yield reduction for P-721 opaque compared with its normal sib line.

Yield of P-721 Derived Lines

Christensen [8] examined the grain yield of advanced breeding lines homozygous for the P-721 opaque gene in comparison with heterozygous and normal, homozygous vitreous sib lines.

The varieties used are F_4 derived bulks from crosses of P-721 by 22 unrelated high-yielding lines from the World Collection of sorghum varieties, and from Purdue University's sorghum breeding programme. The segregating material was handled as part of the regular breeding nursery. The population of inbred lines was selected for short to intermediate height, early flowering and general agronomic desirability. Opaques were selected in the F_3 and F_4 generations, largely on the basis of their acid orange 12 dye-binding capacity. The heads of the 378 lines included in the yield trial were checked for opaqueness by seed sectioning after harvest: 300 lines are opaque; 73 are segregating (indicating that the F_4 head had been heterozygous); and 5 are homozygous vitreous. The selected lines were tested in the F_5 in the two-block randomized complete block design at the Purdue Agronomy Farm during the summer of 1977.

The means for the three genotypes and four check varieties are given in Table II. Opaque lines yield marginally higher than the segregating lines from heterozygous heads, but the difference was not due to the opaque gene itself. This higher yield is almost certainly due to selection for high dye-binding capacity in the F_3 and F_4 generations among the

TABLE 11. MEANS OF CHEMICAL AND AGRONOMIC TRAITS FOR COMPARISON OF GENOTYPES AT THE OPAQUES LOCUS FOR P-721 DERIVED LINES AND HIGH YIELDING CHECKS

	No.	DBC (g X 10 ⁻¹ /l)	Protein (%)	Yield (kg X 10 ² /ha)	100-seed weight (g/100)	Days to flowering	Height (cm)
P-721 genotype							
Opaque	300	7.64	12.51	46.58	2.50	72.8	135
Heterozygous	73	7.04	13.26	44.72	2.89	72.1	148.7
Normal	5	6.78	12.92	41.54	2.87	72.6	156
Checks							
954063 (Inbred line)	4	6.14	11.38	57.32	2.58	68.9	120
RS671 (hybrid)	4	6.18	11.66	56.35	2.30	65.0	111.2
NK300 (hybrid)	4	6.11	10.88	66.70	2.18	64.9	152.5

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heterozygous and normal heads. To be selected for the yield trial, a heterozygous or a normal head would have to have had a very high protein percentage. Since high protein percentages are associated with low yield levels, one would presume that selection against yield occurred in the heterozygous and in the normal heads that were selected. The conjecture is supported by the high protein percentages of the segregating and vitreous lines in the trial. On the basis of this evidence, one would presume that P-721 derived opaque lines would not be higher yielding but should be as high yielding as their normal counterparts.

Seed weight of the opaques is significantly lower than the seed weight of the normal and of the heterozygous lines (Table II). The seed weight of the high-yielding checks is even lower than the seed weight of the opaques. In opaque-2 maize low seed weight is a problem because restricted ear morphology does not allow seed number compensation. Low seed weight is not closely associated with low yield in sorghum. The increased panicle size allows compensation for decreased seed weight by increased seed number.

Discussion

We conclude that the comparison of the yield of P-721 opaque with its isogenic normal sib line shows clearly that the grain-filling period of the opaque kernel is reduced in this particular genetic background. The resultant decrease in seed weight accounts for the reduction in grain yield in that background.

While it is possible that the P-721 opaque gene reduces grain-filling duration in many genetic backgrounds with a consequent decrease in seed weight, the yield data from the F_5 opaque and heterozygous advanced sib lines suggests that selection for yield will result in compensation in

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number of seeds per head and number of heads per unit area. Appropriate genetic backgrounds can be selected that will significantly enhance yield potential of the P-721 opaque gene. The yield differential observed between isogenic opaque and normal lines in sorghum should not be a serious deterrent to the development of high-yielding sorghum varieties with improved protein quality, after the gene has been placed in the appropriate background.

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Final Report of the Research on Sorghum Tannins
Biochemistry Department, Purdue, April 1977 - September 1979
(Adapted from Abstracts of Reports
Presented at the Sorghum Utilization Conference, Wichita, 1979)

Polyphenolic materials such as tannins constitute a natural defense mechanism which in grain sorghum provides protection against weathering, pre-harvest germination, and bird depredation. In some areas these stresses are so severe that low tannin types are difficult to produce. The agronomic advantages of high tannin sorghums are somewhat offset by the nutritional disadvantages associated with these types, which give relatively low weight gains and feed efficiencies in feeding trials with rats and chicks. A major incentive for our work is the resolution of this conflict between sorghum producers and feeders concerning the presence and effects of tannin in sorghum.

When we began to investigate the occurrence and effects of tannins in sorghum we found that the available techniques for quantitating the tannins were not satisfactory. We have therefore developed, or modified from previous techniques, a series of tannin assays which are more suitable. These assays are somewhat independent of each other because they measure different chemical properties of the tannins. Collectively, therefore, they serve not only to determine the amount of tannin present, but also to characterize it to some extent.

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reproducibility and convenience. Perhaps the most significant change is the inclusion of a reagent-free blank which corrects for background absorption. We have shortened the extraction time from 24 hours to 20 minutes, and altered the reaction conditions. The reaction is quite temperature sensitive so the temperature must be controlled. Catechin, although often used as a standard in this assay, gives non-linear standard curves and much less absorption than equivalent weights of tannin. Purified tannin should be used as standard whenever possible.

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Depending on the conditions, purified sorghum tannin can precipitate up to six times its own weight of serum albumin. The protein precipitating power of high tannin sorghum can be assessed in the ground grain without any extraction or other treatment which might modify or fractionate the tannins, by mixing constant amounts of sample with increasing concentrations of ^{125}I -albumin. After centrifugation, measurement of unbound ^{125}I in the supernatant gives a titration curve which shows the protein precipitating capacity of the grain at saturation.

These assays, when applied to mature sorghum grain, all give good correlations with nutritional quality as assessed by rat and chick feeding trials measuring weight gain and feed efficiency. Preliminary studies with immature grain show a much poorer correlation between the results of chemical assays and feeding trials. The reasons for this apparent discrepancy are under investigation.

For maximum accuracy, pure samples of authentic sorghum tannin must be used as standards in tannin assays. In our laboratory, previously described preparations of sorghum tannin are contaminated with protein, which could significantly affect the properties of the tannin with which it is associated. We have devised a purification scheme for sorghum tannin which reduced protein contamination to low levels ($\leq 2\%$).

Several approaches to the problem of antinutritional effects have been considered. Our colleagues at Purdue have removed the tannin from sorghum by extraction with aqueous alkalis or by milling off the surface layers of the grain, but the loss of nutrients which occur with these treatments are significant and probably unacceptable. We have investigated the possibility that sorghum tannins might be modified in situ to forms with reduced antinutritional properties. We have found that sorghum tannins are

readily converted, on addition of small amounts of aqueous ammonia to whole grain at room temperature and atmospheric pressure, to forms which do not respond to any of our chemical assays for tannin. Moreover, feeding trials of this treated high tannin sorghum show that the nutritional quality is correspondingly improved by the treatment, with weight gains on treated high tannin grain equivalent to those observed with low tannin sorghum. The treatment is not specific for ammonia; similar "detoxification" effects have been observed with several dilute aqueous alkalis, including an extract of wood ashes. Both moisture and alkali are required; a variety of heat treatments and conventional cooking procedures have been examined but none showed significant detoxification.

Detoxification of high tannin sorghum, no matter how simple the process, may not be the most desirable long-term solution to the tannin problem. Our goal is a genetic solution: sorghum varieties which have tannins and are thus agronomically superior, but which do not exhibit the antinutritional properties of tannin. During seed maturation some sorghum varieties have quite high tannin levels which diminish on ripening. In the Group II sorghums described by Cummings and Axtell, tannin content diminishes at maturation to undetectable levels, unless special acidic solvents are used to extract the tannin. With Roger Bullard and W. C. Royall of the Denver Wildlife Service, we are examining the bird repellancy, nutritional quality, and other characteristics of selected Group II sorghums throughout the maturation process. Preliminary studies indicate that the tannin of immature grain may have properties somewhat different from that of mature grain. Brief boiling or freezing of immature sorghum followed by drying at room temperature converted the tannin to non-extractable forms, whereas the untreated but similarly dried controls

had extremely high amounts of extractable tannin. These treatments had no apparent effect on the nutritional properties of the tannin. The conversion of tannin to non-extractable forms by these treatments may be due to disruption of tannin-containing structures, permitting cellular components to bind tannins.

EFFECTS OF SORGHUM GRAIN TANNINS ON NUTRITIVE VALUE

J. C. Rogler and W. R. Featherston
Department of Animal Sciences

When several varieties of sorghum grain varying in tannin content were fed to chicks, a high negative correlation ($r = -0.92$) was observed between tannic acid equivalents (TAE) of the grain and growth rate. An identical correlation (-0.92) was noted between TAE and feed efficiency (gain/feed). Supplementing a high tannin sorghum (HTS) diet with ferric chloride (0.5 or 1.0%) to enhance tannin oxidation failed to overcome the detrimental effects of tannin. Similarly, the addition of diethyldithiocarbamate (0.1 or 0.2%) to a HTS diet to inhibit polyphenol oxidase, and thus reduce tannin oxidation, did not affect the tannin toxicity.

Chicks fed HTS diets produced significantly more excreta while consuming less feed than chicks fed low tannin sorghum (LTS) diets. Hence, dry matter utilization of chicks fed HTS was significantly poorer than chicks fed LTS. The growth depression produced by feeding HTS in a sorghum-soybean meal diet could be completely overcome by methionine supplementation. However, the addition of methionine had no effect on dry matter utilization. These results suggest that the growth depression associated with feeding HTS cannot be explained on the basis of reduced digestibility. Since the reduced feed efficiency observed with HTS is not completely corrected by methionine supplementation, lower digestibility appears to be the cause of the poorer feed efficiency.

Other evidence that the detrimental effects of tannins cannot be completely explained by reduced digestibility was afforded by studies where the digestion of all dietary protein, carbohydrate and lipid was

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circumvented by the use of crystalline amino acids, glucose and free fatty acids. When a methanol extract (TAE = 40%) of HTS was added to this diet at a level of 2.5%, a depression in growth rate was still obtained.

Although methionine supplementation is very effective in overcoming the growth depression of chicks fed HTS in a sorghum-soybean meal diet, it is without effect in certain other diets. In a sorghum-crystalline amino acid diet where the essential amino acids are added in the same amounts found in soybean meal and non-essential nitrogen is provided by glutamic acid, HTS reduces chick growth rate and additional methionine is ineffective in overcoming the growth depression. Similarly, the growth depression obtained by adding a methanol extract of sorghum tannins to a purified diet does not respond to methionine supplementation. Why methionine is effective in one case and not in others remains a rather perplexing question.

It has consistently been observed that feeding HTS to chicks results in leg anomalies characterized as a bending or bowing of the leg bones. This was first observed in chicks fed a HTS-soybean meal diet and the incidence of the abnormality usually ranged from 7-10%. The incidence of the condition increased dramatically (60-70%) when HTS was incorporated into a sorghum-crystalline amino acid diet. Increasing levels of vitamins and minerals failed to overcome the bone anomalies caused by HTS. Since bone ash is normal in the afflicted chicks, it is suggested that the defect is in the organic matrix of the bone. Therefore, it was hypothesized that tannins may be absorbed and affecting the bone collagen in a manner similar to their tanning effect in the processing of leather. This involves an increased cross-linking of the collagen fibers due to binding of tannins with collagen. Preliminary studies indicate that there may be an increased

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cross-linkage of collagen fibers in both bone and muscle as indicated by a reduction in the amount of extractable collagen in these tissues from birds fed HTS.

E. RESEARCH PUBLICATIONS (ABSTRACTS)

ABSTRACT

DETERMINATION OF PROTEIN IN TANNIN-PROTEIN PRECIPITATES
Ann E. Hagerman and Larry G. Butler

The amount of protein precipitated by tannin under a variety of conditions is measured by using radiolodinated protein. The protein is mixed with purified tannin, finely ground plant tissue, or an unpurified plant extract; the mixture is centrifuged to remove the insoluble tannin-protein complex. An aliquot of the supernatant layer is counted to calculate the amount of protein precipitated. Complex formation is dependent on the pH and solvent composition. Tannin specific activity is useful for comparing tannin from various sources and for monitoring tannin purification. It is defined here as the ratio between the amount of protein precipitated and the amount of oxidizable material present.

J. Agriculture & Food Chemistry, 1980
Vol. 28: 944-947

ABSTRACT

CONDENSED TANNIN PURIFICATION AND CHARACTERIZATION OF TANNIN-ASSOCIATED PROTEINS

Ann E. Hagerman and Larry G. Butler

The conventional isolation method has been modified in order to minimize protein contamination of tannin purified from high tannin sorghum. The two unique steps of the new procedure are preliminary extraction of the ground grain with ethanol and treatment of the partially purified tannin with phenol to remove traces of noncovalently bound protein.

Tannin-associated protein removed by phenol treatment is not a random mixture of all the seed proteins, but consists of several discrete components which have been isolated and partially characterized. These proteins are quite hydrophobic, and one is rich in proline. With only minor changes, the purification method can be used to isolate tannin from seeds of other plants such as legumes.

J. Agricultural & Food Chemistry, 1980
Vol. 28: 947-952

ABSTRACT

TANNIN CONTENT AS A FUNCTION OF GRAIN MATURITY AND DRYING CONDITIONS IN SEVERAL VARIETIES OF SORGHUM BICOLOR (L.) MOENCH
Martin L. Price, Andrew M. Stromberg, and Larry G. Butler

Twelve varieties of sorghum grain were assayed for tannin content at various stages of maturity. For varieties which were found to contain tannin, maximum values per seed were obtained between 25 and 40 days after half-anthesis. Wide variability in apparent loss of tannin as the grains matured was found between varieties, with tannin contents of mature seed ranging from 3 to 93% of the maximum found in the immature seed. We suggest that varieties with maximum decreases in tannin may be similar to low tannin varieties in the nutritional quality of the mature grain, yet provide bird resistance during immature stages. Drying immature grain at room temperature after boiling for 3 min or freezing caused a drastic reduction in apparent tannin content over untreated controls, but these treatments had little effect on nutritional quality of the grain.

J. Agricultural & Food Chemistry, 1979
Vol. 27: 1270-1274

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ABSTRACT

PROTEIN PRECIPITATION METHOD FOR THE QUANTITATIVE DETERMINATION OF TANNINS
Ann E. Hagerman and Larry G. Butler

The tannin content of crude plant extracts or of purified preparations was determined by adding the sample to a standard solution of protein, isolating the insoluble tannin-protein complex, dissolving it in alkaline solution, and measuring the absorbance at 510 nm after adding ferric chloride. Plots of absorbance as a function of the amount of tannin are linear for tannic acid and partially purified sorghum tannins for amounts of tannin ranging from 0.20 to 1.0 mg. Non-tannin components of crude methanolic extracts of sorghum and cowpeas do not interfere with the assay. The results of the precipitation method are qualitatively similar to results obtained with the vanillin assay. The precipitation assay can be used to study the effects of pH and other parameters on tannin-protein interactions.

J. Agriculture & Food Chemistry, 1978
Vol. 26: 809-812

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ABSTRACT

**A CRITICAL EVALUATION OF THE VANILLIN REACTION AS AN ASSAY FOR TANNIN IN
SORGHUM GRAIN**
Martin L. Price, Steve VanScoyoc and Larry G. Butler

Several parameters of the vanillin assay were examined to determine which must be most closely controlled to ensure accuracy and reproducibility. A 20-min extraction in methanol was found to be adequate. When corrected for background color, the modified vanillin assay was found to give nearly identical values with those obtained with the regular vanillin assay, except with group II sorghum. The reactions of tannin and catechin, the usual standard, with vanillin were found to differ markedly in reaction kinetics. Assays of purified tannin showed that use of catechin equivalents over-estimates tannin content. The assay was found to be extremely temperature dependent. Revised procedures for the vanillin assay are presented which give excellent reproducibility.

J. Agriculture & Food Chemistry, 1978
Vol. 26: 1214-1218

ABSTRACT

OVERCOMING THE NUTRITIONALLY HARMFUL EFFECTS OF TANNIN IN SORGHUM GRAIN BY TREATMENT WITH INEXPENSIVE CHEMICALS
Martin L. Price, Larry G. Butler, John C. Rogler and William R. Featherston

Treatment of high-tannin sorghum grain with moist, alkaline conditions was shown to substantially reduce the amount of tannin as measured by three chemical assays. Chicks fed a high-tannin grain (Savannah III), treated as the whole grain with dilute ammonium hydroxide for 30 days, showed 3-week weight gains and feed efficiencies which were statistically equivalent to those of chicks fed an untreated low-tannin control (RS-610). A shorter treatment of a ground high-tannin grain (BR-54) with a 0.5 M aqueous solution of K_2CO_3 resulted in a comparable improvement in weight gains and a substantial improvement in feed efficiencies. Treatment of the same grain with moisture and CaO gave an improvement of a lesser magnitude. Increases in available protein after treatments did not appear sufficient to account for the nutritional improvements.

J. Agriculture & Food Chemistry, 1979
Vol. 27: 441-445

ABSTRACT

EFFECTS OF HIGH TANNIN SORGHUM, TANNIC ACID, CATECHIN AND SORGHUM TANNIN EXTRACT ON THE PERFORMANCE OF YOUNG CHICKS

J. C. Rogler, W. R. Featherston, R. G. Eikin and R. K. Giles

The inclusion of either high tannin (HT) sorghum, tannic acid or catechin in a sorghum-soybean meal diet depressed growth rate to about the same extent. Supplemental methionine completely overcame the growth reduction caused by HT sorghum, but had little influence on the detrimental effects of tannic acid or catechin. Feed conversion was adversely influenced by all 3 sources of tannin with the greatest effect observed in chicks fed HT sorghum. Additional methionine improved feed conversion of chicks fed HT sorghum or catechin, but had little effect on chicks fed tannic acid. Although HT sorghum depressed growth rate and feed conversion in a sorghum-peanut meal diet, the magnitude of the effect was much less than with a sorghum-soybean meal diet. As with the soybean meal diet, supplemental methionine completely overcame the detrimental effects of HT sorghum on growth and partially overcame the effects on feed conversion in the peanut meal diet. A methanol extract of HT sorghum containing 40% tannic acid equivalents depressed performance of chicks fed a sulfur amino acid-limiting diet where digestion of exogenous protein, carbohydrate and lipid was by-passed by the use of crystalline amino acids, glucose and fatty acids. The addition of methionine to diets with and without tannin extract improved growth rate and feed conversion by about the same percentage, but the methionine supplemented diet containing tannin extract was still inferior to a similarly supplemented diet without extract.

Proceedings of XVI World's Poultry
Congress, 1978
Vol. 7: 1094-1103

ABSTRACT

STUDIES ON THE UTILIZATION BY CHICKS OF SORGHUM GRAINS WITH VARYING TANNIN CONTENTS

W. R. Featherston, J. C. Rogler and R. K. Giles

Studies were conducted on the influence of sorghum grain tannins on weight gain, feed efficiency, dry matter utilization and nitrogen retention by young chicks. Weight gain and feed efficiency of chicks were inversely related to the tannic acid equivalents of the grains in a growth trial utilizing seven varieties of sorghum grain. Significantly ($P < 0.05$) lower dry matter utilization and nitrogen retention were observed with chicks fed high tannin as compared with low tannin sorghum grain. Supplementation of the high tannin sorghum diet with 0.15% DL-methionine or 1% polyvinylpyrrolidone had no effect on dry matter utilization but resulted in a significant ($P < 0.05$) improvement in nitrogen retention. Supplementation of the high tannin sorghum diet with methionine resulted in comparable weight gains as with chicks fed the low tannin sorghum diet similarly supplemented. The ability of methionine to overcome the growth depression noted in chicks fed the high tannin diet without improving dry matter utilization indicates that tannins are exerting their influence in some manner in addition to decreasing the digestibility of the diet.

Proceedings of XVI World's Poultry
Congress, 1978
Vol. 2: 1087-1093

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ABSTRACT

INVESTIGATIONS OF LEG ABNORMALITIES IN CHICKS CONSUMING HIGH TANNIN SORGHUM GRAIN DIETS

R. G. Eikin, W. R. Featherston and J. C. Rogler

Studies were conducted into the etiology of leg abnormalities noted in chicks fed high tannin sorghum grain diets. These anomalies were characterized by a bowing of the legs with a swelling of the hock joints. The incidence of these leg problems was found to be markedly higher when the amino acids provided in the diet by soybean meal were replaced by crystalline amino acids. Supplemental vitamins and minerals had no alleviating effect on the leg problem. Bone mineralization was apparently not influenced by tannins as demonstrated by similar bone ash values for chicks fed high or low tannin sorghums. A possible tannin-caused alteration in the organic matrix of bone is discussed.

High tannin sorghum depressed chick growth and feed conversion when compared with low tannin sorghum in both sorghum-soybean meal and sorghum-amino acid rations. This growth depression was overcome by supplementing the high tannin sorghum-soybean meal diet with 0.15% DL-methionine, but no growth response was observed with a similar supplementation of a high tannin sorghum-amino acid diet.

Poultry Sci., 1978
Vol. 57: 757-762

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ABSTRACT

CURRENT STATUS OF PROTEIN QUALITY IMPROVEMENT IN GRAIN SORGHUM
J. D. Axtell, S. W. VanScoyoc, P. J. Christensen and G. Ejeta

The Ethiopian high-lysine gene is associated with an 80% increase in the lysine concentration of whole sorghum grain. It is generally accepted that the gene reduces yield in all genetic backgrounds. We believe that the Ethiopian high-lysine gene, in its native background genotypes, has greatest applicability as a special purpose grain for people requiring a high-protein food source, i.e. pregnant and lactating women and weaned children, as an alternative to externally supplied supplements. [Caution - Very recent studies by Graham at Johns Hopkins (personal communication to E. T. Mertz) suggest that sorghum flour is quite indigestible by infants and small children. Since this is contradictory to results with laboratory animals, more information is needed before high-lysine sorghums are utilized as weaning foods.] The chemically induced P-721 opaque gene is associated with a 60% increase in the lysine concentration of whole grain in isogenic comparison with the sib line. The lower rate of dry matter accumulation during development on a per seed basis confirms that this gene also is associated with reduced seed weight. However, selection experiments indicate that yield component compensation, particularly in seed number, allows high yielding P-721 opaque lines to be developed. The increment in lysine concentration contributed by the gene in high-yielding genetic backgrounds may not be as great as in the isogenic line.

Proceedings of a Symposium on Seed
Protein Improvement in Cereals and Grain
Legumes, Neuherberg, 4-8 September 1978
Vol. 2:357-365

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ABSTRACT

LYSINE LEVEL IN SOLVENT FRACTIONS OF PEARL MILLET

Christian C. Nwasike, Edwin T. Mertz, Robert C. Pickett, D. V. Glover, B. A. K. Chibber, and Stephen W. VanScoyoc

A typical whole grain sample of normal pearl millet was separated into five fractions by the Landry-Moureaux method. The distribution of proteins among the five fractions resembled that found in corn and not that in sorghum. Complete amino acid analysis of the fractions was made. Of special interest was the low level of lysine in fraction V, which is similar to that found in sorghum. On the basis of this finding, it is predicted that high lysine pearl millet when identified will resemble high lysine sorghum, not opaque-2 corn, in its total lysine content.

J. Agricultural & Food Chemistry,
December, 1979
Vol. 27: 1329-1331

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ABSTRACT

IN VITRO DIGESTIBILITY OF HIGH-TANNIN SORGHUM AT DIFFERENT STAGES OF DEHULLING

Bakshy A. K. Chibber, Edwin T. Mertz, and John D. Axtell

High-tannin sorghum grain was subjected to sequential dehulling to remove tannins. Stepwise removal of tannins gradually increased the percent of nitrogen solubilized by pepsin from 22 to 71% and by trypsin-chymotrypsin mixture from 3 to 35%. These studies suggest that a simple pepsin digestion could be used to predict the biological value of high-tannin sorghums.

**J. Agriculture & Food Chemistry, 1980
Vol. 28: 160-161**

F. THESIS RESEARCH ABSTRACTS

ABSTRACT

GENETIC VARIABILITY IN A BROAD-BASED SORGHUM POPULATION
Thomas Bittinger

Genetic variability was investigated in the diverse, random-mating grain sorghum population, PP9. A Design I mating system was used where 50 random pollen parents were each crossed to a different set of three random seed parents. The 150 resulting progenies were evaluated in a randomized complete-block design over two years at the Purdue Agronomy Farm.

The characters evaluated were days to 1/2 bloom, height, lodging, panicle weight, seed weight, panicle length, and grain yield.

Analysis of variance was performed for each trait, and estimate of the additive genetic variance and the dominance genetic variance were made. Additive genetic variance was greater than dominance variance for all traits except yield. The ratio of dominance variance to additive variance was 1.24 for yield and between 0.18 and 0.66 for height, lodging, panicle weight, and seed weight. Negative dominance estimates were obtained for days to 1/2 bloom and panicle length.

Phenotypic and genetic correlations between the traits were calculated. Yield showed highly significant positive correlations with days to 1/2 bloom and panicle weight. Other significant positive correlations included height with days to 1/2 bloom, height with lodging, panicle weight with days to 1/2 bloom, and panicle weight with lodging.

Expected genetic gains were calculated for various selection procedures and heritabilities were presented.

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The effects of major height and maturity genes on variation for yield was discussed, and separate estimates of genetic variances were calculated for yield after adjustment was made for maturity.

Ph.D. Thesis, 1979
R. P. Cantrell, Professor

ABSTRACT

SELECTION FOR YIELD AND LYSINE CONCENTRATION AMONG OPAQUE P-721 DERIVED GRAIN SORGHUM LINES

Paul Christensen

The expected results of multitrait index selection for yield (YLD) and lysine concentration of sorghum grain, as estimated by acid orange 12, Udy, dye-binding capacity (DBC), are evaluated for a population of F_4 derived lines, homozygous for the P-721 high lysine opaque gene. The lines were tested in the F_5 . The objective for selection is overall improvement of selections from unreplicated nurseries. Optimum selection indexes are evaluated under 4 alternative sorghum grain valuation functions. All value functions give a relative value of 1.0 for normal sorghum, and each gives a relative value of 3.0 at 2 to 4 times normal sorghum DBC, or lysine concentration. The value functions are of the form

$$T = a_{YLD}(YLD) + A_{DBC}(YLD)(DBC-u),$$

where a_{YLD} and A_{DBC} are the relative values for production of nonlysine and lysine components of grain respectively, and where u is a constant.

The first-order approximation to the quadratic merit is good in the relevant value range. Quadratic Index theory contributes to the specification of appropriate approximate first-order values for the measured traits.

Where lysine production received its lowest relative value ($T_1 = 3.0$ when $DBC = 4$ times the normal DBC for sorghum), the optimal index is associated with a decline in expected DBC and an increase in expected yield. Where lysine production received its highest relative value ($T_4 = 3.0$ when $DBC = 2$ times normal), the optimal index is associated with increases in both DBC and yield. By using relative trait values obtained

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form the first-order approximation to the quadratic merit function as index weights one reduces expected gains by less than 10% compared to the optimum index. These relative trait values are good selection index weights.

The reduction in expected gain when protein is removed from the index is only about 2%. Measurement of protein concentration is not necessary in nursery selection. The covariances of protein with DBC and with yield did help specify the best selection index when DBC is relatively valuable.

Optimum selection indexes were associated with reduction in the expected protein concentration. By using restricted selection index theory one can determine indexes which are not expected to reduce protein concentration. These indexes are more likely to increase DBC.

Ph.D. Thesis, 1978
J. D. Axtell, Professor

ABSTRACT

SELECTION FOR GENETIC MODIFIERS THAT IMPROVE THE OPAQUE KERNEL PHENOTYPE OF P-721, HIGH LYSINE SORGHUM (SORGHUM BICOLOR (L.) MOENCH).
Gebisa Ejeta

Vitreous endosperm, high lysine sorghum lines were developed by crossing P-721 opaque with plants in diverse sources of sorghum populations, and by mutation induction using diethyl sulfate (DES).

Approximately 12,000 F₆ heads (from crosses of P-721 opaque with sorghum plants of diverse genetic background) were screened over the light box for vitreous endosperm kernels. A total of 120 heads segregating for vitreous kernel types were selected as possible kernel modifications influenced by so-called modifier genes of the P-721 opaque locus. Further evaluations of opaque and vitreous kernels from these 120 "modified" types resulted in only 15 elite selections with stability in vitreous endosperm phenotype and lysine concentration. There was strong evidence supporting genetic background differences among the family groups evaluated.

Modified endosperm selections were consistently higher in kernel weight and grain yield, and lower in both percent protein and lysine concentration. Total protein yields and total lysine yields of the modified endosperm selections were only 1 percent and 4 percent lower, respectively, than that of their opaque checks.

Kernel weight increased with increase in the degree of kernel modification, with the most vitreous types showing the highest test weight. The trend in percent protein and lysine concentration was in favor of the opaques and the less modified kernel types.

Treatment of seeds of P-721 opaque, high lysine sorghum line with

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diethyl sulfate (DES) also resulted in mutants with vitreous endosperm and high lysine concentration. After five generations of selection, 23 vitreous endosperm, high lysine mutants were identified. Of these, mutants 5589, 5458, and 0226 were found to be stable across generations in kernel phenotype and lysine concentration. On the other hand, a large number of vitreous endosperm mutants showed lysine concentration equivalent to normal sorghums. The reversion to wild-type phenotype (vitreous endosperm and low lysine concentration) of these mutants is speculated to be not in the true reversal of the primary genetic event (a rare occurrence) but possibly in the introduction of an additional mutation somewhere else in the genome masking the expression of the primary mutation, P-721 opaque. These putative suppressor mutants need to be confirmed, however.

In the DES-induced population of mutants, a family of mutant plants with heritable red leaf characteristics was identified. Seeds from the red leaf mutant (5279) plants contain a range in protein concentration varying from 20-28 percent of sample. This red leaf character was found to be due to a single recessive gene.

Analysis of kernels from normal (green leaf) and red leaf mutant plants segregating in an F_2 generation of the cross (PP14(ms_3) x 5279) showed that this mutant is responsible for the high protein concentration in the grain. Two possible explanations for the genetic mechanism involved in the increased concentration of grains from red leaf mutant, 5279, are described.

Both mutation induction and pedigree breeding procedures utilized in this study were effective in generating vitreous endosperm, high lysine sorghum lines. However, the frequency of useful mutants generated from

mutation induction was lower. In addition, the mutation induction approach was more expensive and demanded large number of progenies be evaluated for successful identification of mutants.

Ph. D. Thesis, 1979
J. D. Axtell, Professor

ABSTRACT

EVALUATION OF LEAF ANTHRACNOSE, HEAD BLIGHT AND RED STALK ROT OF SORGHUM
CAUSED BY COLLETOTRICHUM GRAMINICOLA
Alexandra de Silva Ferreira

Of 23 sorghum lines evaluated for resistance to three isolates of Colletotrichum graminicola (Ces.) Wils., nine were susceptible, six moderately resistant, and eight resistant to the leaf blight phase. Some of these lines demonstrated resistance to the head blight phase. The leaf blight phase was more destructive than either the red stalk rot or head blight phases.

No complete resistance to the red stalk rot phase of anthracnose was found, but genotypes varied in the amount of discoloration observed in the pith after inoculation. Resistance to red stalk rot appeared to be independent of the leaf and head blight phases. The stalks of sorghum were not severely rotted and no lodging was observed in the inoculation treatments.

Genotypes varied in their reaction to head inoculation and genotypes could be classified as resistance or susceptible.

Yield in susceptible sorghum genotypes was significantly reduced by leaf blight infection when compared to the non-inoculated control, but yields were not significantly reduced by either the red stalk rot or head blight infections. Yield losses also varied according to genotypes with the head and red stalk rot phases. However, a resistant genotype such as 954206 would be preferable to the highly susceptible genotype 954114.

Sorghum genotypes can be screened for resistance to leaf anthracnose in the greenhouse when plants are inoculated 35 days after planting. The greenhouse inoculations on sorghum lines were highly correlated ($r = 0.87$)

with field inoculations (49 days after planting). The disease was usually more severe in the field than greenhouse. No leaf infection was observed when sorghum plants were inoculated 15 days after planting and a few lesions were found on susceptible genotypes 25 days after planting.

These results indicate that none of the methods are suitable for determining resistance to all three phases of anthracnose, but the leaf blight method is more reliable and can also be used to assay for head blight resistance.

M.S. Thesis, 1979
H. L. Warren, Professor

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ABSTRACT

SORGHUM TANNINS: INHERITANCE, SEASONAL DEVELOPMENT, AND BIOLOGICAL VALUE
Rebecca Hartigan

Sorghum can be separated into three groups according to tannin properties in the seed. Group I sorghum has no tannin and, therefore, no pigmented testa. Group II sorghum grain has tannin extractable only with acidic methanol; group III sorghums contain tannins extractable with absolute methanol as well as acidic methanol. Group II and group III sorghums have a pigmented testa. Group III sorghum tannin developed in the testa throughout the season and remained at maturity. When fed to rats, the group III sorghums significantly reduced weight gain and feed efficiency. Group II sorghums had methanol-extractable and acidic methanol-extractable tannins in the immature seed. The concentration of these tannins was as high in one developing group II variety as in the group II sorghums, but the methanol-extractable tannin disappeared completely by maturity. When fed to rats, group II sorghums gave weight gains and feed efficiencies comparable to the group I sorghums.

Two epistatic genes I_W and I_H controlled tannin characteristics in crosses between group II and group I sorghums. I_W and th produce group II sorghums, tw and I_H produce group III sorghums. I_W is epistatic over I_H and th is epistatic over tw. Additional genes for tannin groupings were indicated in crosses between group III and group I sorghums, but their mode of action is not known.

M.S. Thesis, 1979
J. D. Axtell, Professor

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ABSTRACT

THE INHERITANCE OF RESISTANCE TO COLLETOTRICHUM GRAMINICOLA IN GRAIN SORGHUM, SORGHUM BICOLOR
Ellen Margaret Jones

This study was designed to determine the inheritance of grain sorghum leaf, panicle and grain resistance to the fungal pathogen, Colletotrichum graminicola. Four grain sorghum lines were used in this study: 954206 (R-1), IS 9569 (R-2), 954114 (S-1), and IS 4225 (S-2). All possible resistant by susceptible crosses were made; also the two resistant parents were crossed. The parent R-2 was selected in order to determine if the resistance in this late-maturing line had a genetic component. Leaves of all plants in parent, F_1 , F_2 , F_3 and the first backcross generation (BC_1) were artificially inoculated with a conidial suspension of the pathogen and plants were later given a disease severity rating on leaf, panicle and grain. Segregation patterns from progeny generations were used to postulate genetic models.

Leaf resistance was shown to be controlled by a single completely dominant gene in R-1, while at least two completely dominant, independent genes were present in R-2. No reciprocal differences were noted for the progeny of any cross. It was found that the genetic background of each parent apparently modified the level of leaf susceptibility of its progeny.

Progeny data for panicle and grain disease ratings did not indicate consistent support for any genetic model. A limited quantity of inoculum from leaf lesions as well as unfavorable weather conditions reduced the reliability of disease ratings. However, it was concluded that resistance to panicle and grain infection was dominant to susceptibility, and that relatively few major genes were involved in control of resistance. No

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reciprocal differences were found in progeny data.

Correlation coefficients between diseased parts of F_2 plants were computed. Positive correlations were found between panicle and grain, and between leaf and panicle, but no correlation was found between leaf and grain.

The results of this study indicated that leaf resistance is controlled by relatively few major genes, and therefore, that leaf resistance can be efficiently transferred to other germplasm in a sorghum breeding program. The discovery of at least two different sources of resistance in R-2 offers further breeding opportunities for the incorporation of resistance to C. graminicola.

Ph.D. Thesis,
J. D. Axtell, Professor

ABSTRACT

ROOT AMMONIUM ASSIMILATION ENZYME ACTIVITIES, GRAIN STORAGE PROTEIN AND GRAIN YIELD RESPONSE TO HIGH AMMONIUM FERTILIZER AMONG MAIZE HYBRIDS
Tim Clifford Murphy

A whole-plant hypothesis linking genotypic differences in efficiency of ammonium assimilation and utilization to grain yield under high ammonium fertilizer levels was investigated in genetically diverse maize single cross hybrids. Four hybrids and 5 parental inbreds were grown in the growth chamber and sampled at the 3-leaf stage. Comparisons were made between hybrids and inbreds and within each group for root ammonium assimilating enzyme activity levels, root and shoot dry weights, nitrogen contents, and root protein contents with zero ammonium and 25 mM ammonium treatments.

Glutamate synthase (GOGAT) activity per gram root tissue was significantly higher in inbreds than hybrids in response to 25 mM ammonium but total GOGAT per plant did not differ significantly between inbreds and hybrids.

Glutamate dehydrogenase (GDH) specific activity and activity per plant was significantly higher in hybrids than inbreds as was root and shoot total weight, nitrogen content, and root total protein. Root and shoot nitrogen percents were significantly higher in inbreds.

Glutamine synthetase (GS) did not differ significantly on a per protein or per root tissue basis between hybrids and inbreds. All three enzymes showed increased levels of activity in response to 25 mM ammonium.

Among inbreds response to 25 mM ammonium indicated the root nitrogen percent and root free nitrogen percent correlated significantly negatively with GOGAT specific activity, activity per root tissue and activity per

plant. GDH specific activity and activity per root tissue correlated significantly positively with seedling shoot growth in inbreds.

Among hybrids response to 25 mM ammonium indicated both GOGAT and GDH specific activities correlated highly negatively with shoot total weight at the three leaf stage. Root nitrogen percent correlated significantly negatively with GS specific activity and root growth.

Grain yield in 2 years field trials among the 4 hybrids correlated highly significantly with GOGAT specific activity, activity per gram root tissue and activity per plant in growth chamber seedlings. Grain yield correlated significantly with GDH specific activity and highly positively with GS per gram root tissue. Grain yield also correlated highly significantly negatively with shoot total nitrogen of seedlings. All three activities, GOGAT, GDH, and GS on a per root tissue basis correlated significantly with grain total protein content and highly positively with grain total zein. Grain yield and protein contents were correlated highly negatively with root free nitrogen percent. These results suggest that genotypic ammonium assimilation enzyme differences in activities accentuated under high ammonium stress may correlate with grain yield of genetically elite maize hybrids grown with high ammonium fertilizer in a nearly optimal environment.

Genotypic differences in grain yield and grain total protein, albumin plus globulin, and zein protein fractions among 12 genetically diverse maize single cross hybrids were investigated as sinks for grain dry matter and assimilated organic nitrogen in each of 2 years' yield trials. Yield did not correlate consistently with kernel weight, kernel number, or any of the protein fraction contents among genotypes. However, significant kernel weight differences from the base to the tip of the ears of 12 single cross

hybrids overall did correspond to parallel significant differences in zein storage protein percents. Non-significant differences in non-zein storage percents were found. These results suggest that utilization of the assimilated nitrogen as storage proteins in the grain does not correlate with grain yield among genotypes although the importance of this effect on yield may be confounded with genotypic whole plant differences affecting supply and rate of supply of assimilated carbon and nitrogen to the grain sinks.

Ph.D. Thesis, 1980
J. D. Axtell, Professor

ABSTRACT

MODIFICATION OF THE OPAQUE ENDOSPERM PHENOTYPE OF THE HIGH LYSINE SORGHUM LINE P-721 (SORGHUM BICOLOR (L.) MOENCH), USING THE CHEMICAL MUTAGEN DIETHYL SULFATE
Kay Scott Porter

Acceptance of high lysine sorghum (Sorghum bicolor (L.) Moench) by both growers and consumers has been limited by problems associated with the opaque endosperm of high lysine lines. In this study, vitreous high lysine mutants of sorghum were induced by treating seeds of an opaque high lysine line, P-721, with the chemical mutagen diethyl sulfate (DES). Putative mutants were identified by screening M_2 seeds from individual self-pollinated M_1 heads over a light box. Vitreous segregates in otherwise opaque heads were considered to be possible modified types and were subsequently evaluated for protein quality and degree of vitreousness.

Putative mutants were grown in head rows in the M_3 and M_4 generations and self-pollinated heads were harvested. Individual heads in each row were kept separate at each generation. Non-viable mutants or "mutants" classified as opaque in the M_3 generation were discarded.

Only the most vitreous mutants in each generation were evaluated chemically. The remaining (less vitreous) ones were carried to subsequent generations for analysis. Possible mutants were evaluated for either percent protein and lysine (as percent of protein and percent of sample), or dye-binding capacity (DBC) and percent protein. Vitreous segregates at the M_2 and M_3 generations were compared to opaque samples from the same heads. Mutants at M_4 were compared to opaque P-721 and its normal-lysine, non-opaque sib.

Three hundred and thirty-five vitreous endosperm mutants which were similar to their opaque checks in protein quality were identified on the

basis of initial chemical evaluations. Approximately 70% of the mutants evaluated initially in either the M_2 , M_3 or M_4 generations had lysine as percent of protein greater than 2.5%.

Mutants were evaluated in subsequent generations to assess the stability of both protein quality and modified endosperm phenotype. Fifty-six mutants were superior in protein quality (lysine greater than 2.5% or DBC greater than 40) in at least two generations. Thirteen of the 56 mutants were homozygous vitreous at M_4 and were selected. Lysine content (percent of protein) of the selected mutants averaged 2.65% at M_3 and 2.61% at M_4 . Lysine as percent of sample averaged 0.36 in M_3 , but reduced protein in all mutants at M_4 resulted in slightly lower values (0.29) in that generation.

Mutants varied considerably in the degree of vitreousness in the endosperm. Kernel maturity (influenced by the duration of the growing season) seemed to be the factor most important in altering the degree of vitreousness. Immature kernels were always less vitreous than fully mature kernels which may have resulted in the discarding of later maturing types.

Most modified mutants with protein quality approaching that of their opaque counterparts were intermediate in vitreousness. A high degree of vitreousness was most often associated with relatively poorer protein quality although exceptions were found. Of the 13 homozygous vitreous mutants selected, two were similar in appearance to the non-opaque, normal sib of P-721. Seven mutants were intermediate in the degree of vitreousness and four were only slightly modified. Mutants having slightly poorer protein quality but with more vitreous phenotypes were also selected.

Mutation induction was effective in generating a large number of

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mutants in an opaque, high lysine background. Mutants approaching opaque in protein quality (lysine content) but with modified, vitreous endosperms, were selected for use in conventional breeding programs. Such mutants may prove useful in overcoming some of the problems associated with the soft opaque kernel phenotypes, and may improve grower and consumer acceptance of grain sorghums with high protein quality.

Ph.D. Thesis, 1977
J. D. Axtell, Professor

ABSTRACT

VARIATION FOR STALK CHARACTERISTICS AND THEIR ASSOCIATION WITH LODGING AND HEAD WEIGHT IN A DIVERSE SORGHUM POPULATION.
Thomas Prest

Variation for stalk characteristics and their association with lodging and head weight were analyzed in a diverse sorghum population, PP9, using 80 S₂ lines. These lines were categorized into three height groups, the tall (greater than 170 cm), medium (110-170 cm), and short (less than 110 cm). The stalk characteristics studied included, rind thickness, rind puncture, third internode density, third internode diameter, and stalk juiciness. The stalk measurements were taken at physiological maturity.

Significant differences between lines within height groups were observed for internode diameter, density, and rind thickness. Differences in rind puncture between lines in the medium and short groups were significant in 1977 but not in 1978, whereas differences in rind puncture in the tall group were significant in 1978 but not in 1977.

Significant differences in lower stalk breakage were observed between lines within the medium and tall groups. Such breakage was negligible in the short group. However, differences in peduncle breakage were nearly significant.

Third internode diameter, rind thickness, and third internode density had significant negative correlations with lower stalk breakage in the tall group. Within the same group, rind puncture was not significantly correlated with stalk breakage.

Third internode diameter and rind thickness showed significant negative correlations with lodging in the medium group. Rind puncture and

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Internode density were not significantly correlated with lodging.

None of the characteristics of the lower internodes were significantly correlated with peduncle breakage in the short group.

The correlations were generally low in all groups. Thus, the given stalk measurements, when taken at physiological maturity, should not be considered as equal replacements for field estimates taken later on in the season. However, selection for increased diameter and/or increased rind thickness may still be effective in increasing lodging resistance.

Lower stalk breakage was not significantly correlated with head weight in either the tall or medium groups. Correlations between head weight and the stalk characteristics under study were generally not significant. Pertaining to PP9, these data suggest that recurrent selection for increased yield without selection for lodging resistance may not decrease lodging resistance in the selected populations. Concurrent selection for both traits is still advised.

M.S. Thesis, 1979
R. P. Cantrell, Professor

ABSTRACT

THE EFFECT OF TANNINS ON NUTRITIONAL QUALITY OF DRY BEANS, PHASEOLUS VULGARIS L.

Richard Ronnenkamp

Tannin compounds are found in many foods and often are associated with a bitter taste. In sorghum, tannins are a factor in the nutritional quality of the grains. Dry beans have toxic factors that influence the nutritional value, but tannins are not included.

From the dry bean collection, 69 entries were selected from a variety of locations and seed color. The entries were analyzed for tannin content. A white, black, and red bean were selected for low, medium, and high tannin content. These beans were used to prepare eight diets for evaluation with weanling rats. The testae were removed or extra testae were added to change the tannin content of the diets. The influence of tannins on the albumin and globulin portion of the protein for the beans used in the diets and for other sources of white, black, and red beans was determined. The association of tannin content with color was observed in the F_2 generation of crosses of white and red beans.

A range of tannin content was found in the entries. White beans were zero and colored beans ranged from very low to 2.5 catechin equivalents. The highest values were found in red beans. The weight gain and protein efficiency ratio of rats was improved when the red testa of the red bean was replaced with a white testa and the tannin content was reduced from 2.5 CE to almost zero. The weight gain and protein efficiency ratio of rats decreased as the tannin content of the beans in the diets increased.

The tannin content did not influence the distribution of the albumins and globulin fractions of the bean protein, but changes in these fractions

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were observed between bean types.

The use of initial rat weight as a covariate in analyzing the data was significant for the dependent variables weight gain and food consumption, but not for the protein efficiency ratio.

The F_2 segregation of tannin content was not controlled by the genes for testa color. A range of high and low values was observed within a given color.

Ph.D. Thesis, 1977
J. R. Wilcox, Professor

ABSTRACT

INFLUENCE OF SEED PROTECTANT ON THE TOLERANCE OF GRAIN SORGHUM TO ALACHLOR AND RELATED COMPOUNDS

Joao B. da Silva

The advent of herbicidal antidotes has opened a new field of research and created possibilities for the use of herbicides in grain sorghum (Sorghum bicolor (L.) Moench) that otherwise would be unsafe for the crop. The influence of the seed treatment with 1,8-naphthalic anhydride on the tolerance of three sorghum hybrids, RS-610, B-35 and NK-180, to three triazine herbicides, EPTC, either alone or in combination with the herbicidal antidote R-25788 (N,N-diallyl-2,2-dichloroacetamide), alachlor, metolachlor, propachlor and four experimental chloroacetamide herbicides, was studied in nine field experiments carried out in the 1975-1977 period, in three different locations of the state of Indiana. The influence of other factors such as soil type, soil temperature, sources and means of protection, herbicidal rates and seed protectant rates on the tolerance of the crop to alachlor was studied in four greenhouse experiments.

Results obtained from the field experiments allowed many important observations concerning the use of 1,8-naphthalic anhydride as a seed treatment to protect grain sorghum:

1. Independently of the seed treatment, damage to grain sorghum caused by alachlor varied with the rate employed, and means of application, the soil type and among sorghum hybrids. When grown on the Chalmers Silty Clay Loam, RS-610 showed more tolerance to alachlor than both B-35 and NK-180.

2. Metolachlor and RE-19790

[3-chloroacetyl-4,4-cyclohexane-spiro-(2,2-dimethyl-1,3-oxazolidine)] were

the most promising herbicides among the new chloroacetamides in both weed control and selectivity to grain sorghum. GCP-5544

(N-benzyl-N-isopropyltrimethyl acetamide) was the most selective to the crop but it failed to control weeds especially redroot pigweed (Amaranthus retroflexus L.).

3. Atrazine and Cyanazine, applied alone or in combination with propachlor did not cause any significant injury to grain sorghum and they were unaffected by the seed treatment.

4. R-25788 (N,N-diallyl-2,2-dichloroacetamide) did not give any significant protection to grain sorghum when applied in tank mixture with EPTC. This herbicide was always injurious to the crop and its damaging action was partially hindered by the seed treatment with naphthalic anhydride. Although the protective action of the seed treatment was statistically significant, it was not great enough to give sorghum plants conditions to produce as much as the handweed plots in many cases.

5. As a general rule the seed treatment with naphthalic anhydride was beneficial any time its protective action was necessary. This was especially true for the chloroacetamide herbicides which were not injurious to grain sorghum all the times. When its protective action was not required, the effect of the seed treatment was unapparent or prejudicial. This negative action of naphthalic anhydride was noticed at least in three different cases, all three recorded on the Bedford Silt Loam.

Experiments carried out in the greenhouse were complementary to the field experiments and corroborated field observations. The trials provided information on the influence of soil temperature which was found to affect

the herbicide but not the seed treatment with naphthalic anhydride, and they also showed that sorghum protection from alachlor can be obtained from both naphthalic anhydride and R-25788 but only as a seed treatment.

Ph.D. Thesis, 1977
J. L. Williams, Jr., Professor

ABSTRACT

AGRONOMIC AND BIOCHEMICAL EVALUATION OF THE SORGHUM (SORGHUM BICOLOR (L.) MOENCH) HIGH-LYSINE P-721 OPAQUE GENE IN ISOGENIC AND DIVERSE GENETIC BACKGROUNDS

Stephen VanScoyoc

Yield and accumulation of panicle seed weight, number of seeds, seed weight, volume, and density, and black layer formation were compared during development of endosperm mutant P-721 (P-721-0), its normal sib line (P-721-N) and hybrids RS671 and NK300 grown at 4 populations. Performance under a stress environment was poorer and yield was reduced 15-20% in P-721-0 compared with P-721-N. Yield reduction was attributed largely to smaller seed weight and density. Increased population acted as a stress environment and resulted in decreases in seed weight per panicle, seed numbers and panicles per plants for lines and hybrids. One hundred seed volume, weight and density were unaffected by increased population. Completion of black layer formation in all seeds coincided with physiological maturity, as indicated by maximum seed weight per panicle, in P-721-N at the lowest population but not in P-721-0. At high populations, date of completion of black layer was not a good predictor of maximum seed weight accumulation for any of the lines or hybrids.

F₆ opaque endosperm lines derived from crosses of P-721-0 with vitreous lines, hybrids and populations and selected for yield, dye binding capacity, lodging and disease resistance as well as food grain quality were compared in a one year trial with 12 high-yielding, widely adapted vitreous endosperm controls. Eighteen opaque lines had yields equivalent to the vitreous controls (above 7000 kg/ha dry weight) while containing, on the average, one percent more protein and 0.5% more lysine as a percent of

protein. Improvement in yield of the best P-721-0 derived opaque lines was due to increases in all yield components and not to height.

Changes in seed weight, protein, oil, and amino acid profiles; and the proportions, amino acid patterns and electrophoretic mobility of Landry-Moureaux protein fractions of developing endosperms of P-721-0 and P-721-N grown at a low population were followed at weekly intervals from 10-59 days after half-bloom (DAHB).

Patterns of seed weight, volume and nitrogen content were similar to those of accumulation of seed weight per panicle. Levels in both lines increased steadily from 10 to 31 days, at which time rates were reduced for P-721-0 but not P-721-N. By 38 DAHB, accumulation had ceased in P-721-0 but continued through 45 DAHB for P-721-N. Whole seed protein content did not change during development but P-721-0 endosperm protein increased 1.2% while that of P-721-N fell 0.7%.

Landry-Moureaux fraction I albumins and globulins differed little among lines. Fraction II plus III total kafirin accumulation plateaued at 24 DAHB for P-721-0 but continued to increase rapidly through 38 DAHB for P-721-N. Fraction IV plus V total glutelin synthesis ceased beyond 24 DAHB for P-721-N but increased steadily through 45 DAHB for P-721-0 when expressed either on a percent of total protein or on a N per endosperm basis.

Shifts in Landry-Moureaux protein fraction amino acid profiles during endosperm development were generally small and did not differ greatly among lines. Lysine content was highest in fraction I, intermediate in fractions IV and V and lowest in fraction II and III.

SDS-PAGE of endosperm protein fractions during development indicated few or no differences in banding patterns for any of the fractions and only

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occasional differences in staining intensities between line bands. Ten DAHB whole seed patterns were, however, very different from those later times in development for all fractions. Kafirin bands were absent at 10 DAHB.

Optimal conditions for a rapid fractionation technique for sorghum endosperms were established. This method would be useful in a screening program for mutants with high lysine content of glutelin.

Ph.D. Thesis, 1979
J.D. Axtell, Professor

ABSTRACT

RELATIONSHIP OF TANNIN AND SEED COLOR TO SEEDLING VIGOR IN SORGHUM BICOLOR
(L.) MOENCH
Billy Woodruff

A 2-year study was conducted to determine the relationship of tannin and seed color with germination and emergence. Fifty-seven lines were selected from the World Sorghum Collection based on tannin content and seed color. Seedling emergency under field and laboratory conditions was used as a measurement of seedling vigor. Warm germination percentages of high tannin and dark brown-seeded lines of seed harvested in 1975 were equal to seed harvested in 1977. Germination of 1977 seed showed no difference between tannin or color groups. High tannin lines of 1975 seed had a significantly higher emergence than low tannin lines. High and low tannin lines of 1977 seed were not significantly different in seedling vigor but medium tannin lines had a significantly lower vigor. Dark brown seeds were higher in emergence than light brown or nonbrown seeds under cool, wet conditions. Significant differences were observed among lines within groups for seedling vigor. Based on this data, the cold testing of dark brown-seeded lines might serve as a starting point in a breeding program interested in screening a large number of lines for increased seedling vigor.

M. S. Thesis, 1978
R. P. Cantrell, Professor

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ABSTRACT

INHERITANCE OF TANNIN QUANTITY IN SORGHUM
Billy Joe Woodruff

The vanillin assay was used to estimate the tannin content of 13 F_2 populations. The analysis revealed an intermediate to high broad-sense heritability for tannin quantity. Similar F_2 segregation patterns and F_1 's in many crosses suggested a few genes. High tannin was found to be dominant to low tannin. A very high tannin line indicated the presence of at least one partially dominant gene. Classification of the F_2 based on the parental and F_1 ranges of catechin equivalents suggested one or two genes were segregating in most crosses, although different gene loci or alleles appeared to exist. Fitting the expected distribution within each phenotypic class to the observed F_2 distributions indicated one or two gene models were inadequate to explain the data.

Ph.D. Thesis, 1980
R. P. Cantrell, Professor

G. SEMINARS, CONFERENCES, NEWSLETTER CONTRIBUTIONS, AND INVITED LECTURES

Breeding for Improved Nutritional Quality. Plenary Invited Paper presented at Plant Breeding Symposium II, Iowa State University, March 12-16, 1979. (In Press, Iowa State University Press, 1980).

Elkin, R. G. "Influence of sorghum grain tannins on methionine utilization in chicks." Poultry Science Association meetings, Auburn, Alabama, August 8-12, 1977.

Featherston, W. R. "Studies on the utilization by chicks of sorghum grains with varying tannin contents." XVI World's Poultry Congress, Rio de Janeiro, Brazil, September 17-21, 1978.

Rogler, J. C. "Effects of high tannin sorghum, tannic acid, catechin and sorghum tannin extracts on the performance of young chicks." XVI World's Poultry Congress, Rio de Janeiro, Brazil, September 17-21, 1978.

Featherston, W. R. "Sorghum grain tannins and protein utilization." AGRISCOPE, WTHR Channel 13, March 1, 1978.

Price, M. L., A. Hagerman, S. VanScoyoc, and L. G. Butler "Improvements in analytical techniques for rapid determination of the tannin content of sorghum." Reunion Internacional de Sorgo, Buenos Aires, March 1978, published in Proceedings, p. 428-450.

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"Overcoming the Antinutritional Effects of Tannin in Sorghum", Conference of General Collaborators, Northern Regional Research Center, USDA, Peoria, Illinois, October 24, 1978.

"Tannins in Sorghum Grain: Problems, Solutions, and Opportunities", American Seed Trade Assoc., Hybrid Corn and Sorghum Research Conference, Chicago, December 14, 1978. (text published in Proceedings, Publication No. 33, p. 190-201).

"Improving the Nutritional Quality of High Tannin Sorghum", 11th Biennial Sorghum Utilization Conference, Wichita, Kansas, February 28, 1979.

"Biochemical Effects of Sorghum Polyphenols", Symposium on Polyphenols in Cereals and Legumes, 39th Annual Meeting, Institute of Food

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Butler, L. G., A. E. Hagerman and M. L. Price, Analysis of Tannins in Sorghum, Proceedings of Eleventh Biennial Grain Sorghum Research and Utilization Conference, Wichita, Kansas, March 1979, p. 31.

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DATE: March 15, 1979

memorandum

REPLY TO
ATTN OF:

DS/AGR/FCP, Robert I. Jackson

SUBJECT:

Status Report of Sorghum/Millet Research

TO: DS/RDM, Dr. Miloslav Rechcigl

Three universities are conducting research on sorghum and one on millet under AID contracts. All of the research under these four contracts will be transferred to the Collaborative Research Support Program for Grain Sorghum and Pearl Millet (CRSP-GS/PM) once the CRSP becomes operational.

1. Purdue University initiated the Research Project, "Inheritance and Improvement of Protein Quality and Content in Sorghum bicolor (L.) Moench", in 1966. The contract is now being extended for six months, through September 30, 1979 at a cost of \$172,000.

Considerable progress has been made in the development of techniques which reduce the tannin score to low levels as assayed by tests. Chemical treatment with small amounts of mild aqueous alkali effectively alleviates the antinutritional properties associated with tannin, but more conventional cooking processes apparently do not. These conclusions are somewhat tentative, being based on studies with only a few sorghum varieties, and must be evaluated more thoroughly during the extension period. Preliminary studies with the sorghum types which contain tannin, extractable only with acidic solvents suggest that the antinutritional effects of this type are not significant. Some of the sorghum varieties of this type contain high amounts of tannin during seed development, and Purdue is evaluating their bird resistance in extensive trials in this winter's nursery in Puerto Rico in collaboration with the Denver Wildlife Center.

Sorghum lines which have improved protein quality and also a vitreous (hard) endosperm needed for human acceptance are now available and need to be incorporated into advanced breeding materials.

2. The University of Nebraska has just completed its fifth year of the AID contract for "Sorghum Yield Physiology". The contract has been extended to August 1979 when it will become part of the CRSP-GS/PM.

The principal research efforts of the contractor have included the following:



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- a. Continued evaluation of breeding methodology for use with random mating populations, $-S_1$ progeny testing appears best.
- b. Incorporation of greenbug resistance, twin seeded character and yellow endosperm into one population.
- c. Demonstrated minimum till sorghum yields equal conventional till yields with fewer trips.
- d. Determined that genotypes selected for drought tolerance were the least adversely affected by drought and responded the best to a single water application at bloom.
- e. A new water gradient application system was initiated to evaluate plant reaction to different levels of water stress.
- f. Differences in mineral uptake and utilization efficiency were demonstrated. P-efficient plants grew well under phosphorus stress and Al-toxicity conditions. P-efficient plants did not do well under Fe and Cu stress conditions. Martin and KS35 were determined to be P-efficient.
- g. Further developed and confirmed the utility of the germination and early growth cool temperature screening test.

3. The Project, "Development of Improved High Yielding Sorghum Cultivars with Disease and Insect Resistance" is carried out by Texas A & M University. This contract has also been extended for six months at which time it will become a part of the CRSP-GS/PM.

Elite sources of germplasm generated in the Texas programs from introduced, converted and partially converted exotic sorghums were distributed to sorghum workers throughout the world. Disease and insect resistant, dwarf height, photoperiod insensitive sorghums were combined in random mating populations both for easy distribution to sorghum improvement programs in LDCs and as research tools for improvement of grain sorghum in temperate regions. Development of greenbug resistant hybrids, the discovery of high levels of midge resistance and possible resistance to spider mites are new major breakthroughs in sorghum improvement. Some of these disease and insect resistant sorghums have extraordinary general adaptation. During the past year, several lines and hybrids possessing tropical adaptation were grown in Africa and South America. The potential for these types of sorghums is excellent.

Kansas State University has recently completed its first contract year for the project "Improvement of Pearl Millet". This contract will continue through September 1980.

The main accomplishments from this past year are:

a. Development of several millet populations, composites and dwarf, early-maturing hybrids and inbred lines. Several dwarf millet hybrids with maturity requirements similar to the sorghum checks produced yields, plant heights and lodging scores comparable to hybrid grain sorghum.

b. Data from investigations on water use and leaf temperature of millet from one season indicate that the genotypes having the highest leaf temperatures produce the greatest grain yield.

c. Preliminary determinations of the nutritional quality and digestibility of pearl millet grain by analyzing protein in germplasm introductions and determining magnitudes of variation in lysine content were made. These investigations will be continued.

d. A bulk population of millet was used to determine the susceptibility of infestation by certain species of stored grain insects. Of the 15 major insect pests used in the test, rice and maize weevils were responsible for the greatest loss, 44 and 43 percent.

Inheritance and Improvement of Protein
Quality and Content in Sorghum bicolor - Purdue
University

The report of Dr. Peterson, Chairman of the subcommittee that included Drs. Ehrenreich, Moss, and Mr. Ludington was summarized as follows:

The request is for an extension of a project initiated in 1966 with Purdue University. The project has had periodic review and previous extensions with total expenditures of \$2,684,486 up to March, 1978. It is (or was) coordinated with three other AID sponsored sorghum improvement projects, one with the University of Nebraska initiated in 1974 with expenditure commitments to 1980 totaling \$683,000; one with Texas A & M University funded in 1980 for \$884,946; and one with the University of Puerto Rico which was terminated in May, 1977 and with expenditures of \$214,700.

If the proposed extension is approved to March 31, 1979 as requested, AID's investment in sorghum breeding by 1980 will total \$5,397,525. Original objectives of the research have been covered several times in the past and will not be repeated here. Also, since this project was started in 1966, ICRISAT had grown about 15,000 accessions of sorghum, had identified some of the better lines for regional testing, the emphasis being on drought tolerance, quality for human food, and insect resistance.

As some of the RAC members will recall, the early excitement over discovery of high lysine lines was dampened somewhat by the problems of the floury, opaque endosperm of high lysine lines. Problems were somewhat analogous to those encountered with maize

high lysine lines. By means of induced mutations, the Purdue investigators have identified a few mutants that have both improved protein quality and vitreous grains. Three such mutants were identified as having high lysine, a vitreous endosperm, and good agronomic characteristics.

A survey of Ethiopian lines has turned up a line with 20 percent protein, with normal appearing plump seeds. The problem is to incorporate these quality characteristics with good agronomic types. Crosses for this purpose have been made and some lines are ready for evaluation at ICRISAT and in Brazil. Also a high lysine random-mating population is being prepared for distribution and use in recurrent selection programs in LDC's. The negative correlation between yield and protein content is almost universal in cereals and so the transfer of high protein to high yielding varieties is questionable.

The problem of tannins in sorghums, although long known, has been extensively studied as a part of this project. The problem as RAC may recall is that the tannins are beneficial in providing some measure of bird control, a very serious problem where sorghums are grown. However, these compounds are nutritionally harmful in reducing digestibility of the proteins, and as such, tend to off-set benefits from breeding for higher protein values. These are phenolic compounds of which there are many and possibly of variable reactivity with proteins. Accordingly, there is a problem of identifying the various fractions, determining their anti-nutritional qualities, and finding genetic or chemical means for reducing their harmful effects.

Objectives of the proposed extension of the project are as follows:

1. Identify and evaluate sorghum lines or mutants with improved nutritional quality using both chemical and biological methods (Six separate activities are proposed under this objective).
2. Identify the chemical nature and composition of the protein fractions of selected high lysine sorghum genotypes and correlate the grain fraction composition with nutritional quality. Also investigate the biochemistry of sorghum tannins and their interactions with sorghum proteins. Develop simple processing techniques to alleviate the anti-nutritional properties of tannins in grain sorghum. (Six different activities under this objective).
3. Determine the nutritional value of sorghum varieties differing in amino acid composition, carbohydrate composition, and/or tannin content for the monogastric animal. (Chicks and rats) (5 activities).

The issues regarding the proposed extensions include the following

1. Have the investigators made satisfactory progress considering the duration and level of funding?
2. Will the extension to March, 1979 see the project through to a satisfactory conclusion?
3. If so, to what use will be the knowledge and materials produced from this project?

Response to these questions

1. Is progress satisfactory?

As RAC will recall, the project got off to a questionable beginning with a lot of activity but not much direction or sharply focused goals. This was corrected after a few years with a reorganization of the project. To the best of my knowledge, everyone has been well satisfied with the leadership and direction of the project although progress has been slower than anticipated. Mr. Ludington, in particular, questions the advisability of future support of research in breeding for quality, at least as it relates to amino acid balance. The problem is not a limitation of the investigators or the support, but that increasing lysine in the protein continues to have very difficult side-effects.

2. Can the project be brought to a satisfactory conclusion in one or more year?

This seems very doubtful, but of course, plant breeding tends to be a never-ending activity. On the basis of the written reports, it seems reasonable that the breeding materials could be turned over to ICRISAT after another year but they are not in a position to evaluate lines for protein quality at present. Inheritance studies for protein quality also require several years but these studies have already begun. The studies of various phenolic compounds and their anti-nutritional qualities would appear to require several years to complete.

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3. Future value and use of the project.

I have always been troubled by the fact that Purdue is well outside the general adaptation of grain sorghums. Therefore, inclusion of Nebraska and Texas in the project seemed a desirable step. Funneling materials from Purdue to these states and to ICRISAT therefore helped correct this deficiency. The Nebraska and Texas projects have two more years. It appears that the one-year extension would be useful in getting these materials transferred to these on-going sorghum breeding programs. However, facilities for protein quality and phenolic compound studies are at Purdue. Although the method developed at Purdue could be relocated elsewhere, it would be at considerable expense in time, equipment, and methodology.

It might be necessary to continue longer some of the analytical work at Purdue on protein quality and phenolic compounds but it would seem desirable to phase out the breeding work by the end of another year. On this basis, a motion to approve the extension seems in order.

Dr. Ehrenreich supported the one year extension of the project. However, he asked the AID staff to provide information on several points: 1) why if the AA/TA approved the termination of the project in March 1978, is a one year extension requested? 2) why does the experimental design not anticipate the closing of the project? 3) the budget asks for \$161,000. How are these approximately eight science man/years accounted? and 4) is this request a means of bridging the gap until Title XII funding becomes available?

Dr. Moss added that \$87,000 was included for the identification of mutants. How long will we continue to work with amino acids? Enough has been invested in this area to pursue more work in tannins. It would be logical to cut the budget.

Mr. Ludington suggested that the book should be closed on research in tannins. He suggested that Dr. Burns from Southwest Georgia University, who is the leading authority in this field, be contacted for advice. Drs. Schweigert and Peterson thought there was yet much research to be done on tannins. The technical appendix to the project proposal, which references Dr. Burns, leaves that impression.

Dr. Long (DSB) thought the problem of the one year extension would be clarified when BIFAD decides what kind of sorghum-millet program will make sense. This based on the assumption that a group of institutions will continue working on sorghum.

Dr. Yohe (DS/AGR/CP) explained that with sorghum there is no problem of shrunken endosperm as in barley. In less optimal conditions, yield would not be less for improved lysine content. Several donors are interested in investing money in the tannin problems. For some reason, grain with high tannin content appears to be resistant to the quela bird attack. There is a need to understand this to a greater extent. Purdue expected to ask for a Title XII grant. The one year extension would enable Purdue to bridge the gap. Purdue expects to carry out 12.5 man years of research.

Dr. Yohe remarked that he had requested Purdue to develop a state-of-the-art paper. They probably drew on available resources and therefore, is not exhaustive. Some RAC members agreed that the study was not exhaustive.

Dr. Peterson proposed the following motion:

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Motion:

That the proposal be approved as submitted for funding for one year.

Dr. Moss pointed out that AID has probably supported work on amino acids in cereals long enough. He suggested that additional protein from other foods, bean, for example, might have greater payoff.

Dr. Peterson commented that in monsoon countries sorghum is important and that a sorghum variety improved for its amino acid content probably would be a better approach than working with beans or other food sources.

Vote: Motion was approved with one negative vote.

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UNITED STATES GOVERNMENT

Memorandum

TO : TA/RES, Miloslav Rechcigl, Jr.

DATE: February 3, 1977

FROM : TA/AGR, Leon F. Hesser *LFH*

SUBJECT: Report for March 31-April 1, 1977 - RAC Meeting, Purdue Maize Research

RAC, at its meeting March 20-21, 1975, specified that "a progress report is to be made at the end of two years" on the maize protein research project, contracted with Purdue University.

The attached report is submitted to meet this requirement. It was prepared by our Crops Division and is based on a review of the project at Purdue, December 13-14, 1976, as well as on the most recent annual reports.

Enclosure
a/s



5010-110

Buy U.S. Savings Bonds Regularly on the Payroll Savings Plan

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Report on Project "Inheritance and Improvement of Protein Quality in Maize"
- Purdue University. Extension approved at RAC meeting, March 20-21, 1975.
Contract in force until March 31, 1978.

- - - - -

At the RAC meeting referenced above, the following recommendations were included in the approval motion:

1. Emphasis on genetic studies should be increased, particularly of double and multiple combinations of endosperm types.
2. The breeding work might be decreased.
3. Genetic materials should be made freely available.
4. A progress report is to be made at the end of two years.
5. The project should be phased out by the end of the three-year extension.

This report is being presented to satisfy the requirement indicated in 4.

The project was most recently reviewed by a team from AID/W on December 13-14, 1976. An annual report, covering work during the period April 1, 1975 - March 31, 1976 is on file and available for examination.

A. Genetic, Developmental, and Biochemical Studies.

1. Double Mutant Types.

Special emphasis has been placed on study of endosperm mutant combinations, particularly the opaque-2 + Sugary-2 double mutant. Lysine content of the double-mutant is as high or higher than that of the single-mutant opaque-2 types, and the endosperm texture is more satisfactory. However, the double-mutant kernels are even smaller, compared with fully normal types, than are the o₂ kernels. There appear to be some differences in this weight loss in different backgrounds, but on the average, o₂ types are about 85 per cent of normal and su₂ o₂ types about 82 per cent of normal in kernel weight.

Further studies have shown that this decrease in kernel weight is the result of early termination of grain filling (dry matter increase) in the o₂ and su₂ o₂ types. Whereas normal endosperm types, under the usual conditions in Central Indiana increase in weight of dry matter until about 50 days after pollination, the o₂ and su₂ o₂ types

slow down in weight increase after about 38-40 days, and kernel filling stops before the normal time. This suggests that the observed decrease in kernel size (and resulting yield decrease) is a developmental phenomenon, which may be very difficult to overcome by breeding procedures.

2. Genetic and Environmental Stability of Modified and Non-modified Synthetics.

Genetic and environmental stability was studied in several opaque-2 synthetics. High and low lysine versions of HMO were selected for modification, and Temperate A and Temperate B synthetics were selected for both opacity and modification giving eight different synthetic versions. Plant characteristics measured were ear height, per cent lodged plants, and ear yield per plant. Kernel characteristics measured were per cent modified kernels, modification index, weight, and volume of 100 kernels, specific gravity, and per cent protein and per cent lysine in protein on a whole kernel basis. The following conclusions were made:

- a. A location effect was found for per cent modified kernels and modification index. Values were higher at locations where cooler temperatures prevailed. Kernel weight and volume and lysine content were higher at Lafayette than at the other two locations.
- b. The interactions location x population and planting date x synthetic were significant for both kernel weight and volume.
- c. Selection for higher lysine content in HMO synthetics was effective, and this difference was constant throughout the subsequent random mating generations. Lysine values were also constant through these generations.
- d. Selection for higher lysine content resulted in decreased protein content, frequency of modified kernels, modification index, and specific gravity, and increased kernel volume.
- e. Selection for higher modification was effective in increasing both per cent of modified kernels and modification index in all synthetics.

- f. Selection for higher modification resulted in increased specific gravity and decreased kernel volume in all synthetics. Kernel weight was decreased as an effect of selection for high modification only in the HMO synthetics.
- g. Lysine in protein was decreased as an effect of selection for higher modification and the change was largest in the Temperate A and B synthetics.

3. Nitrogen Distribution and Amino Acid Composition.

The endosperm proteins of inbred C103 near isogenic for su_2 , o_2 , and su_2o_2 and the normal counterpart were fractionated using the Landry-Moreaux procedure. Normal and su_2 had high zein levels (49.9% and 43.8%, respectively), whereas o_2 and su_2o_2 had low zein levels (18.8% and 13.2%, respectively). Opaque-2 had a high albumin and globulin level (24.1%) and su_2o_2 had a high glutelin level (34.2%). The lysine levels of the mutants was explained by the relative abundance of the different protein fractions and not by changes in the amino acid composition within the protein fractions.

4. Characterization of Key Proteins in High Lysine Endosperms.

A detailed electrophoretic characterization of the polypeptides in the endosperm of normal, opaque-2 and double mutants with opaque-2 has indicated significant differences between the zein and zein-like polypeptides of these mutants.

Two polypeptides in Fraction II and one in Fraction III are of special interest because of their amounts in opaque-2 and opaque-2 genotypes. Preliminary efforts have been made to obtain purified preparations of these polypeptides in quantities large enough to determine their chemical composition, and the results have been encouraging. These polypeptides will now be isolated by a combination of ion exchange and gel filtration chromatography. The purified polypeptides will be characterized by amino acid analysis.

B. Breeding Work.

The development of composites and other source material carrying the o_2 or double-mutant factors, has been deemphasized in the past two years. The stocks already developed have been maintained and are being further selected for improved agronomic characteristics.

Six related single crosses from the diallel among Oh43, W64A, B37, and C103 inbreds and the three-way hybrid (Oh43 x B37) x C103 each near isogenic for the o_2 , su_2o_2 and their normal counterparts were evaluated for agronomic yield in a replicated yield test on the Purdue Agronomy Farm, in only one season. Three normal commercial single crosses and their o_2 counterpart hybrids were also grown as checks. The six related single crosses and the three-way normal hybrid ranged from 144 to 187 bu/acre; the opaque hybrids ranged from 113-163 bu/acre; and the sugary-2 opaque-2 hybrids ranged from 113-143 bu/acre. The commercial normal hybrids ranged from 171 to 206 bu acre and their counterpart opaque-2 hybrids ranged from 136-177 bu/acre. Over all the opaque-2 hybrids ranged from 74 to 93% of their normal counterpart hybrids, while sugary-2 opaque-2 hybrids ranged from 74 to 87% of their normal counterpart hybrids. The mean protein per cent over all the hybrids for the normal, opaque-2, and sugary-2 opaque-2 near isogenic varieties was 9.523, 9.831, and 10.584%, respectively; while mean lysine as a per cent of protein values were 2.99, 4.29, and 4.50; and the mean lysine as a per cent of sample values were 0.285, 0.419, and 0.476 for normal, opaque-2, and sugary-2 opaque-2, respectively. In each case the genotypes were significantly different at the 0.01% level.

C. Distribution of Germ Plasm.

Source germ plasm of modified opaque-2 of HMo $_2$ Synthetic, Mod. Syn. Ao $_2$, Mod. Syn. Bo $_2$, Temp HAO $_2$, Temp HBO $_2$, Colus o_2 , Temp HASu $_2o_2$. Temp HBSu $_2o_2$ materials are available and have been distributed to many individuals representing several countries. In addition, Temp HAWx o_2 , Temp HBWx o_2 , Temp HAbt $_2o_2$, and Temp HBbt $_2o_2$ materials are available as source germ plasm. Genetic materials of inbred line conversions of 27 elite inbreds to opaque-2, sugary-2, and sugary-2 opaque-2 will be made available and a planned release is proposed. Some of these materials have already been formally released. Genetic materials of opaque-2 and sugary-2 have been made freely available in a few inbred backgrounds.

D. Coordination with CIMMYT.

Purdue project personnel have continued to hold coordination meetings with CIMMYT on an annual basis. The most recent joint session was held at CIMMYT Headquarters during the last week of January 1977. Present and future cooperation in research were discussed, with emphasis on use by CIMMYT of a modified o_2 system in breeding maize for developing countries. There was also discussion of possibilities for developing a coordinated, interdisciplinary program for consideration by the Title XII Board. The proposed program would include participation by DC institutions and would involve outreach activities as well as research.

E. Status of Knowledge.

It is clear that the opaque-2 system, including various modifying factors, can be utilized to sharply enhance lysine content of maize grain, and it is also clear that this enhanced amino acid value makes maize a more satisfactory cereal for nutrition of monogastric animals, including humans. Agronomic disadvantages, especially those intrinsically related to the o_2 character, make it likely that the use of high-lysine maize with its improved nutritional value must be considered as a specialty crop for certain LDC situations where maize is a staple of the human diet.

Though the yields of "modified" opaque-2 and sugary-2 opaque-2 are not comparable to normal counterpart materials, nonetheless they could play an important role in helping to alleviate human nutritional problems in selected areas of the world.

F. Further Research Needs.

Basic research is needed to generate information on the molecular and developmental biology of maize relative to protein synthesis. Future areas of research on protein synthesis mechanisms should include the molecular biology of cereal grain protein synthesis, amino acid biosynthetic pathways, and storage protein synthetic mechanisms. In particular, the "trade-offs" between altered protein/amino acid content and other kernel components need to be elucidated and the possibilities for optimal combinations should be worked out.

At the conclusion of the review on December 13-14, 1976, project leaders and administrators at Purdue were informed of the intention to phase out this project March 31, 1978. It is possible that Purdue, or perhaps other institutions as well, may submit proposals for more fundamental research on protein/amino acid content in cereal grains.

Mr. Blumgart stated that Africa Bureau is deeply concerned with the rural employment and he felt, therefore, that this project would provide information for program planning and development; and since Africa represents a diversified area a study in one country might not produce information needed by the African countries.

Dr. Bernstein discussed the changes in A.I.D.'s approach to research especially that which concerns itself with social science. With respect to this particular proposal from Michigan State University he felt that a joint planning and collegial participation in the project activity might require some changes in methodology. Dr. Bernstein also recalled the RAC's reservation expressed at the time when the project was submitted for RAC review last year. At that time RAC felt that the project statement failed to show collaboration and consultation with Africa.

Dr. Bernstein observed that past experiences tend to indicate that, if LDCs are pressed too hard, then it does not take long before all the commitments start falling apart.

Motion: That the project be approved with the following recommendations: (1) if feasible, it should focus on one country; (2) both unrelated activities and personnel should be deleted; (3) graduate program and training should be aligned to conform with research objectives; (4) due attention should be paid to models; and (5) the budget should be modified to reflect the above changes.

(Motion carried; unanimous)

5. Inheritance and Improvement of Protein Quality and Content in Sorghum - Purdue University

Dr. Brady invited the Chairman of the subcommittee to present the review. Dr. M. Peterson presented the report on behalf of himself and Drs. Whitney and Schweigert. He opened the discussion with a brief history of the project so as to acquaint the new RAC members with this particular activity. Dr. Peterson explained that the project underwent several reviews by RAC and A.I.D. (with the help of RAC members and outside experts) the last extensive review took place on October 29, 1971. He felt that the pertinent recommendation of the review teams are incorporated in this research proposal. After briefly discussing project accomplishments, Dr. Peterson offered some observations and suggestions pertaining to the future activities of this project:

(1) laboratory analyses are too far behind, (2) sorghum is viewed by A.I.D. and Foundations as an important crop, (3) breeding effort should center in sorghum growing countries (LDC's) or States (U.S. activity), (4) quality program should link with breeding program but they do not have to be located in the same place, (5) A.I.D. should plan to organize and fund international sorghum leadership to operate a research network.

Dr. Whitney felt that the revised project reflects the changes suggested by all the numerous reviews. He thought that the present project objectives are realistic and the project is useful. He said that the bioanalyses of protein of about 1,000 lines represent an enormous undertaking by Purdue. He explained that sorghum is the most heterogenous crop. This is exemplified by the many interactions between protein, amino acids and environment. He felt that yields per acre should be improved. Present production of five bushels/acre obtained in some LDCs, is totally inadequate. Dr. Schweigert mentioned that he also was involved in the intensive review of this sorghum project. He feels that biological quality of protein is very important and thus he would like to see a nutritionist on the staff of the project.

Dr. Kelley commented briefly on the A.I.D. participation in the establishment of the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) which will work on sorghum, millet, legumes and other upland crops. Purdue sorghum people will backstop the ICRISAT activity, he said.

Dr. Litzenberger replied to some of the questions raised by the RAC. The project accumulated vast amounts of information which is being processed for publication in scientific journals. He mentioned that tannin found in sorghum grain represents a new problem which will be investigated by the contractor. To Dr. Smuckler's observation that, when wheat becomes available, people turn to it instead of continuing with eating sorghum. Dr. Kelley responded in affirmative but, he added that sorghum and millet will be grown in drier areas and thus local people will consume it. Dr. Breitenbach commented that TA/AGR is discussing the possibility of integrating the sorghum research between Purdue, USDA, U.S. universities and private industry. A.I.D. anticipates being able to report to RAC in about one year on the future course of sorghum work.

Motion: That the project be continued for three years with the provisos; (1) the project be reviewed annually and (2) that TA/AGR report to RAC one year hence as to the future course of the project.

(Motion carried; unanimous)

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AGENCY FOR INTERNATIONAL DEVELOPMENT

RESEARCH ADVISORY COMMITTEE

Minutes of the Thirty-Second Meeting
(January 28-29, 1971)

Dr. Long opened the meeting and welcomed those present. He explained that several of the RAC members unexpectedly were unable to attend including the Chairman, Dr. Brady, who was prevented from coming by a snowstorm. Dr. Long introduced Dr. Bernard S. Schweigert of the University of California at Davis, whose nomination as a member of the RAC is in process. He then turned the meeting over to Dr. Montgomery who acted as Chairman in the absence of Dr. Brady.

Project Review

1. Inheritance and Improvement of Protein Quality and Content in Sorghum Vulgare -- Purdue Research Foundation

Dr. Peterson presented the report of the review subcommittee consisting also of Drs. Milner and Ruttan. He described the background and objectives of the project and the progress to date. He pointed out that a team of A.I.D. and USDA personnel had conducted an on site review of the project in December, 1970. This team recommended extension of the project for an additional three years with a review scheduled at the end of the second year subject to redesigning the project in order to resolve some of the problems which have been encountered. One problem has been the size of the world sorghum collection and the resultant difficulty in completing the screening of the collections for high protein and amino acid balance, as well as publishing the results of these screenings. Modifications are also needed in the breeding program to place more emphasis on agronomic characteristics including yield, as well as greater efforts to evaluate for protein quality. The subcommittee members felt that the distribution system was too elaborate for the breeding program backing it up, and suggested that the number of cooperators be reduced. Regarding the lack of data returns from observational nurseries Dr. Peterson suggested that returns could be increased if cooperators knew that summaries of findings would be published and distributed promptly. The subcommittee also felt that the program was too large to be handled by a half-time project director.

Dr. Milner felt that the project had too many objectives, and he also was concerned about the nutritive aspects. He observed that the wide range in protein availability of the various lines studied, as well as the related apparent lack of correlation between essential amino acid levels and nutritive value, raised questions as to whether the biochemical/nutritive work at its present level is supplying adequately

useful guidance to the plant breeders. He asked, since this work is meant to be of primary benefit to the LDCs and since in many countries sorghum is used as much as a forage crop as for grain production, what is being done to determine the effects of breeding on forage nutritive quality, composition and toxic constituents. He also was concerned about the apparent scarcity of publications in connection with the project.

In the ensuing discussion Dr. Ruttan reiterated the subcommittee view that field activity was overextended relative to laboratory activity. Dr. Milner elaborated on his concerns about nutritive factors, while Dr. Schweigert was concerned about acceptability of the food beyond just the matter of nutritive qualities. Dr. Kelley said that most of the questions raised at this meeting also had been raised during the previous A.I.D. review in December, and that his staff was taking steps to resolve them. For example, a seminar on the problem of biological availability of protein will be held in the next few months. Additional full-time scientific manpower will be assigned to the project, and copies of publications will be sent to the RAC as these become available. Also, measures are being taken so that field activity will not be overextended relative to laboratory activity.

Decision: That the extension be approved for one year only with the provisos that (1) the program be redesigned in accordance with the RAC's recommendations including modifications in the breeding program, greater efforts to evaluate for protein quality, and limitation in number of field sites, (2) that the objectives should be sharpened and decreased in number, and (3) that this redesigned project be submitted to the RAC before the end of the one year extension authorized,

2. Development of Weed Control in Less Developed Countries
-- Oregon State University

Dr. Peterson commented on this project on behalf of Dr. Hagen, the chairman of the review subcommittee, who was absent and Dr. Ruttan the third member of the review subcommittee. Dr. Peterson presented the background of the project, listed the field sites and reviewed the objectives. He then summarized his conclusions based on his in-depth review and project site visit in September, 1970. He said that progress toward the stated objectives of the project has been excellent, and recommended continuation of the project along the present lines for another five years. He added that major improvements are being made in finding practical answers to the problems with weeds and in developing research capability within cooperating countries. In addition,

scientists. Dr. Kelley described the difficulties to date in conducting the field work as a result of A.I.D. limitations on the number of permanent U.S. personnel stationed in the field. However, this difficulty is being remedied. In addition, there is a provision for the consortium to have a minimum of two meetings a year for coordination purposes. Dr. Ayers described the over-seas activities to date, particularly in Brazil. Dr. Montgomery questioned whether two or three meetings a year were adequate to provide proper coordination, and recommended that the A.I.D. Office of Development Administration be asked for advice on the design and administration of the project. He and other RAC members wanted to see the political science and economics components of the project strengthened. Dr. Peterson volunteered his services to help evaluate the project. Dr. Kelley suggested that Drs. Peterson, Reville and Montgomery and perhaps one or two others visit Colorado State to review the project.

Decision: Approved for A.I.D. support subject to the provisos that (1) continuation beyond one more year would be subject to review and approval by the RAC subcommittee, and (2) that the advice and guidance of the A.I.D. Office of Development Administration be obtained regarding the design and administration of the project.

7. Inheritance and Improvement of Protein Quality and Content in Maize -- Purdue University

Dr. Brady presented the report of the review subcommittee which also included Drs. Milner and Montgomery. He presented the background of the project and the reasons for adding the research on maize to the work already being done at Purdue on sorghum. He believed Purdue was well qualified to carry out this work, but asked whether in the development of this project, there had been sufficient coordination with Ford, Rockefeller and others who are also working in this field. Dr. Brady was in favor of the proposal, especially the genetic plant breeding component.

Dr. Milner pointed out that the aim of this proposal really is to create another world center in maize breeding for nutritional improvement which will be directly parallel to the work being done at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico. This should be understood more clearly. Dr. Milner also stated that several major commercial firms are now routinely

producing and selling high lysine corn as well as carrying on extensive research in this field. With regard to the "coupling function" discussed previously by the RAC, Dr. Milner requested that the A.I.D. staff consult with CIMMYT, as well as any other appropriate organizations, in order to decide what type of commitment by A.I.D. is really necessary and how it will relate to other work being done in this field. He favored approval of the proposal provided the foregoing coordination could be accomplished satisfactorily. Drs. Caton and Ayers indicated that much of this coordination already had been done and that more was underway.

Decision: Approved for A.I.D. support with the recommendation that a RAC subcommittee consisting of Drs. Brady, Milner and Peterson work with the A.I.D. staff to develop, with Purdue and representatives of the USDA and the International Maize and Wheat Improvement Center (CIMMYT), a coordinated course of action.

Dr. Brady then said that he and his special subcommittee would revise their recommendations on the RAC's contribution to research planning and evaluation in A.I.D. in the light of the intensive discussion by the RAC members earlier in this session as well as the comments of Mr. Poats and others. He stated that he would send copies of the revision to the RAC members, possibly in time for discussion at the April 29-30 meeting.

The Chairman thanked the members and staff and adjourned the meeting.

TA/RUR:M.L.Becker:4/2/70

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FINAL REPORT

on

**Development of Improved
High Yielding Sorghum Cultivars with
Disease and Insect Resistance**

Contract No. AID/t-a-c 1384

March 1, 1979 - September 30, 1979

Prepared by

**Texas Agricultural Experiment Station
Department of Plant Sciences
Department of Entomology
College Station, Texas
and
Texas Agricultural Experiment Station
Lubbock, Texas**

Submitted to

**Agency for International Development
Department of State
Washington, D.C.**

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Keith Lahr	Research Associate
Leslie Kay Smith	Technician I
Janell McCullough	Technical Assistant II
Diane Giles	Student Technician
David R. Senter	Student Worker III
Ronald T. Smith, Jr.	Student Worker I
Linda Greenwood	Student Worker I
Abdel-Azim Abdalla	Graduate Student, Sudan
J. Alberto Betancourt	Graduate Student, Mexico
John W. Clark	Graduate Student
San San Da	Graduate Student, Upper Volta
Dan H. Meckenstock	Graduate Student
German Jose Perez	Graduate Student, Venezuela
Geoffrey L. Thomas	Graduate Student, Australia
Jose Armando Valdez	Graduate Student, Mexico

L. W. Rooney

Cheryl F. Earp	Research Associate
Lisa Blakely	Graduate Teaching Assistant
Patricia Howard	Technical Assistant II
John Akingbala	Graduate Student, Nigeria
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REPORT SUMMARY

Project Title: Development of Improved High Yielding Sorghum
Cultivars with Disease and Insect Resistance

Contract Number: AID/t-a-c 1384

Key Investigators: Richard A. Frederiksen
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Contract Period: March 1, 1977 - September 30, 1979

Period Covered
by Report: March 1, 1979 - September 30, 1979

Total AID funding of contract to date: \$894,946

Total expenditures and obligations through
previous contract year: \$894,946

Total expenditures and cumulative costs
for current year: \$225,000

Estimated expenditures for next contract period: -0-

NARRATIVE SUMMARY

During the past year some 48 technical articles, papers, and presentations were made on sorghum and sorghum improvement. These papers covered a wide range of topics on sorghum improvement in general, host resistance, cereal chemistry, physiology, and basic genetics. In 1979 two separate programs related to advanced training of scientists from developing countries were conducted by Texas A&M researchers. At Corpus Christi the Annual Grain Sorghum Workshop was highlighted by an interdisciplinary discussion on the merits, definition, and deployment of the trait known as "tropical adaptation." Other aspects of this workshop included a tour of the research nurseries at Berclair, Beeville, Corpus Christi, and Robstown. A technical paper session was used to review on-going research programs and graduate student problems. The International Short Course on Breeding Plants to Resistance to Pests was conducted over a two-week period and coordinated by the faculty at College Station. Essentially all of the project leaders on sorghum improvement participated in this program which included the preparation of a techniques paper. Two releases in 1979 included a twin-seeded pollinator line, Tx431, and a population TP15RB which is a broad base composite developed from converted exotic sorghums. Three major developments were noted on sorghum downy mildew: (1) a new race of the pathogen was found in South Texas, (2) low temperature environments were found to predispose some downy mildew resistant sorghums to the disease, and (3) a new technique was developed in cooperation with Brazilian scientists on the identification of individual plants in F_2 populations segregating for resistance to sorghum downy mildew. Considerable progress was made on the determination of mechanisms of resistance to grain mold to sorghum. Joint observations made by Latin American and African scientists support the hypothesis that there are distinctly

different races of the fungus causing anthracnose of sorghum but some lines possess resistance to all of the isolates.

Midge resistant sorghum varieties that are also resistant to anthracnose, downy mildew, and head smut and low in tannins were released and distributed. These varieties contain both restorer and non-restorer lines enabling the production of midge resistant hybrids.

A line has been identified that is resistant to greenbugs in the adult plant stage but not in the seedling stage. The line is not bloomless, indicating a source of resistance not previously identified.

ANNUAL RESEARCH REPORT

ANNUAL RESEARCH REPORT

A. General Background

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the principal food and feed commodities of the world. Sorghum is considered by many authorities as the third most important food grain, exceeded in utilization for food only by wheat and rice. Furthermore, sorghum is well adapted to cultivation under wide extremes in environmental conditions including arid, semi-arid, and sub-tropical areas of the world where other food commodities are often less well adapted.

In Southwestern USA, where sorghums are a principal agricultural commodity, serious disease and insect problems have occurred repeatedly. During the past few years, there have been grave losses caused by head smut (*Sphaeolotheca reiliana*); downy mildew (*Peronosclerospora sorghi*); maize dwarf mosaic (MDMV); anthracnose (*Colletotrichum graminicola*); charcoal rot (*Macrophomina phaseolina*); Fusarium stalk rot (*Fusarium moniliforme*); and a number of common foliage diseases.

Insects causing significant yield losses in grain sorghum annually are the sorghum midge (*Contarinia sorghicola*) and greenbug (*Schizaphis graminum*). Banks grass mite (*Oligonychus pratensis*) is a severe problem in the Great Plains region. Economically injurious insects that appear to be increasing in severity and distribution are white grubs (*Phyllophaga crinita*), sugarcane rootstalk weevil (*Anacentrinus deplanatus*), corn leaf aphid (*Rhopalosiphum maidis*), and yellow sugarcane aphid (*Sipha flava*). Consequently, the need for superior sources of disease and insect resistance in sorghums is urgent in Texas as well as throughout the world.

In 1963, the Texas Agricultural Experiment Station and the United States Department of Agriculture initiated a program whereby exotic sorghums from

the World Sorghum Collection were converted from tall, late-maturing, photoperiod-sensitive types to shorter, earlier-maturing, less-photoperiod-sensitive lines. Conversion is accomplished by a crossing and backcrossing program using the short winter days of Puerto Rico for crossing and growing F_1 's, with selection for short, early plants within segregating populations during the long summer days in Texas. Many exotic lines have been reported to be resistant to a number of diseases and insects, but until converted or partially converted, most of these lines are not available for use in temperate areas of the world. During the conversion process, we have been able to evaluate resistance from some partially converted exotic sorghums to diseases and insects prevalent in the United States. Some of these sorghums possess extraordinarily broad adaptation across both temperate and tropical areas of the world, excellent cultural traits, as well as high levels of disease and insect resistance.

B. Statement of Project Objectives

1. To identify and catalog sources of disease and insect resistance;
2. To develop high yielding agronomically desirable sorghum varieties and lines;
3. To develop elite breeding populations of sorghum with high levels of resistance to disease and insects;
4. To develop efficient resistance screening techniques;
5. To evaluate populations of plant pathogens for their range or changes in virulence;
6. To identify characteristics useful in seed production such as cytoplasmic sterility factors, apomixis and crossing traits.

C. Continued Relevance of Objectives

Clearly, the objectives are part of an on-going program which has been actively developing at the Texas Agricultural Experiment Station for the past 14 years. These activities involve an integrated interdisciplinary approach toward the development of sorghums for cultivation in temperate and tropical regions. The work plan stresses ecologically sound host plant resistance as a means of combating diseases and pests. Until very recently, the limited germplasm resources have restricted sorghum improvement; however, the recent development of converted and partially converted exotic sorghums at the Texas Agricultural Experiment Station has opened tremendous opportunities for sorghum improvement in Texas that has relevance throughout the world. The finding of photoperiod insensitive sorghums with wide adaptation across both temperate and tropical zones, makes the Texas program very useful to the tropical environments of the developing countries of the world.

These objectives are a sound and vital component of sorghum improvement for agriculture. They represent areas that need research and the findings and progress of this research will have application to crop improvement throughout the world.

D. Accomplishments to Date

During 1979 several unique disease problems were encountered. The season itself was late, cooler than average, and had a very wet, early growing season followed by a dry summer. Initially downy mildew developed at Port Lavaca in a Tx430 hybrid, but only at the earlier planting date, confirming in part other's observations that there is a cool temperature breakdown of Tx430 resistance to downy mildew. Later a distinctly new pathotype of *Peronosclerospora sorghi* was identified from Mathis, Texas. This is a first for the species in Texas. New races can be a mixed

blessing because, while it is almost inevitable that pathogens will vary, this variation tends to erode the value of host resistance. New isolates, however, can be used to define levels of resistance and the nature of variation present in the pathogen. Both bacterial stripe in South Texas and bacterial streak on the High Plains developed to unusually high levels. While neither appeared to cause extensive losses, their dramatic appearance caused considerable alarm to some growers, particularly those who grew hybrids susceptible to bacterial stripe.

A new disease, tentatively named sorghum wilt, caused by a *Phialophora* sp. was identified. This disease develops vigorously in the greenhouse and following inoculation in the field. Further work on sorghum wilt is justified.

THE 1979 DISEASE NURSERIES

Berclair - Head Smut

Grown principally as a head smut nursery for screening for resistance to races 3 and 4 of *Sphaelotheca reiliana*. About 10,000 entries from various programs were evaluated at this site in 1979. The level of disease was lower than usual, although fair evaluations for smut, rust, grey leaf spot, and late season stresses were possible. "Pink sheath rot" caused by *Sclerotium rolfsii* developed on a few entries.

Beeville - Head Smut and Downy Mildew

At Beeville, the nursery is divided into sections based on the types of alternate year spreader crops used to encourage disease development. During 1979 the downy mildew area had an excellent disease severity. Screening of exotic introductions, evaluation of Rid-o-mil® for chemical control and extensive early generation of breeding material was possible. A larger area is devoted to head smut screening; however, in 1979 head

smut was not as high as it was in 1978. Generally, leaf diseases develop extensively at this site, but because of cool weather during the early season and relatively dry flowering period, leaf diseases were moderately severe. Bacterial stripe was generally prevalent, along with moderate levels of grain mold, Fusarium head blight and grey leaf spot. Extensive selections and observations by the breeders were made at this location in 1979.

Corpus Christi

A major epidemic of bacterial stripe developed on the station at Corpus Christi. Good levels of downy mildew and head smut, zonate leaf spot were also present.

Edna - Head Smut

Fair head smut, excellent grain mold, and fine agronomic plant growth characterized the 1979 Edna head smut nursery. Because of the similar race pattern of smut at Berclair and Beeville we elected to abandon this nursery site for 1980.

Robstown

At Robstown, excellent downy mildew permitted evaluation of DMR sudan grass selections as well as the commercial hybrid evaluation program. Low levels of other foliar diseases permitted an ideal environment for controlled inoculations with *Colletotrichum graminicola* and *Gloeocercospora sorghi*.

College Station

In this area little naturally occurring sorghum diseases develop. In 1979 the environment permitted modest control of inoculation, but when augmented by field foggers excellent anthracnose, zonate leaf spot, and grain mold developed.

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Lubbock and Halfway

A naturally occurring epidemic of bacterial streak and superb inoculations with maize dwarf mosaic were the major problems associated with the nurseries at these locations. The occasional plant with sorghum wilt was observed.

Chillicothe-Vernon

At this site we evaluated entries for Periconia root rot. Three dates of planting of Colby demonstrated that disease develops aggressively in this susceptible cultivar throughout the season.

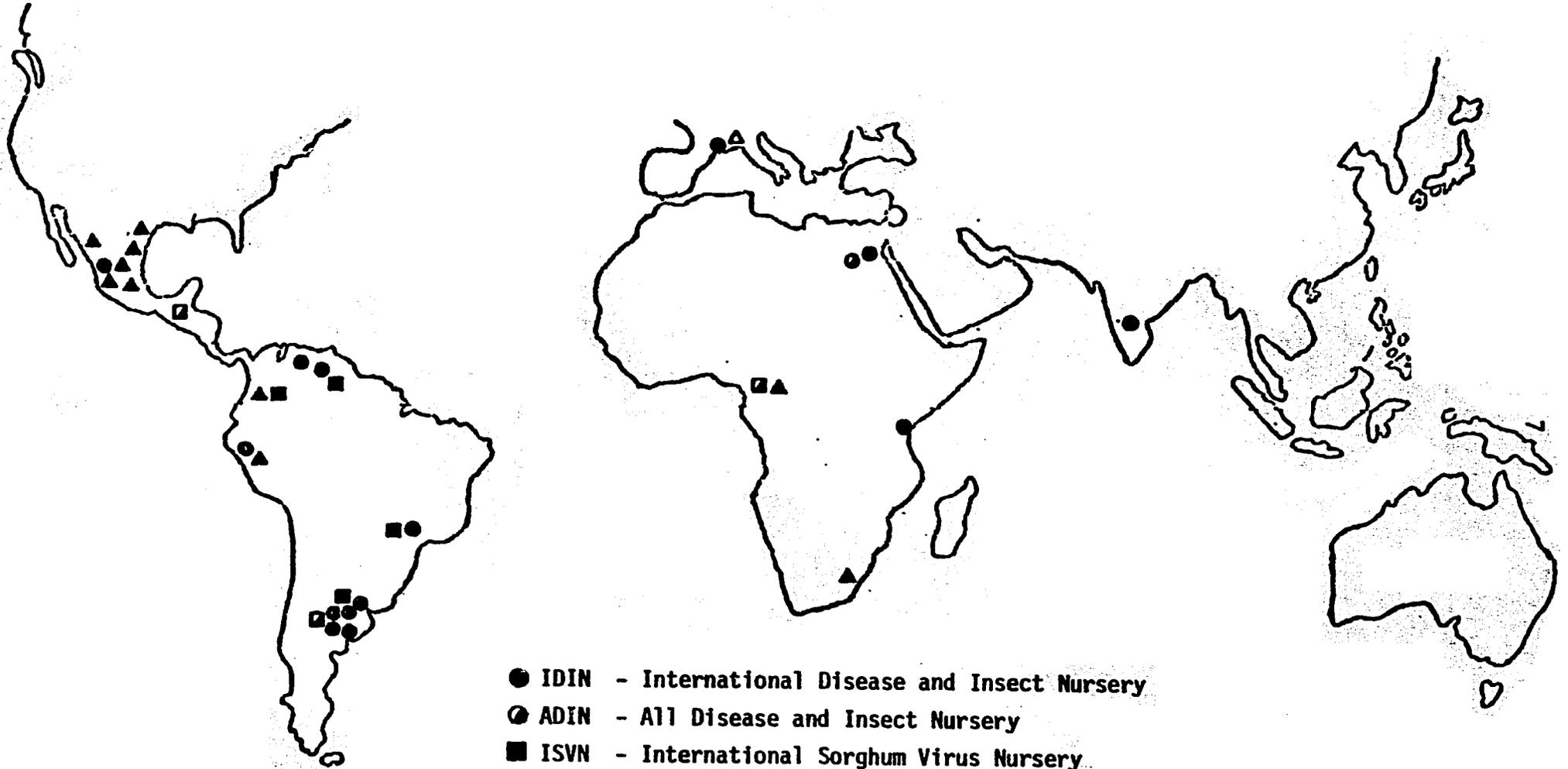
IDIN

The 30-entry International Disease and Insect Nursery included seven new entries in 1979 (Table 1). They included a grain weathering resistant line, three agronomically improved midge resistant lines, one greenbug resistant line, a charcoal rot and lodging resistant line, and a tropically adapted B line. The IDIN has been distributed to 14 cooperators in nine foreign countries (see map p. 7) during 1979 (Table 2). Hopefully this continued distribution will assure exposure to the primary disease and insect pests encountered throughout the world's major sorghum growing areas.

Results from recent data indicate severe levels of bacterial stripe and MDMV infestation at locations in Argentina and Venezuela, respectively (Table 3). Very susceptible reactions to bacterial stripe were noted on SC175-14, SC110-14, SC173-14, and SC112-14 while SC599-6, TX7078, SC599-11E, 77CS1, SC376-6, and 120 x 7000 scored very low or resistant-type ratings. With respect to the MDMV reaction, BTx398, B1778, and SC279-14 displayed very good resistance at all locations while, as in 1978, QL3 received a susceptible rating indicating the possibility of a new strain of the virus.

GLOBAL DISTRIBUTION OF TEXAS A&M SORGHUM NURSERIES

1979



- IDIN - International Disease and Insect Nursery
- ADIN - All Disease and Insect Nursery
- ISVN - International Sorghum Virus Nursery
- ISAVN - International Sorghum Anthracnose Virulence Nursery
- ▲ TAT - Tropical Adaptation Test
- △ UHSN - Uniform Head Smut Nursery

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Table 1. International Disease and Insect Nursery (IDIN) - 1979.

Entry No.	Designation	IS No.	Variety, Kind or Group	Plot No.	
				Repl I	Repl II
1	SC 103-12	2403	Caudatum	14	41
2	SC 110-14	12610 C	Zerazera	4	50
3	SC 170-6-17	12661	Zerazera	21	39
4	SC 170-14	12661 C	Zerazera	1	42
5	Sc 173-12	21664	Zerazera	26	54
6	SC 175-14	12666 C	Zerazera	19	32
7	SC 326-6	3756	Nigricans	7	59
8	SC 414-12	2508	Cau-Kaf	11	49
9	SC 599-6 (9247)	17459	Cau-Nig (Rio)	28	46
10	SC 599-11E	17459	Cau-Nig (Rio)	3	38
11	SC 748-5	3552	Cau-Guin	15	53
12	SC 630-11E	1269	Caffr	6	40
13	R 5388	-	(SC599-6XSC110)der	25	34
14	6 BH 4613 (MR 4)	-	2566(3197X170-6)der	13	56
15	6 BH 4654 (MR 4)	-	2566(3197X170-6)der	18	31
16	B 447	-	B2752X(3197X170)der	2	43
17	Tx 2771 (MR 4-R line)	-	2566(3197X170)der	29	52
18	1790 E	-	(SC56XSC33)der	8	36
19	B Tx 623	12661 der	(B3197X170-6)der	23	48
20	R 1750	12661 der	(B3197X170-6)der	5	33
21	77 CS 1	-	(IS2930XIS3922)der	20	58
22	TAM 428	12610 der	Zerazera	10	45
23	Tx 430	12661 der	(Tx2536XSC170-6)der	27	60
24	GPR-148	-	CSV-5	17	35
25	CS 3541	-	CSV-4	22	55
26	QL3 sel	-	C. Kaf. der	30	44
27	B Tx 378	413	Redlan	9	57
28	B Tx 398	412	Martin	24	36
29	Tx 7078	415	Comb. 7078	12	47
30	Tx 2536	10542	Y. D. Fet. der	16	51

Table 2. IDIN distribution and disease severity rating¹

Location	Cooperator	#IDIN	Sorghum downy mildew	Anthraxnose	Rust	Grey leaf spot	Leaf blight	Zonate leaf spot	Charcoal rot	Grain weathering	IDIV	Bacterial stripe
Venado Tuerto, Argentina	Rodolfo Rossi Federico Llobet	2	-	-	-	-	-	-	-	-	+	±
Rojas, Argentina	Elba B. Alessandrini	1	-	-	-	-	-	-	-	-	-	±
Pergamino, Argentina	Eduardo Teyssandier	1	-	++	++	-	+	-	+	+	+	+++
Chidayo, Peru	Marco A. Neciosup	1	-	-	-	-	-	-	-	-	-	-
Aragua, Venezuela	Hector Mena	3	+	+	+	+	-	++	-	++	+++	-
Gaborone, Botswana	Max Boling	1	-	-	-	-	-	-	-	-	-	-
Giza, Egypt	S. I. Salama	1	-	-	-	-	-	-	++	-	-	-

¹Disease rated according to severity where + = low level of infection, ++ = moderate, +++ = high.

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Table 3. Cumulative disease reaction mean of IDIN entries.

	SDM	Anthr.	Rust	Green leaf spot	Leaf blight	Zonate leaf spot	Char- coal rot	Grain weather	MDMV	Bact. stripe
SC175-14	1.8	1.1	0.8	1.5	0.5	1.3	1.4	1.9	1.6	3.3
SC110-14	1.0	1.1	0.9	1.5	0.1	1.7	1.2	2.0	1.7	3.0
R1750	-	0.6	0.8	-	0.8	1.5	1.7	1.5	1.0	2.5
SC599-6	1.5	2.4	0.8	1.5	0.8	1.9	1.2	2.2	2.0	0.8
GPR-148	1.0	1.9	1.8	2.0	0.5	1.9	1.1	2.0	1.7	1.8
BTx398	1.5	1.7	1.8	2.3	0.8	2.5	1.8	1.9	1.3	1.3
TAM482	1.0	1.3	1.8	1.8	1.0	2.0	1.3	1.8	1.7	2.8
SC170-6-17	1.0	2.8	1.7	1.5	0.8	1.9	1.2	1.5	1.7	2.0
SC414-12	1.0	2.0	1.3	1.5	1.0	1.8	1.3	2.0	1.6	1.8
B1778	-	2.5	1.9	-	1.5	3.0	1.0	2.2	1.1	1.1
SC173-12	1.5	1.8	1.1	1.8	1.0	2.3	1.2	1.7	1.6	2.9
SC103-12	1.8	2.6	1.8	1.0	2.0	2.0	1.2	1.7	1.6	1.1
(120 x 7000) sel.	-	3.3	1.7	-	0.3	2.0	1.3	1.4	1.6	0.8
BTx378	1.5	1.0	1.8	1.3	0.3	2.1	1.3	1.7	2.1	0.9
Sc748-5	1.5	1.5	1.7	1.5	0.3	1.8	1.4	1.7	1.7	1.9
Tx7078	2.0	2.4	1.8	1.8	1.3	2.2	1.1	2.1	1.9	0.8
SC599-11E	2.3	2.0	1.8	1.8	0.8	2.0	1.1	1.5	1.7	0.7
SC170-14	1.3	2.2	1.6	1.3	0.5	1.8	1.2	1.7	2.0	2.2
SC279-14	1.8	1.8	0.9	1.5	1.0	1.3	1.6	1.3	1.4	2.6
BTx624	1.8	1.1	1.3	1.3	1.0	1.8	1.9	1.8	1.6	2.5
QL3 sel.	1.7	1.2	1.7	1.3	1.3	2.3	1.4	1.4	1.6	1.0
Tx430	1.0	2.2	0.8	1.5	1.3	1.5	1.3	1.4	1.6	2.4
Tx2536	3.0	1.9	1.2	1.8	1.0	2.3	1.3	2.2	2.2	2.0
SC112-14	1.8	1.8	0.9	1.0	0.5	2.0	1.6	1.8	1.6	3.1
77C51	-	3.0	1.0	-	1.0	1.5	1.2	1.6	1.5	0.8
SC326-6	2.0	0.9	1.1	2.0	0.5	2.3	1.4	1.4	1.7	0.5
CS3541	-	2.5	1.3	-	0.8	1.0	1.1	1.7	1.1	1.3
SC237-14	2.0	1.3	0.9	1.5	0.3	1.5	1.2	1.7	1.8	2.3
Tx2748	-	1.8	1.4	-	0.8	2.0	1.1	1.3	1.8	2.3
R5388	-	2.8	0.8	-	0.8	1.5	1.3	1.6	1.3	1.8

Research is currently under way to select a new set of host differentials to characterize the strains.

Grain weathering continues to pose a threat to maturing grain in the humid tropics, often being one of the principal factors limiting production. The IDIN presently includes genotypes with good levels of resistance to grain weathering; however, under optimal weather conditions this resistance can be overcome by the pathogen complex. Thus, there is a need to expand our screening program by introducing the nursery in areas where high levels of disease pressure are present.

The entries 77CS1, GPR148, CS3541, Tx430, R1750, SC110-14, and SC175-14 continue to display good to excellent general leaf disease resistance although leaf anthracnose ratings were slightly higher on several of these lines, notably 77CS1, GPR148, CS3541, and Tx430.

Data for charcoal rot from Argentina and Egypt helped to identify several possible sources of resistance. The consistent performance of these lines may provide the genetic background to control this disease.

ADIN

The 70-entry All Disease and Insect Nursery (ADIN) included 22 new entries in 1979. They included grain mold (weathering), lodging, greenbug, midge, rust, and anthracnose resistant lines with improved agronomic traits, as well as lines with tropical adaptation, tan plants, white pearly grain, and good general leaf disease resistance from the TAES breeding nurseries.

All of the IDIN entries are included in this nursery. Thirty sets were grown at 18 locations in the U.S. and Mexico (Table 4). Evaluation was made on all of the major disease problems of sorghum in the Americas, particularly head smut, downy mildew, anthracnose, maize dwarf mosaic, foliage diseases, and Fusarium head blight. Excellent evaluations were

Table 4. 1979 ADIN (All Disease and Insect Nursery) Locations*

Location	No.	Characteristics Evaluated
Texas		
Beeville	1	Downy mildew, head smut, bacterial stripe, grey leaf spot, zonate, Fusarium head blight, adaptation.
Berclair	1	Downy mildew, head smut, grey leaf spot, zonate, rust, adaptation
Big Spring	1	Drought tolerance
Chillicothe	1	Periconia root rot, drought, bacterial stripe
College Station	3	Leaf blight, insecticide burn, adaptation, MDM
Corpus Christi	1	Bacterial stripe, head smut, downy mildew
Edna	1	Head smut, downy mildew, seed weathering, Fusarium head blight
Halfway	4	Rust, MDM, adaptation, bacterial streak, drought, corn leaf aphid
Lubbock	7	Lodging, drought, bacterial streak, MDM, adaptation
Pecos	2	Mites, drought
Robstown	1	Downy mildew, zonate
New Deal (Texas Tech Farm)	1	Drought
Weslaco	1	Insecticide burn, adaptation
Georgia	1	Anthracnose, foliage diseases
Kansas	1	Chinch bugs
Mississippi	1	Anthracnose, foliage diseases
Mexico		
Rio Bravo	1	Downy mildew foliage diseases
Tampico	1	Foliage diseases, adaptation

* The ADIN contains the 30 IDIN entries plus 40 additional entries.

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obtained for grain mold, downy mildew, bacterial stripe, and anthracnose. Bacterial streak reactions were obtained for the first time on ADIN entries. As in previous years certain lines are highly resistant to several of these disease problems. None are highly resistant to all. Generally, the environments within Texas and the cooperating states provide an excellent means for thorough testing of these advance entries.

DOWNY MILDEW

A. Cultural Control

In 1977, two studies were initiated to determine the feasibility of reducing initial inoculum (oospores) of *Peronosclerospora sorghi* through use of cultural methods. The objective was to determine correlations between inoculum density (numbers of oospores per unit of soil) and the actual incidence of SDM. Previous work by other researchers determined that deep plowing to a depth of 12 inches reduced SDM incidence the following season, but our deep plow studies at two locations also analyzed the inoculum density of oospores in the soil profile.

Deep plow plots had less SDM incidence (26% or greater reduction) and a lower inoculum density in the upper 20 cm of the soil profile than normal tillage plots. However, both deep plow and normal tillage plots had approximately the same total inoculum density in the top 40 cm of soil profile. This indicated that deep plowing diluted the current season's oospores throughout the 40 cm of profile instead of just the upper 20 cm as with normal tillage.

A related study conducted at three locations utilized an SDM-susceptible sorghum in continuous culture to stimulate growing of SDM-resistant sorghum and crop rotation practices. This simulation was achieved by continuously removing and rogueing (R) the systemically-infected SDM plants from one-half

of the plot and the other half was nonrogued (NR) as a control treatment. Uniform SDM incidence occurred across both halves of each plot in year I, but during years II and III, R plots had a 50% less incidence of SDM than NR plots. Inoculum densities in the upper 20 cm of the soil profile were uniform across each plot in year I, but R plots had inoculum densities 25% less than NR plots in year II and 58% in year III.

There was not complete correlation of inoculum density with SDM-incidence between plots nor in the same plots in successive years indicating that differences in soil environment due to soil type or other atmospheric variables have profound effects on efficiency of initial inoculum.

B. Chemical Control

An experimental systemic fungicide (CGA48988) developed by Ciba-Geigy was evaluated as a seed dressing for control of sorghum downy mildew (SDM) caused by *Peronosclerospora sorghi* at three locations in South Texas. SDM incidence six weeks after planting was 8-28% in plants from nontreated seed and 0% in plants from seed treated with CGA48988 at rates greater than 0.5 g a.i./kg of seed. At one location there appeared to be reduced local lesion development on plants from seed treated with any rate of CGA48988.

Under SDM favorable conditions CGA48988 used at seed dressing rates of 0.1 to 3.0 g a.i./kg of seed on susceptible sorghum reduced early season SDM, increased number of grain bearing heads, and significantly increased yields. Although high seed dressing rates reduced seedling stands, they were greater than those from nontreated seed.

This chemical has demonstrated a potential for use as a control of SDM in sorghum and represents a needed complimentary and alternative control to the already-implemented plant resistance.

C. Local Lesion Assa

The use of local lesions as a downy mildew screening tool was proposed as early as 1969 but until recently little effort was directed toward using this method. During 1979 Dr. Nelson Gimenez F. determined that IS 12661C, IS 2508C, IS 12612C, CS 3541, and Tx 430 had "resistant" local lesions. F₂ progenies of Tx 430 by susceptible Tx 7078, 77CS1, IS 3757 der., and IS 12610C were evaluated. In all cases parental classes were recovered but ratios of resistance, intermediate and susceptible classes differed considerably (Table 5). F₃ analysis of the F₂ tests are planned. F₁'s tended to be resistant. Trials using other DMR sources will be evaluated in 1980.

Table 5. Frequency distribution of F₂ sorghum seedlings of local lesion reaction classes following inoculation with P. sorghi

Parents	Reaction Classes ^{a]}		
	R (1-2.9)	I (3-3.9)	S (4-5)
IS 12610 x Tx 430	48	39	13
Tx 430 x IS 3757 der.	72	11	17
(IS 2930 x IS 3992) x Tx 430	13	61	26
Tx 430 x Tx 7078	63	25	11

a] Based on a 1-5 scale, type 1 local lesions are essentially not visible. Type 2 cause distinct necrosis and remain small. Type 3 are larger, may have some chlorosis generally not sporulate. Type 4 and 5 permit rapid colonization of the leaf with death in the case of 5.

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D. Affect of Environment on Downy Mildew Development

During 1978 an unusually high incidence of downy mildew (30%) was observed in hybrids utilizing Tx 430 as a downy mildew resistant parent. Extensive testing showed that no new race was present in this field. However, it appeared that lower than normal temperatures during the early portion of the growing season may have predisposed the plants to infection. In 1979, planting date with the coolest soil temperatures had the highest level of downy mildew in the Tx 430 hybrids. However, some genotypes showed a differential response to temperature as the incidence of disease went up in late plantings. This discovery makes it imperative that we examine known sources of resistance to downy mildew at lower than normal testing temperatures to see if they also are subject to these reaction changes.

E. Race 2 of *Peronosclerospora sorghi*

In 1979 sorghum downy mildew was observed in a commercial hybrid known to have had resistance to downy mildew in South Texas for nearly a decade. Subsequent evaluation of isolates of *P. sorghi* from this field with isolates having been used in the laboratory for the past 5 years showed them to differ significantly on three varieties. A paper describing this new race has been submitted for publication.

Physiologic specialization of this pathogen is not unlike that of any other pathogen. We can expect some variation in pathogenicity any time host resistance is used as a means of controlling the disease. We are fortunate that the more popular sources of resistance used in South Texas at this time are not subject to attack by this new race. Nevertheless, only a limited number of sources of resistance have been evaluated to the new race of downy mildew and extensive testing is planned for the summer of 1980.

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HEAD SMUT

A population study on sorghum head smut was designed to determine rate at which particular host genotype permits the selection of a particular genotype of the pathogen. Sorghum head smut populations had been observed to shift very rapidly following the growing of specific host varieties. This study was conducted by artificially inoculating 20 monoteliospore cultures of race 3 and race 4 isolates of *Sporisorium reilianum* in four commercial hybrids (RS 610, Pioneer 8311, NK 262, and NK 266). Sori produced from plants of each hybrid were bulked and used as a new population for another cycle. Currently this study is at the 4th cycle. At the 5th cycle cultures of the original race 3 and race 4 isolates will be compared with the isolates produced after five generations on selected differentials.

The potential of using leaf symptoms as a tool for evaluating resistance or susceptibility to head smut was studied. Unlike corn head smut, correlation between sori production and leaf symptoms was poor. Artificially inoculated plants in the Uniform Head Smut Nursery (UHSN) during the 1979 spring field plantings failed to produce plants exhibiting leaf symptoms even though several entries produced smut sori at flowering. Similarly, greenhouse plants inoculated during the hot summer months (June and July) did not produce leaf symptoms but later produced sori.

Chlorotic flecking was observed in sorghum seedlings artificially inoculated in January, 1979 and December, 1979. The slower growth rate of plants at cooler temperatures may influence leaf symptom expression in sorghum. Even so, some plants in variety ATx 618 exhibited heavy chlorotic flecking but did not produce smut sori at flowering.

The causal organism of head smut of corn and sorghum belong to the same species, *S. reilianum*. Except for host specificity, knowledge is very scarce regarding differences between them. Results showed that there was no

significant difference in spore morphology (size, shape, color) between the two smuts. The germ tube or the promycelium of corn head smut was, however, significantly longer (1.25μ) than sorghum head smut. Germination of freshly harvested corn smut spores ranged from 4.54 to 23.91% as compared to 0.71 to 5.62% in sorghum head smut. The time for germination varied within corn and within sorghum head smut collections and it was not correlated with percent germination nor with length of storage of smut spores. Corn head smut spores are more benomyl tolerant than sorghum head smut spores. Transmission electron microscopy of cross-sections of spore walls revealed no significant differences. Corn head smut teliospores produced sporidia, although poorly, when grown in a medium composed of heat-killed sorghum head smut sporidia. Sorghum head smut teliospore germinated, also poorly, but did not produce sporidia in this medium.

Several experiments were conducted in an attempt to increase the germination of smut spores. The presence or absence of continuous or discontinuous light did not affect the time of germination, length of promycelium, type of growth (sporidial or mycelial) nor percentage germination of corn and sorghum head smut spores. Similarly there were no differences in spore germination when spores were plated on potato dextrose agar with 0.75%, 1.0%, and 1.5% agar to vary the moisture content of the medium. Oven drying the spores at $35\text{ C} \pm 1$ at different time intervals up to 72 hours did not increase nor decrease spore germination.

Temperature and relative humidity (RH) greatly influenced viability of spores. There was a significant decrease in germination when sorghum smut spores were kept at 53% RH or higher at room temperature after 9 months of storage. At 3-5 C, however, only those kept at 100% RH had decreased spore germination.

Ultrastructural and histopathological studies of the host-parasite relations of sorghum head smut were conducted. Intercellular and intracellular mycelia were observed in host tissues. Intracellular hyphae from developing sori and parenchymatous cells of older plants behaved like haustoria. There was penetration of the host cell wall, invagination of host plasma membrane, and formation of an encapsulation between the host plasma membrane and the fungal cell wall. The encapsulation was eventually completely ensheathed, i.e., the host cell wall was deposited in the encapsulation.

The cytoplasm of *S. reiliana* contained the usual complement of cellular organelles found in other eukaryotic cells. Differences between vegetative and reproductive mycelia were noted.

ANTHRACNOSE

During 1979 the International Sorghum Anthracnose Virulence Nursery (ISAVN) was distributed to the following locations: Guatemala, Venezuela, Brazil, Indonesia, Nigeria (Table 6). The ISAVN was also planted in College Station, Texas; Rohnstown, Texas; Meridian, Mississippi; and Experiment, Georgia. In addition, anthracnose evaluations were conducted on the IDIN and CLAT nurseries in Brazil. A new entry, SC 748-5, was added to the ISAVN during 1979.

Data obtained from the IDIN grown in Sete Lagoas, Brazil indicated that the *Colletotrichum graminicola* attacking sorghum in that country differed from that of the United States. SC 110-14, SC 112-14, SC 170-14, SC 173-12, SC 175-14, SC 237-14, SC 599-6-3(9247), SC 599-11E, and TAM 428 are traditionally anthracnose resistant lines, but they were susceptible in Brazil. BTx378 and QL3 sel., which are susceptible in the U.S., had little or no anthracnose in Brazil. SC 103-12, SC 326-6, and SC 748-5 showed very good anthracnose resistance at all locations.

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Table 6. Distribution of 1979 International Sorghum Anthracnose Virulence Nurseries (ISAVN).

Location	Number	Cooperator
Argentina	1	
Brazil, Sete Lagoas	2	F. T. Fernandes
Brazil, Sete Lagoas	4	R. Schaffert
Guatemala, Cuyuta	3	A. Plant
Indonesia, Tiupon	2	I. S. Suwelo
Nigeria, Zaria	1	N. V. Sundaram
Venezuela, Aragua	2	H. A. Mena
United States		
Experiment, Georgia	2	R. R. Duncan
Meridian, Mississippi	2	N. Zummo
College Station, Texas	1	M. A. Pastor-Corrales
Robstown, Texas	1	M. A. Pastor-Corrales

The IDIN, CLAT, and ISAVN were evaluated for anthracnose at Experiment, Georgia where excellent anthracnose developed. The most significant finding was that TAM 428, a sorghum line which was traditionally resistant to anthracnose throughout the continental United States was attacked by *C. graminicola*. The symptoms developed in both the foliage and panicle. This indicates that there are variations in the pathogen population attacking sorghum in Georgia and a new pathotype of *C. graminicola* is present.

The ISAVN data obtained from Samaru, Nigeria was valuable. Two distinct types of anthracnose symptoms were observed. Type 1 consisted of isolated spots and type 2 consisted of patchy spots. The traditional resistant lines, Brandes, Rio, SC 599-6(9247), SC 167-14, SC 328, and SC 748-5 had the type 1 symptoms while TAM 428 had the type 2 symptoms. Previously SC 748-5 has been shown to be anthracnose resistant at all locations.

ZONATE LEAF SPOT

Investigations into the disease, zonate leaf spot of sorghum, were conducted for a second year, both in the field and in the lab. Field research included the screening of various sorghum lines for resistance to the causal pathogen, *Gloebocercospora sorghi*. Observation of disease progression on various lines throughout the growing season indicated that disease develops more rapidly on plant material and the effectiveness of the systemic fungicide benomyl as a chemical control was confirmed. Also, individual lesion development was observed and measured daily. Laboratory research was involved with various factors influencing the survival and germination of the conidia and sclerotia. Such factors included the effect of temperature and relative humidity on the survival of the propagules and the influence of different sorghum exudates on the germination of conidia and sclerotia in the soil.

SORGHUM VIRUS PROGRAM

At the International Sorghum Disease Workshop in Hyderabad, India in December 1978 the Sorghum Virus Committee composed of R. W. Toler, USA; D. S. Teaxle, Australia; E. E. Teyssandier, Argentina; M. Ricelli, Venezuela; and S. C. Dalmacio, Philippines developed an international sorghum virus nursery. The nursery is composed of ten lines that have varying degrees of resistance and susceptibility to the sugarcane mosaic virus group which includes the Johnsongrass strains.

The inbreds are to be maintained in Australia by R. G. Henzell and in the U.S. by Fred Miller. The nursery will be sent to anyone requesting seed from either Australia or the U.S. International Virus Nurseries were sent to Argentina, Brazil, Colombia, and Venezuela in 1979 from the U.S.

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A uniform data collection sheet with procedures for taking notes was developed and will accompany each nursery. The nursery is composed of OKY8, Tx 3197, Atlas, Rio, Martin, SA8735, NM31, SC9907, Q7539, and QL 11. The nursery approach to identifying of the viruses is to be backed up by electromicroscopy, serology, and other techniques.

The development of an international nursery was prompted by the recent occurrence of a virus or strain observed in Venezuela that infects and causes disease losses in sorghums that have tolerance, resistance, and immunity to SCMV-J (MDMV) from the U.S. and Australia.

At the present we are evaluating the reaction of the differentials in the nursery in the greenhouse to strains A, B, C, E, and F of maize dwarf mosaic virus, brome mosaic virus, and strains A and H of sugarcane mosaic virus.

In 1979 a joint study of inheritance of resistance to MDMV using mechanical inoculation was conducted with Dr. D. T. Rosenow at Lubbock. The inheritance of maize dwarf mosaic reaction involved the following lines in F_1 and F_2 combinations:

1. Martin (TBx398) - Tolerant (takes virus but is not affected adversely)
2. Redlan (BTx378) - Susceptible - Shows "red leaf" necrosis under cool temperatures only
3. Rio deriv (B-SC599 der.) - Susceptible - Shows "Rio reaction" (severe stunting and chlorosis (not red leaf, not dependent on temperature. Under field conditions, normally only a low percentage of plants show symptoms)
4. QL3 - Resistant - Resistant to infection, both natural and artificial.

The following has been determined to date:

- a. Martin tolerance is completely dominant over Redlan susceptibility with a single dominant gene indicated.
- b. Rio susceptibility is dominant over Martin tolerance and Redlan susceptibility with more than one gene indicated.
- c. QL3 resistance is completely dominant over all three. May be a single dominant gene.

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DATA SHEET FOR INTERNATIONAL TESTING OF SORGHUM DIFFERENTIAL LINES
FOR REACTION TO SUGARCANE MOSAIC VIRUS

Country:Cooperator:Address:Locality of testing site:Environment of test:Type of inoculation:Height above sea level:Glasshouse
Growth cabinet
Field
(Tick one)Natural (aphid)
Manual
Air-blast
(Tick one)Temperatures during test:Original host of virus:Planting date:MaximumMinimumVirus strain:Date results assessed:

Line	% Infection	Symptoms (describe and take color photographs)	
		Inoculated leaves	Systemic symptoms.
OKY8			
Tx3197			
Atlas			
Rio			
Martin			
YEF x SSK Wx SA8735			
NM31			
SC0097			
Q7539			
QL11			

Additional comments:

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FUSARIUM HEAD BLIGHT

Fusarium head blight (FHB) occurred on grain sorghum grown throughout Central and Southern Texas in 1979. Disease surveys determined that 43% and 91% of the fields in Central and Southern Texas had plants with symptoms of FHB. Weight and size of kernels from FHB panicles were significantly reduced when compared with kernels from normal-appearing panicles. Kernel weights were reduced an average of 12% (ranging from 4 to 22%) in Southern Texas. FHB did not reduce quality of the harvested grain. Based on estimated yield figures, FHB probably reduced yields in Texas by 32 to 72 thousand MT resulting in a \$3.2 to \$7.1 million loss to farmers. FHB occurrence appears to be related to below normal temperatures and above normal rainfall during the period following anthesis and, possibly, to above normal temperatures and below normal rainfall during the March-April period.

GRAIN MOLDS

The value of artificial inoculation for identifying grain mold (GM) resistant lines was further substantiated. SC0719, a line resistant to grain weathering, appeared fairly susceptible to GM fungi under artificial and natural conditions. *Gibberella fujikuroi* perithecia were found on molded kernels of SC0719; the sexual stage of *Fusarium moniliforme* (FM) may be important in overseasoning and variation of the pathogen.

Histopathological studies were initiated with *Curvularia lunata* (CL) and FM. Macroscopic observations indicated that the basal area of the floret and kernel is the area of colonization by FM and that the glumes and pericarp are the colonized tissues with CL. Microscopic observations indicate that the lemma, palea, and lodicules are the first tissues extensively colonized by FM and CL (within 5 days after inoculation at anthesis). Fungi proceeded to colonize the developing kernels.

GRAIN WEATHERING (GRAIN MOLD)

The 25 entry replicated Grain Weathering Test (GWT) was planted at nine locations in Texas in 1979, as well as in Mexico, Puerto Rico, Georgia, and Brazil. Significant weathering developed in South Texas. Several lines possess excellent resistance, with two entries (IS9530 and SC650-11E) ranking in the top six. A summary of reactions from Robstown, Beeville, Berclair, and Edna is as follows:

<u>Designation</u>		<u>Rating</u> ^{1/}
SC719-11E	IS7013 der.	1.6
SC279-14E	IS7419C	1.8
SC566-14E	IS7254C	2.0
IS9530	IS9530	2.1
SC748-5	IS3553 der.	2.2
SC650-11E	IS3856 der.	2.3
SC630-11E	IS2856 der.	2.3
BTx398	(Martin)(check)	2.8
B2219	(check)	2.8
CS3541	(check)	3.0
TAM428	(check)	3.1
Tx2536	(check)	4.6

^{1/} Rated on 1-5 scale. 1 = no mold or weathering, 5 = completely destroyed

SORGHUM QUALITY

Sorghums with and without undercoats were grown to determine changes that occur in the testa and pericarp during maturation of the kernels. Tannins in the grain at differing stages were analyzed by several methods. Lines with dominant B₁B₂ genes had pigmented integuments at or near anthesis. Tannin content was low for all kernels without a testa and for those with

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a pigmented testa without dominant spreader gene. In sorghums with dominant B_1B_2 's the tannin content expressed as catechin equivalents reached a maximum at about 20 days after anthesis and decreased afterwards but contained significant quantities at physiological maturity.

Grain from the 1978 GWT at Corpus Christi and Lubbock were analyzed by the leachette test to relate conductivity ratings to subjective ratings of grain weathering. The samples were badly weathered and insect damaged. The variability of the data was so great that the leachette test was of no use. Samples of sound grain with no weathering from India gave repeatable conductivity values but the correlation with observed differences in mold resistance was not encouraging. Samples of grain with no weathering were harvested from the 1979 GWT at Lubbock which will permit a good evaluation.

LODGING, CHARCOAL ROT, DROUGHT

This program in 1979 emphasized resistance to moisture stress during the late stage of grain development which, in effect, gives drought tolerance and results in resistance to lodging.

Planted at several locations in 1979 were three lodging tests of breeding lines; a 25-entry Statewide Lodging Test (SLT); a 36-entry Advanced Lodging Test (ALT); and a 49-Entry Preliminary Lodging Test (PLT). Also planted were two tests to evaluate specifically late season (post-flowering) drought stress tolerance and early season (pre-flowering) drought stress. These were a 25-entry Drought Line Test (DLT) and an 81-entry Drought Hybrid Test (DHT). Moderate pre-flowering drought stress developed only at Big Spring and Chillicothe. Severe post-flowering stress developed at both Lubbock and Halfway, resulting in excellent evaluation of resistance to such drought stress. Of significance was the outstanding post-flowering drought resistance that SC35 imparted to its hybrids. Also, hybrids involving the lines

1790E, 1790L, SC56-6, and SC56-14 showed good levels of drought tolerance. Post-flowering drought tolerance is expressed by the plants remaining green, developing grain to normal size, and maintaining good stalk strength. Susceptibility is expressed by premature plant senescence, reduced grain size and grain yield, susceptibility to charcoal rot, and susceptibility to lodging.

GROWTH REGULATORS

All of the sorghum maturity genotypes identified by Quinby were grown under controlled conditions with a 12 hour photoperiod and growth, developments, and date of floral initiation determined. Determination of the IAA and ABA levels in these genotypes is in progress. Studies on the hormonal regulation of tillering were completed and at least three groups of substances, gibberellins, auxins, and cytokinins were implicated in regulation of tillering. A means was devised to promote tillering of plants in the field by simultaneous applications of gibberellins and cytokinins. Work has continued to verify the identity of IAA and ABA from sorghum leaves, and initial work with the gas chromatograph-mass spectrometer has been done.

GENETICS

Frequency of apomictic seed formation was not increased in F_1 progeny of facultative apomicts. A population of apomictic germplasm is being developed to recombine genes for apomixis from several sources. Additional backcrosses were made to introduce the nucleus of TAM428 into A2 cytoplasm and thus produce a cytoplasmic-genic male sterile of TAM428. TAM428 is a restorer in A1 (milo) cytoplasm. Each of the new steriles derived in the reciprocal crossing program were crossed to B398 to establish isocytoplasmic lines for definitive tests. Test-cross progeny of the KS steriles were observed in Nebraska and Texas to determine cytoplasmic differences.

GREENBUG

A new source of greenbug resistance has been identified that is resistant in the adult plant stage but susceptible in the seedling stage of plant growth. Crosses have been made with this line to determine inheritance of the resistance and to combine it with sources previously identified into one line. This line and a newly developed greenbug resistant "B" line appear to be more resistant to late season drought stress than greenbug resistant lines previously identified.

SORGHUM MIDGE

Host Plant Resistance: Based on ratings from previous years, 30 of the resistant converted exotic sorghums were reevaluated in "summary trials in 1979. Most of the 30 lines had lower midge damage ratings than the susceptible standard checks except when midge density was extremely high (200-400 midge adults/head/day). Mean damage ratings for replications (4) and locations (4) showed that the following seven converted lines suffered less than 40% seed loss due to sorghum midge: IS 7064C, IS 2579C, IS 3071C, IS 2549C, IS 8233C, IS 8231C, and IS 12666C.

Most midge resistant lines discovered have been "R" lines and the development of good midge resistant "B" lines has been the drawback in the production of hybrids. However, screening trials conducted during 1979 show that progress has been made in the development of agronomically improved midge resistant "B" lines. Of 67 "B" lines screened at Corpus Christi and Beeville, 10 lines suffered 35% or less midge damage. The least damaged "B" line received only 12% damage under extremely high midge density. In a College Station test of a different group of "B" lines only two suffered less than 50% midge damage. Resistance is obviously midge density dependent.

Good progress has been made in developing agronomically acceptable midge resistance "R" lines and such germplasm has been released. On 20 "R" lines tested in replicated trials at four locations (37 entries total) one-half of the lines suffered 44% midge damage or less (as little as 25% damage). Susceptible standard checks suffered 78% midge damage.

It is encouraging to see the advancement being made in the development of midge resistant sorghum hybrids. Hybrid screening trials conducted at four locations indicated that resistance is midge density dependent. In tests where midge numbers averaged approximately 5/head/day, hybrids suffered little damage. For example, of 65 hybrids screened at Beeville, five hybrids suffered less than 25% damage (some suffered less than 10% damage) while susceptible hybrids suffered from 70-100% damage. Some of the least damaged hybrids included: ATx 2761 x TAM 2566, ATx 2757 x TAM 2566, ATx 2753 x TAM 2566, ATx 2756 x TAM 2566, and ATx 2761 x TX 2374.

Biology and Population Dynamics: An understanding of the factors influencing diapause termination is essential to accurately describe the population dynamics of the sorghum midge. In an attempt to elucidate these factors, field collected diapausing larvae were subjected to differing combinations of temperature, photoperiod and humidity in the laboratory. It appears that the number of hours of daylight within each 24 hour period has little or no effect on adult emergence. Temperature; on the other hand, has a profound influence on the number of midge and the number of days required for adult midge to emerge from diapause. At optimum temperatures of 25° to 30° C, adults begin to emerge in as few as 15 days, while a higher and lower temperature, 30 to 40 days are needed. Using regression analysis, an emergence threshold was estimated to be 15.3° C. Exposure to high levels of moisture seems to be essential for the termination of diapause. This moisture can be in the form of free water or as high relative humidity. No

adults emerged from sample spikelets not subjected to a prolonged period of high moisture conditions.

From field studies, overwintering sorghum midge began to emerge from spikelets in soil on April 25 which exactly coincided with the first flowering of johnsongrass in the area. The entire emergence period lasted ca. 50 days, with peak emergence on May 15 after which numbers gradually declined and approached zero about June 11.

The activity of adult sorghum midge in relation to emergence of non-diapausing forms and subsequent oviposition in flowering panicles is an important area of study and is needed in support of host plant resistance studies, planting dates, sampling, and insecticidal control. Several factors influence this activity, i.e., temperature, humidity, radiation, etc. Data collected in 1979 indicate that males emerge 1-3 hours before females. Females mate soon after emergence and migrate to flowering panicles. Males remain swarming around the panicles. Peak oviposition occurs during mid-day in most cases, but this may be delayed and prolonged on cool, overcast days. On rainy days adult activity and emergence usually, significantly declines.

Developmental duration of sorghum midge from egg to adult requires 14 to 21 days with most developing in 17 days during the hot days of summer. During cooler periods, development is slowed and it is not uncommon for 20 to 24 days to be required for eggs to hatch and adults to develop and emerge. Temperature also effects adult longevity. During hot days 50% of both males and females confined in plastic cages had died after only 7 hours. On cooler days males (50%) lived 10 hours while females (50%) lived 24 hours. In most cases it appears that females live twice as long as males, but seldom more than one day. Consequently, sorghum is attacked by a new infestation of sorghum midge adults each day.

At least three species of parasites attack sorghum midge. Total parasitism increases as the season progresses, but the population dynamics of the parasite species differ substantially. Also, these parasites significantly influence midge population dynamics; especially numbers of overwintering midge larvae.

Advanced sorghum midge resistant germplasm, improved for agronomic quality was evaluated during 1979. "Summary" nurseries of four trials were screened at at least three locations. Of approximately 200 converted exotic lines, 30 were selected for final evaluation. Likewise, nurseries containing advanced "B" line germplasm and "R" line germplasm and nurseries of these in hybrid combination were screened. Data indicated that good progress is being made in transferring midge resistance into good agronomic parental lines and hybrids. Resistance, however, is midge density dependent.

Knowledge of sorghum midge biology and population dynamics has advanced from studies conducted in 1979. Midge diapause is terminated by exposure of larva to favorable temperature and moisture as indicated by bioclimatic chamber and field experiments. Adult midge oviposition lags behind adult emergence from infested panicles by several hours. Only females migrate to flowering panicles after mating with males which remain near the panicle from which they emerged. Many factors influence adult midge emergence. Females live twice as long as males but longevity for either is usually less than one day. Egg to adult developmental time is 17 days, but this increases as temperature decreases. At least three parasite species attack sorghum midge and this greatly influences midge population dynamics; especially numbers of overwintering midge larvae.

Economic threshold levels for four species of panicle infesting bugs were refined during 1979. Alternate host plants of these bug species were also identified.

PANICLE INFESTING BUGS

Damage to sorghum seed by four species of bugs was assessed during 1978. Seed weight and percent germination generally decreased as numbers of rice stink bugs, southern green stink bugs, leaffooted bugs, and conchuela stink bugs per panicle increased. Mean number of feeding punctures per seed and percent punctured seed generally increased as bug populations increased, especially at the highest infestation level. To some extent, weight of damaged seed decreased as the incidence of punctures increased. Damage to sorghum grain was largely due to direct feeding on seed by bugs, but indirect damage resulted from bugs feeding on panicle stems and rachis branches. Bugs were more injurious to grain in the milk and soft dough stages of development than to grain in the hard dough stage.

TROPICAL ADAPTATION

In 1979, a 30-entry standardized Tropical Adaptation Yield Test which included both parental lines and an array of hybrids were planted at several international and stateside locations. This array of materials was selected to cover the range of items known to have either specific or wide adaptation, as well as a broad base of base metabolic temperature differences. BTx3197, BTx378, and RTx7000 have high base temperatures and are described as more temperately adapted whereas BTx623, RTx430 and RTAM428 have substantially lower base temperatures and are adapted more to tropical areas producing sorghum. As previously described, the tropically adapted (TA) sorghums are those photoperiod insensitive types which have the capacity to produce a high and stable yield in short-day and high nighttime temperature areas.

Tropically adapted materials have performed extremely well in Mexico, Guatemala, El Salvador, Brazil, Tanzania, Ethiopia, Sudan, and Nigeria. In

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1978-79 ATx623 hybrids with RTx430, SC0326-6, 74CS5388, and ADN55 were very high yielding. These hybrids were all white or cream colored and should be useful for human food. In Mexico, the twin seeded TA hybrids under dryland conditions performed most desirably. The hybrids A1399 x SC3541 and ATx623 x SC0326-6 have shown very broad ranges of foliar disease resistance in addition to the high yield and wide adaptation.

Further laboratory data have been collected which demonstrates that the variations in base temperature are heritable. When two diverse lines were crossed (4.6°C x 11.5°C) the F^2 segregation indicated a near normal curve ranging from 5.3°C to 14.8°C base temperatures. These observations were based on 183 observations.

It was determined from phenological development studies that the tropically adapted materials spend proportionally more of their growth time in CSI and this is reflected in higher leaf numbers and substantially higher leaf areas. This advantage in leaf area gives the TA plants the ability to produce higher yields in areas of high night-temperature, as they do not become source limited but rather have sufficient leaf area to supply both the plants respiratory requirements and fill the grain as well.

INTERNATIONAL TROPICAL ADAPTATION TEST (TAT) - 1979

Texas Agricultural Experiment Station
(In Cooperation with USAID - ta-c-1384)
College Station, Texas

Year: _____

Location: _____

Entry No.	Designation ^{1/}	Parent or Hybrid	Variety Name, Hybrid, Pedigree	Seed Source	Plot Number ^{4/} Replication		
					I	II	III
1	BTx3197	P	Combine Kafir-60	FS	104	210	301
2	BTx378	P	Redlan	FS	104	210	309
3	RTx7000 ^{2/}	P	Caprock	78CS928	106	212	327
4	BTx623 ^{2/}	P	(BTx3197 x IS12661) sel.	FS	117	205	326
5	RTx430	P	(Tx2536 x IS12661) sel.	FS	114	201	308
6	RTAM428	P	BC ₁ of IS12610	FS	127	221	325
7	RS610	H	ATx3197 x RTx7078		125	207	318
8	ATx378 x RTx7000	H		TE/79	109	220	307
9	ATx399 x RTx430	H		Warn	112	209	311
10	ATx378 x RTx430	H		WAC	128	224	316
11	ATx623 x RTx430	H		Cros/79	126	217	323
12	ATx623 x RTx7000	H		78CS928	121	225	313
13	ATx622 x RTAM428	H		78CS923	102	204	310
14	ATx622 x RTx2536	H	is (Tx2536)	78CS857	107	227	305
15	ATx623 x SC0326-8	H	is BC ₁ of IS3758	1099*	110	202	320
16	ATx623 x 74CS538H	H	is (SC0599-6 x SC0110-9) sel.	1018	105	206	312
17	ATx623 x SC0599-11E	H	is BC ₁ of IS17459 (Rio)	893	122	213	306
18	ATx622 x 76CS388	H	is (Tx2536 x IS12661) sel	1026	103	229	308
19	ATx622 x 76CS500	H	(Tx414 x SC0120-8)-3-2-2-1-1	1637	108	215	302
20	ATx622 x 76CS478	H	(Tx414 x SC0108-)-15-1-2-1-1	1610	118	230	302
21	ATx623 x 76CS490	H	(Tx414 x SC0108-)-15-2-3-6-1-R	1631	115	203	303
22	ATx622 x 76CS510	H	(Tx414 x SC0120-8)-3-2-2-1-3	77TS046	119	208	302
23	ATx622 x ADN56	H	(SC0120-6 x Tx7000) sel	78CS1700	123	211	329
24	ATx622 x 76CS5274	H	Twin Seed, dominant	996	129	226	324
25	ATx622 x 72T123-1	H	Twin Seed, dominant	866	130	216	315
26	ATx623 x 76CS4409	H	Twin Seed, dominant	961	124	219	330
27	A1399 x CS3541	H			101	222	304
28	A1399 x 77CS1	H			113	228	322
29	A1399 x 77CS3	H			116	214	328
30	A4R x RTx430 ^{3/}	H			111	223	314
31	Local ^{3/}				131	232	331
32	Local ^{3/}				132	231	332

** 8 grams seed per envelope (250-300 seed for 2 short (10-15 feet) or 1 long (20-30 feet)) row. Seed is treated with Captan and Chlordane.

^{1/} This test contains several hybrids (and some parental lines) with good "tropical adaptation".

^{2/} Female pedigrees of ATx622, ATx623 and A1399 is BTx3197 x SC0170-6 (IS12661), ATx399 is Wheatland, A4R is A(BTx406 x Rio).

^{3/} Please add local varieties or hybrids meaningful to the test. Two hybrids with 50 grams each (ATx399 x RTx430, and ATx378 x RTx430) are enclosed for use as border rows or for use in the two open entries if you have no local entries.

^{4/} Test should be planted using these numbers as plot numbers.

^{5/} Please take following data if possible:

- | | |
|--|--|
| a. date (days) to flower | g. disease ratings on any diseases in test (see rating scheme) |
| b. plant height (and head exertion) | h. insect damage ratings if occur |
| c. number of harvested heads | i. grain weathering ratings |
| d. threshed grain weight | j. any other notes such as bird damage, stand %, etc. |
| e. 100 kernel weight | |
| f. overall desirability rating (based on yield and overall adaptation) | |

^{6/} We normally hand harvest heads from portions of each plot (as little as 6 feet, 6 inches (of 40 in. row) = 1/2000 of an acre)

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Table 7. 1979 locations of TAES hybrids and lines for yield, adaptation and growth and development evaluations (includes International Tropical Adaptation Trials, yield observations, and variety evaluation).

Location	No. trials	No. entries	Cooperator
Brazil	1	12	R. Schaffert
Colombia	1	30	A. Grobman, CIAT
Egypt	3	30	S. I. Salama
El Salvador	1	30	R. Clara
Guatemala	1	295	A. Plant
Indonesia	1	33	I. S. Suwelo
Nigeria	1	30	H. Van Arkel
Nigeria	1	30	N. V. Sundaram
Peru	2	21	M. A. Neciosup
Somalia	1	6	M. A. Dukseyeh
South Africa	2	30	H. M. Muller
Mexico			
Celaya, Guanajuato	1	30	G. Vega
Culiacan, Sinaloa	1	30	c/o G. Vega
La Barca, Jalisco	1	225	S. Medina
Rio Bravo, Tampico	1	30	H. Williams
Tampico, Tampico	1	30	L. Sottero
Zacatepec, Morales	1	30	A. Avila
United States			
Texas: College Station	1	50	F. R. Miller
Corpus Christi	1	25	L. Reyes
Dallas	1	25	B. Simpson
Lubbock	1	25	D. T. Rosenow
Lubbock	1	50	D. T. Rosenow
Thrall	1	25	F. R. Miller
Weslaco	1	50	R. Creelman
Nebraska: Lincoln	1	25	J. Eastin

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POPULATIONS

Population development in 1979 involved the following populations:

1. TP15RB - broad base conversion - released
2. TP17R - yellow endosperm - third random mating
3. TP18RB1 - low smut (head) - second random mating - select S_1 's for head smut evaluation in 1980
4. TP20RB - weathering resistant - first random mating following selection of half-sibs for weathering resistance from 1978 random mating
5. TP21RB - food type (white or colorless pericarp) - second random mating following selection of S_1 's for weathering resistance and non-stained grain from Lubbock, 1978 planting of TP21RB₀₂.
6. TP22RB - leaf disease resistant - fourth random mating - select S_1 's for evaluation in 1980

Half-sibs from the low smut population, TP18RB, were inoculated with head smut to begin a selection scheme based on half-sib evaluation. Remnant seed from resistant half-sibs were bulked for random mating in Puerto Rico during the winter, 1979-80.

A new population, TP23B, a B-line population with midge resistance was formed and sent to Puerto Rico for its first random mating during the coming winter of 1979-80.

Another new population, TP24R, with white or colorless seed and only tan plants, was developed by bulking seed of various lines and breeding selections. It will be random mated in Puerto Rico during the winter of 1979-80.

Summary of releases during
AID Contracts ta-c-1092 and ta-c-1384

Lines and Germplasm Stocks

TAM 428*	Disease resistant pollinator line - tropically adapted
Tx 430	Disease resistant pollinator line - tropically adapted
Tx 431**	Twin-seeded pollinator line
Tx 622	Disease resistant A & B line - tropically adapted
Tx 623	Disease resistant A & B line - tropically adapted
Tx 624	Disease resistant A & B line - tropically adapted
TAM 2566*	Midge resistant line
TAM 2567*	Greenbug resistant yellow endosperm pollinator
TAM 2568*	Greenbug resistant yellow endosperm pollinator
120 converted lines*	Also temperate and tropical bulks
TAM 2574-TAM 2713	Gaines-Karper nursery breeding stock lines
Tx 2714-Tx 2733	Disease resistant zerazera derivative restorer lines
Tx 2734-Tx 2748	Greenbug resistant restorer lines
Tx 2749-Tx 2752	Greenbug resistant A & B lines
Tx 2753	New cytoplasmic-genic sterile A & B lines (A2 cytoplasm)
Tx 2754-Tx 2761	Midge resistant A & B lines
Tx 2762-Tx 2781	Midge resistant lines
ISR-1	Midge resistant R line (cooperative with Brazil)

Germplasm Bulks

TAM Bk-43*	Greenbug resistant B-line bulk
TAM Bk-44*	Greenbug resistant B-line bulk
TAM Bk-45*	Downy mildew resistant yellow endosperm zerazera bulk
TAM Bk-46*	Downy mildew resistant yellow endosperm zerazera bulk
TAM Bk-47*	Downy mildew resistant yellow endosperm zerazera bulk
TAM Bk-48*	Downy mildew resistant yellow endosperm zerazera bulk
TAM Bk-49	Downy mildew resistant sudangrass germplasm bulk
TAM Bk-50	Downy mildew resistant forage germplasm bulk
TAM Bk-51	Maize dwarf mosaic virus resistant germplasm B-line bulk
TAM Bk-52	Maize dwarf mosaic virus resistant germplasm R-line bulk

Populations

TP1R	Greenbug resistant
TP8R	Midge resistant
TP11	Twin-seeded population
TP15RB**	Broad base, conversion lines
PR2BR	Day length insensitive, ms ₃ (cooperative with SEA, AR, MITA, & UPR)
PR4BR	Day length insensitive, ms ₇ (cooperative with SEA, AR, MITA, & UPR)
RSP3BR	Downy mildew resistant sudangrass germplasm (cooperative Kansas, Texas, and MITA - Puerto Rico)

* Developed prior to beginning of AID contract, but officially released after contract started.

** Released in 1979.

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INTERNATIONAL SHORT COURSE ON BREEDING
PLANTS FOR RESISTANCE TO PESTS

A short course on breeding plants for resistance to pests was conducted from July 23 to August 12, 1979 for plant protection specialists and plant breeders from developing countries. The short course was conducted under the joint sponsorship of Texas A&M University and the University of California/AID Pest Management Project. Drs. G. L. Teetes and R. A. Frederiksen served on the Planning and Implementation Committee along with Dr. G. A. Niles and M. K. Harris (chairman).

The first phase of the short course (2 weeks in Texas, July 23 to August 3) included lectures, laboratory exercises, and field demonstrations of the principles and techniques for breeding cotton, sorghum, rice, corn, peanuts, forage grasses, and certain grain legumes resistant to arthropod and disease pests. The second phase of the short course included participation in the IX International Congress of Plant Protection, Washington, D.C. (August 5-12).

Instruction was by professional plant protection specialists and crop breeders with overseas experience and familiarity with agricultural conditions in developing countries. Subject matter for the workshop included lectures on principles of host plant resistance to pests, the integrated approach to pest management and crop production, economics of pest management, and application of plant genetics to breeding for plant resistance. Practical exercises demonstrated techniques for breeding and evaluating plants for pest resistance. Field trips were made to show how insect and disease resistant varieties of cotton and sorghum must be managed on commercial farms to produce maximum profits.

A proceedings of the short course lectures will be published.

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SORGHUM WORKSHOP

On June 25-26, 1979 a sorghum workshop was held at the Texas A&M Research and Extension Center at Corpus Christi. The workshop provided visiting sorghum research scientists and others representing commercial companies and public institutions from the U.S. and Mexico with an overview of sorghum research being conducted by Texas A&M University with special emphasis on the Coastal Bend region.

The workshop was initiated June 25 at the sorghum nursery site near Berclair, Texas where workshop participants heard Drs. R. A. Frederiksen, F. R. Miller, J. W. Johnson, and G. N. Odvody discuss the various research projects contained within the Coastal Bend sorghum nurseries. A tour of the Berclair and Beeville, Texas sorghum nurseries followed with special attention to the insect and disease reactions.

On the evening of June 25 a technical papers session was held at the Research Center in Corpus Christi. Staff and graduate students from Texas A&M discussed results of their research on sorghum physiology, breeding, diseases, and insects.

Workshop participants toured the sorghum disease nursery at the Perry Foundation, Robstown, Texas on June 26. The nursery included disease research on anthracnose, zonate leaf spot and other foliar diseases, host resistance, cultural and chemical control of sorghum downy mildew, and grain mold and grain weathering research. Participants returned to the Corpus Christi Research Center and toured the Corpus Christi sorghum nursery.

On the afternoon of June 26 the workshop concluded with a panel discussion entitled "Environmental Adaptation in sorghum. Sources: Parameters? Limits?" Dr. H. E. Joham, Head of the Department of Plant Sciences, Texas A&M University, acted as moderator. Initial presentations were made by

panel members Dr. F. R. Miller, sorghum breeder, Texas A&M University; Dr. R. A. Creelman, sorghum physiologist, Texas A&M University; Dr. Jerry Eastin, sorghum physiologist, University of Nebraska; and Dr. Gene Dalton, Sorghum Research Director, Pioneer Hi-Bred International, Plainview, Texas. After the initial presentations an open discussion was held amongst panel members and all workshop participants. Portions of the sorghum workshop were attended by Dr. Perry Adkisson, Vice President for Agriculture and Renewable Resources, Texas A&M University.

" . . .we find sorghum germplasm from Texas very useful in our breeding program. Breeding materials which have done well and are being widely used in our breeding program are the A&B lines of Tx 622, 623, 624 and recently TS 7701. The R line Tx430 continues to look very promising under our conditions. I feel continuing germplasm exchange and reciprocal visits of staff between our two programs could continue to be mutually advantageous."

from a letter written by
Brhane Gebrekidan to F. R. Miller
on June 12, 1979.

PERSONS REQUESTING AND RECEIVING SEED OF DISEASE AND INSECT
RESISTANT SORGHUM LINES OR GERMPLOSM IN 1979

INTERNATIONAL:

Argentina:	Arnalda, D. E. Llobet, F. Mustapic, A. H. Pacagnini, H. J.	Mexico:	Aranza, E. Camplis, J. V. Guiragossian, V. Santiago, Median D.
Australia:	Hare, B. W. Henzell, R. G.	Nigeria:	Sundaram, N. V.
Bangladesh:	Auch, D.	Peru:	Neciosup, M.
Brazil:	Borgonovi, R. Fonseca, J. N. L. Giacomini, F. Rossetto, C. J. Schaffert, R.	Philippines:	Dalmacio, S. C.
Canada:	Major, David J.	Senegal:	Chantereau, J. Gahukar, R. T.
Egypt:	Salama, S. I.	South Africa:	Muller, H. M.
El Salvador:	Andrews, K. L. Clara, R.	Tanzania:	Mukura, S. Z.
Ethiopia:	Gebrekidan, B.	Thailand:	Samphantharak, K.
France:	Cogat, M. Kaan, F.	Upper Volta:	Bonzi, S. N.
Honduras:	Peairs, F. B.	Uruguay:	Artola, A. P.
India:	House, L. R. Jotwani, M. G. Mehra, K. L. Narasimhamurty, K. Nath, Bholi Prasada, M. N. Rao, K. E. P.	Venezuela:	De Cordova, O. R. Riccelli, M. Romero, F. T.
Indonesia:	Suwelo, I. S.		
Japan:	Hoshino, T.		
Kenya:	Dabrowski, Z. T. Pinto, F.		
Korea:	Kim, S. K.		
Malawi:	Beck, B. D. A. Manda, R. B.		

U.S. PRIVATE BREEDERS:

ACCO Seed	Kramer, N. W.
Asgrow Seed Co.	Wilson, J. M.
Battelle	Kidd, G.
Cargill, S. A.	Barberia, C.
Coffey Seed Co.	Coffey, L. C.
Cokers Ped. Seed Co.	McDonald, Lynn
Conlee Seed Co.	Lacy, M. D.
Crosbyton Seed Co.	Boardman, N. R.
DeKalb AgResearch, Inc.	Lambright, L., Maunder, A. B.
Funk Seed International	Rogers, Rod
Gold Kist Inc.	Glueck, James
Growers Seed Assoc.	Nelson, Mike
Harpool Seed Co.	Dean, L.
NC+ Hybrids	Foster, J. H.
Northrup King Co.	Sidwell, Raymond
PAG	Berry, C. D.
Pioneer Hi-Bred Int.	Kidd, H. L., Faber, Joe
Ring Around Seed	Elsworth, R. L.
Taylor-Evans Seed	Allison, James
Texas Triumph Seed Co.	Koepp, Noble
WAC Seed Co.	Ramey, Payton
Weather Master Seeds	Greenley, K. L.
R. C. Young Seed Co.	Arnold, Keith, Melton, H. L.
7700, Inc.	Longoria, J.

U.S. PUBLIC BREEDERS:

University of California	Worker, G. F.
University of Georgia	Duncan, R. R.
Iowa State University	Atkins, R. E.
Kansas State University	Clafin, L., Mize, T. Kissel, D.
Mississippi State University	Gourley, Lynn
University of Nebraska	Ross, Bill
Oklahoma State University	Weibel, D. E.
Purdue University	Axtell, John
Texas A&M University	Hossner, Mustain, Vietor, Sullivan
Texas Tech University	Rao, Arelli; Clegg, Max
USDA, SEA-AR, New Orleans, LA	Neucere, Joe
USDA, SEA-AR, College Stn. TX	Bashaw, E. C.; Craig, J.
USDA, SEA-AR, Florida	Chourey, P.S.
Arizona	Dobrenz, A.
Arkansas	York, J.
California	Worker, G.
Colorado	Hinze, Greg
Georgia	Duncan, R.
Kansas	Hackerott, H.
Nebraska	Clark, R. B.; Eastin, J.
Texas	Reyes, Lucas

PERSONS REQUESTING AND RECEIVING SEED OF TAM Bk-51 and TAM Bk-52

U.S. PRIVATE BREEDERS

ACCO Seed Company	Plainview, Texas
Asgrow Seed Company	Plainview, Texas
Conlee Seed Company	Waco, Texas
Coffey Seed Company	Plainview, Texas
Coker's Pedigreed Seed	Lubbock, Texas
Continental Grain Company	Chicago, Illinois
Crosbyton Seed Company	Crosbyton, Texas
DeKalb AgResearch	Glennvil, Nebraska
DeKalb AgResearch	Lubbock, Texas
Funk Seeds International	Lubbock, Texas
Gold Kist	Ashburn, Georgia
Growers Seed Association	Lubbock, Texas
Harpool Seed Company	Denton, Texas
Hoegemeyer Hybrids	Hooper, Nebraska
Land O'Lakes	Webster City, Iowa
NC+ Hybrids	Hastings, Nebraska
Northrup, King & Co.	Eden Prairie, Minnesota
Northrup, King & Co.	New Deal, Texas
P-A-G Seeds	Lubbock, Texas
Pacific Oilseeds	Woodland, California
Pioneer Hi-Bred International	Plainview, Texas
Ring Around Research	Plainview, Texas
Taylor-Evans Seed Company	Tulia, Texas
Texas Triumph Seed Company	Ralls, Texas
WAC Seed Company	Hereford, Texas
George Warner Seed Company	Hereford, Texas
R. C. Young Seed & Grain	Lubbock, Texas

U.S. PUBLIC BREEDERS

University of Nebraska	W. M. Ross
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INTERNATIONAL BREEDERS

Pacific Seeds	Australia
SARH INIA CIAN	Mexico

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PERSONS REQUESTING AND RECEIVING SEED OF TP 15RB**U.S. PRIVATE BREEDERS**

Continental Grain Company	Chicago, Illinois
Hoegemeyer Hybrids	Hooper, Nebraska
Land O'Lakes	Webster City, Iowa
NC+ Hybrids	Hastings, Nebraska
P-A-G Seeds	Lubbock, Texas
Taylor-Evans Seed Company	Tulia, Texas
Texas Triumph Seed Company	Ralls, Texas
WAC Seed Company	Hereford, Texas
R. C. Young Seed & Grain	Lubbock, Texas

INTERNATIONAL VISITORS
to the
Texas Agricultural Experiment Station and Its Personnel

NORTH AMERICAMexico:

Chaudhary, Hans R.
Garza, Jose
Ramos, Guillermo Federico

Guatemala:

Plant, Albert N.

Cuba:

Castro, Luid Lago

SOUTH AMERICAArgentina:

Casagrande, Miguel
Mendez, Federico
Scantamburlo, Jose L.
Scheidl, Guillermo H.

Brazil:

Borgonovi, Fenato Antonio
Fernandez, Nelson Gimenes
Giacomini, Fredolino
Schaffert, Robert

Venezuela:

Calabria, Jose Miguel
Giral, Helio Campos
Mores, Atilio A. H.
Suarez, Antonio
Taborda, Felix

EUROPEEngland:

McNeil, D. G.

France:

Kaan, Francois

ASIAChina:

Botian, Li
Jiusi, Zhu
Ke, Fu
Qisheng, He
Silin, Tang

India:

Gill, S. S.
Nath, V. Ravindra
Ragnunathan, V.
Reddy, K. V. Seshu
Sahni, V. N.
Sant, M. V.
Seetharama, N.
Williams, Robert J.

Japan:

Hoshino, Tsuguhiro

Nepal:

Pickett, D. R.

AFRICAEthiopia:

Brahani, Dr.
Gebrekidan, Brhane

Guinea-Bissau:

Barbos, Ahala Zamora
Da Silva, C. S.

Nigeria:

Sundaram, N. V.

Somalia:

Duksiyeh, M. H.
Dule, A. A.
Fardi, A. J.

Sudan:

Ali, Mahmoud

Upper Volta:

Nacro, Mouhoussine
Quattara, Karamokotie'Boura
Suchet, L. Louis
Toguyeni, Yembila A.

Zambia:

Kapooria, R. G.

South Africa:

McOmie, J. B.
Rensburg, N. J.
Cowie, Trevoa

SUMMARY TRIP REPORTS

CHINA

The following are observations of Dr. Frederick R. Miller, member of the U.S. Germplasm Team, August 13, 1979 to September 13, 1979, in the People's Republic of China.

The mission of the United States Germplasm Team was to study and arrange for the exchange of germplasm with the People's Republic of China. Those crops to be considered were soybeans, sorghum, millets (setaria) and vegetables. Research scientists in both countries share the belief that plant germplasm provides the foundation for a productive agriculture both now and into the future, and that both countries as well as other peoples will benefit from cooperative exchanges of such germplasm.

The team was very well received throughout China where we travelled. Research scientists, especially agronomists and plant breeders, were available to each institute that was visited to explain the respective programs and to discuss exchange of germplasm. Each breeder or commodity worker gave a general statement of the magnitude of each program including data on ranges of diversity of specific traits. The breeding programs were very much similar to those of the U.S. or other areas, especially in their objectives. These were given as higher yields, disease and insect resistance, and some attention to quality or end use characteristics. Discussions generally centered on the amount of diversity, its collection, maintenance, use and exchange. There was particular attention given to the commodities which did not originate in China or where major improvement had been made in the U.S.

Broadly, diseases were not a major problem except in certain commodities such as viruses on tomatoes, peppers and in some areas on maize and sorghum.

Head smut of maize was observed in the Northern areas of Liaoning Province and common smut below Beijing. There were problems with insects but the breeders were vigorously looking for host resistance. Head smut of sorghum and loose kernel smut were present as well as MDMV and in some areas there were yield reductions associated with them.

Arrangements were made to make contacts for seed through Dr. George White's office - Dr. George White, Plant Introduction Officer, Building 101, Room 322, BARC-West Germplasm Resources Laboratory, Beltsville, MD 20705. As seed becomes available, it will be distributed to anyone who wants it. Similarly, requests for seed from China should be directed to Dr. White, who will coordinate and assist in routing the requests to the correct Chinese scientists.

Those persons especially concerned with sorghum are as follows:

1. Xu Yun-Tain, Deputy Director of the Crop Germplasm Institute
Chinese Academy of Agricultural Sciences
Beijing, People's Republic of China
2. Yen Shi-Sheng, Associate Researcher, Sorghum Breeder
Agricultural Academy of Heilungjiang Province
Harbin, People's Republic of China
3. Kou Xin-San, Senior Researcher in Sorghum
Henan Provincial Agricultural Academy
Zhenzhon, Henan, People's Republic of China
4. Chen Bao-Chin, Head, Sorghum Laboratory
Northwest Water Conservation Institute
Shaanxi Provincial Agricultural and Forestry Academy
Wu Kung, Shaanxi, People's Republic of China
5. Mrs. Chang Xie-Ning, Sorghum Researcher
Shandong Provincial Agricultural Academy
Jinan, Shandong, People's Republic of China
6. Chao Kai-To, Sorghum Breeder, Director of Crop Breeding Institute
Liaoning Provincial Agricultural Academy
Shenyang, Liaoning, People's Republic of China

GUATEMALA

During May 15 to 18, 1979, Dr. Jerry W. Johnson, Mr. James Phillips, and Dr. George L. Teetes traveled in Guatemala to evaluate sorghum midge resistant sorghums. Seed for these nurseries had previously been sent to Dr. Albert Plant, USAID, Sorghum Program Coordinator. The nurseries were planted on the Cuyuta Experiment Station of the Instituto De Ciencia y Tecnologia Agricola (ICTA). The Cuyuta Experiment Station is south of Esquintla which is 55 kilometers south of Guatemala City.

Most of the time was spent in the nursery at Cuyuta. A sorghum midge resistant sorghum hybrid test was evaluated. The test contained 25 entries with four replications. The agronomic qualities of the hybrids were good and the resistance level comparable to that observed in the USA. Sorghum midge density was less than desired, but appeared to be adequate for the second planting which was just past flowering at the time of our visit. One of the technicians at the Cuyuta Station, Ing. Edgar Oliva, assisted in rating the midge resistant hybrid test. He will rate the later planted test and is certainly qualified to do so.

Over 500 selections were made in 750 rows of sorghum breeding material. Selections were made in F_2 and F_3 generation material segregating the midge resistance and agronomic quality. Most of the material in the nursery looked extremely good under Guatemalan conditions in this "off season" planting. Especially encouraging was the quality and yield potential of several midge resistant B-lines. It is hoped that Dr. Plant will begin the process of sterilizing these B-lines as rapidly as possible.

Much of the yellow endosperm material derived from AF-28 x Tx 2536 types had weathered poorly. However, the midge resistance level of AF-28 looked good, but the line is far too tall.

This nursery allowed the opportunity to make some decisions concerning which A-lines to use in future midge resistant hybrids. Special attention was given to white seeded sorghum since much of the sorghum in Guatemala will be destined for human food.

Three technicians from the Cuyuta Station observed the selection process. They were Ing. Jorge Fuentes, Ing. Victor Salguero, and Ing. Hector Villedo. Each of these men appeared well trained, interested, and enthusiastic. It is apparent that Dr. Plant has spent a considerable amount of time with these young men.

It was encouraging to note the progress that the Texas sorghum material has made in regard to tropical adaptation. Much improvement has been made in the last two years and sorghum yield potential in Guatemala is extremely good. Sorghum midge resistant sorghums would certainly add to the stability of yield in that country.

After evaluation and selection in the nursery was completed, a tour was made into the southern, drier areas of Guatemala toward the Pacific Coast. The tour was made to observe local sorghums and production techniques. In the area, most of the small farmers had already planted sorghum and it looked extremely good. Recent rains had provided ideal growing conditions. The "rainy" season had just begun. The tour took us through Taxisco and Chiquimulilla and back to Guatemala City.

In Guatemala City, we had the opportunity to confer with Dr. Carl Koone, Rural Development Officer for the local USAID Mission, and Clem Weber, Assistant Rural Development Officer. The cooperative (Texas-Guatemala) sorghum research activities were reviewed. It was apparent that these activities are mutually beneficial and should continue. Dr. Koone was interested in the Sorghum/Millet Title XII project. He was brought up to date on recent developments and prospects.

MEXICO

From July 10 to July 14, 1979, Victor Bozzuffi travelled in Northern Mexico to observe USDA/APHIS disease monitoring plots and to establish contact with local sorghum workers. Cooperative research activities were discussed with personnel at the Agricultural Research Station located in Rio Bravo Tamaulipas. Among those considered were chemical control of sorghum diseases (sorghum downy mildew and rust) as well as germplasm exchange.

The remainder of the trip was spent observing disease monitoring plots installed at Rio Bravo, Zaragoza and Matamoros, Coahuila and Apodaca, Nuevo Leon. All diseases (grey leaf spot, sorghum rust, MDMV, zonate leaf spot, rough spot, and head smut) had previously been reported in Texas. Commercial sorghum growers were in the midst of harvesting operations, yields being good to excellent (3-4 tons/hectare) due to favorable weather throughout the growing season.

Contacts were made at Rio Bravo with Ing. Enrique Calles, station director and Ings. Rodolfo Giron and Julio Aguirre, pathologists. At the Zaragoza station contact was made with Dr. Raul Flores and Ricardo Lozano, station director and pathologist, respectively. Contact was also made with Ing. Victor Manuel Valdez, Matamoros station director, and Dr. Teodoro Herrera, pathologist. At the University of Nuevo Leon a brief summary on sorghum diseases and our activities in Mexico to graduate students in plant pathology was shared with Ing. Ciro Valdez and Dr. Jose de la Garza.

From August 17 to August 19, 1979, Victor Bozzuffi travelled to CIMMYT headquarters at El Batan, Mexico. He met with Dr. Vartan Guiragossian, ICRISAT sorghum breeder stationed at CIMMYT. A tour was made of

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Dr. Guiragossian's upland (high elevation) nursery where he is selecting for cold tolerant, early maturing genotypes. This was followed by a trip to Poza Rica, Veracruz to observe lowland nursery material. Specific screening at Poza Rica includes army worm and stem borer resistance, food types, as well as disease resistance (foliar and grain mold). Objectives here include selection of breeding lines with wide adaptation to tropical and subtropical conditions.

From September 2 to 8, 1979, a follow-up trip was made to CIMMYT at El Batan to observe and evaluate disease reactions of sorghum breeding material at Poza Rica. Cercospora sorghi, Puccinia purpurea, and Gleocercospora sorghi were the evident foliar pathogens while species of Curvularia, Fusarium, Olpitrichum, Phoma, and Penicillium were found in abundance on the grain. Mold due to the latter organisms was extremely severe while few entries manifest significant levels of resistance. Among those with low (1.2-1.5 on a 1-5 rating scale) were M36368, M36285, 1319A x CS3541, 1319A x 77CS1, ATx611 x GPR148, 1778A x GPR148, SC170-14-1, (IS145 x SC3541)-29-1 L.P., CENTA S-1-76-1.

Material was selected based on plant type, desirability, disease resistance, etc., which will be included in a tropical sorghum yield trial to be distributed to cooperators in Central and South America. Lines with wide adaptation will subsequently be returned to CIMMYT for further crossing to enhance that particular character.

From September 23 to September 25, 1979, October 9 to October 11, 1979, and November 26 to 30, 1979, Victor Bozzuffi travelled to Guadalajara, Jalisco. Contacts were made with local seed company representatives as well as INIA sorghum workers in install chemical control plots for sorghum.

rust. Applications were made during the first two visits while harvesting and final evaluations were made during the final trip at which time a disease survey was conducted in the Cienaga region.

Results obtained from the test plots failed to indicate differences between treated and untreated entries. Disease pressure was severe in the area and as a result it was concluded that the product offered no control of the organism. Further studies may possibly be made at a future date. INIA workers were involved in the project and will be encouraged to assume leadership now that the proper techniques have been established.

A disease survey of the Cienaga region (approximately 95 km southeast of Guadalajara) revealed a widespread incidence of sorghum leaf blight. Actual damage could not be accurately assessed due to a severe drought throughout most of the central sorghum production region. Losses in some areas are estimated at more than 50% of potential yield which in optimal years can reach 9 to 10 tons per hectare.

Restricted outbreaks of sorghum rust were also encountered, however, they appeared to be much less damaging than those due to leaf blight. All production fields visited were found to be infected with sorghum downy mildew although the incidence was variable among the locally grown hybrids, ranging from a trace to 15% infected plants. Grey leaf spot, bacterial stripe and head smut were also observed although their incidence was considered minimal.

BRAZIL

From February 7 to February 17, 1979, M. A. Pastor-Corrales travelled to Brazil to conduct a disease evaluation, with emphasis on anthracnose, of the TAES nurseries planted in some of the sorghum growing areas of Brazil. Dr. Robert Schaffert, Sorghum Project Leader and Sorghum Breeder for the Brazilian Corn and Sorghum National Project (SNPMS/EMBRAPA) was in charge of the nurseries and coordinated the trip. F. T. Fernandes and N. de Almeida (sorghum pathologists) and R. A. Borgonovi and F. Giacomini (sorghum breeders) assisted with my visits to the field.

I arrived at the Corn and Sorghum National Research Center located in Sete Lagoas, M. G. on February 7, 1979. The following day I departed for Riberao Preto, S. P. where I evaluated some of the Brazilian National Sorghum Trials. Sorghum germplasm from the TAES has been incorporated in some of the sorghum lines tested here. The following nurseries were evaluated: The Sweet Sorghum National Trial (ENSS), The Grain Sorghum National Trial (ENSG), and the Commercial Grain Sorghum National Trial. Anthracnose was present throughout the fields and several lines and hybrids susceptible to the anthracnose pathogen were severely attacked by it. Some of the sorghum material which is resistant to anthracnose in the United States was susceptible here, i.e., sorghum lines Rio and Wiley. Brandes, a sorghum line from the U.S. and its progeny were very resistant to anthracnose. In Sao Paulo I also visited the sorghum field of Sementes Contibrazil Ltd., a seed company located in Cravinho. Much of their concern in this company is the incorporation of resistance in sorghum to anthracnose.

On February 12 I returned to Sete Logoas where I evaluated the International Disease and Insect Nursery (IDIN), The Converted Line Anthracnose Test (CLAT), and the National trials for sweet and grain sorghum, and the Sorghum Disease National Nursery. Anthracnose was more widespread and

severe here, due perhaps to environmental conditions (lots of rain and humidity) very conducive to anthracnose development. Here, again, some of the U.S. resistant sorghum lines were severely attacked by the anthracnose pathogen [TAM428, BTx624, SC110-14, SC112-14, SC170-14, SC173-12, SC175-14, SC237-14, SC599-6(9247) and SC598-11E]; however, some of the traditionally U.S. susceptible lines were not attacked (BTx398 and QL3). Some other diseases observed were: grain molds (*Fusarium* sp., *Curvularia* sp.), rust (*Puccinia purpurea*), zonate leaf spot (*Gloeosporium sorghi*) and sheath blight (*Sclerotium rolfsii*).

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FUNGAL ANTAGONISM AMONG FOUR SORGHUM GRAIN MOLD FUNGI: IMPORTANCE OF SCREENING PROGRAMS

L. L. Castor, R. A. Frederiksen, K. N. Rao, and R. J. Williams

ABSTRACT

During the 1978 rainy season, 10 Indian grain sorghum lines were grown at ICRISAT Center, Hyderabad, India. Heads were inoculated at flowering with spores of *Fusarium moniliforme* (FM), *Fusarium semitectum* (FS), *Curvularia lunata* (CL), and mixtures of FM-FS (F), and FM-FS-CL (FC). *Phoma sorghina* (PS) occurred naturally on the grain and was uniformly distributed throughout the experimental nursery. Data were taken during grain development and on harvested grain to determine the relative importance and interactions of the 4 fungi. FM and CL were more damaging than FS. FM and CL reduced moisture content, weight, size, and germination of the kernels. FM and CL increased percent molded kernels while FM increased pre-harvest sprouting. FS caused little or no damage compared with the controls. Damage was less with F and FC mixtures indicating an antagonistic interaction with FS. Inoculating with FS at flowering protected the developing grain from FM and CL through harvest. Significantly greater PS occurred on the uninoculated grain compared with grain from fungal inoculated heads. Natural FS inoculum may interfere with the expression of susceptibility to FM and CL in screening programs. FM, FS, and CL appear to interfere with the expression of susceptibility to PS. Resistance to FM, CL, and PS seems to be polygenic and independent.

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SCREENING FOR STRESS TOLERANCE IN SORGHUM

L. E. Clark, D. T. Rosenow, and A. J. Hamburger

ABSTRACT

Grain sorghum lines and hybrids with early season stress tolerance were identified in tests at Chillicothe, Texas in 1978. Sorghum was under moisture stress compounded by extremely high temperatures from planting through the flowering stage. These conditions resulted in stress symptoms ranging from extreme leaf rolling, bleaching of leaves, leaf firing, blasting of panicles, and delayed flowering. Subsequent rainfall during the following flowering permitted those plants which more effectively tolerated the early stress to respond favorably and produce yields in excess of 4500 kg/ha. Hybrids that flowered in 70 days or less were among the highest yielding, but some exceptions existed. Some hybrids that flowered in 75 to 85 days tolerated early stress and responded to mid and late September rains. Another important factor associated with adaptation of hybrids to these conditions was the rate at which grain drying occurred during October. Grain from many of the higher yielding hybrids was less than 15% moisture at harvest compared to values greater than 20% for other hybrids.

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THE QUANTITATIVE RELATIONSHIP OF *SPHACELOTHECA REILIANA* TELIOSPORES AND
INFECTION OF MAIZE HYBRIDS AND INBREDS
J. H. Foster and R. A. Frederiksen

ABSTRACT

The relationship of the number of *Sphacelotheca reiliana* teliospores and infection of maize hybrids and inbreds was investigated using sterilized soil selected from fields with a history of maize head smut and without a history of head smut. Teliospores were harvested from smutted plants and sifted through a #400 mesh screen. Teliospores were added to the sterilized soil with a history of head smut for a 10^{-1} dilution (1 part teliospores to 10 parts soil on volume to volume ratio). Serial dilutions were made through a 10^{-10} dilution. Susceptible maize hybrid P3369A and inbreds SC229, C123 and LH20 were used to determine the relationship between densities of teliospores and incidences of head smut. The resulting infection levels demonstrated a linear relationship between the number of teliospores present and the infection percentages ($r = -0.9642$). When a soil without a previous history of head smut was used, significantly fewer infections occurred in P3369A. This was interrupted as a fungistasis action in the soil without a previous history of head smut. There was no significant difference between the 10^{-1} , 10^{-2} , and 10^{-3} dilutions probably because of teliospore saturation. Significant differences for percent infection occurring at the selected dilutions demonstrated that there were differences in the relative susceptibility of the three inbreds.

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EFFECT OF NIGHT TEMPERATURE ON PHENOTYPIC PERIODS AND YIELD COMPONENTS OF
GRAIN SORGHUM

J. R. Gipson, J. W. Johnson, and D. T. Rosenow

ABSTRACT

A temperate hybrid (RS 671) and a tropically adapted hybrid (A Tx 623 x Tx 430) were grown from emergence to maturity under 14 and 22 C night temperature regimes in the field growth chambers in 1977 and 1978. In addition, night temperature control was maintained from emergence to bloom on RS 671, A Tx 623 x Tx 430, and RS 610 in 1977 and on the same three plus A Tx 378 x Tx 430 in 1978. Of the three phenological periods, emergence to panicle initiation (GS-1), panicle initiation to bloom (GS-2), and bloom to maturity (GS-3), the greatest temperature response occurred in GS-2. Although length of both GS-1 and GS-3 were modified by temperature, the overall effect was not as great as in the GS-2 period. Of the cultivars studied, the tropically adapted hybrid, A Tx 623 x Tx 430, was the least sensitive to temperature, while the temperate hybrid, RS 671, was the most temperature sensitive. There were no temperature effects on yield components when treatments were terminated at bloom, but temperature effects were apparent when treatments were imposed from emergence to maturity, with number of seed/head and wt. of seed/head reduced at the higher temperature.

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INFLUENCE ON NIGHT TEMPERATURE ON PHENOTYPIC PERIODS AND YIELD COMPONENTS OF
TEMPERATE AND TROPICALLY ADAPTED TYPES

J. R. Gipson, J. W. Johnson, and D. T. Rosenow

ABSTRACT

Night temperature regimes of 14 and 22 C were imposed on temperate and tropically adapted sorghum hybrids growing in the field in 1977 and 1978. Temperature control was maintained on a temperate hybrid (RS 671) and a tropically adapted hybrid (A Tx 623 x Tx 430) from emergence to maturity each season. In addition, temperature control was maintained from emergence to bloom on RS 671, A Tx 623 x Tx 430, and RS 610 in 1977, and on the same three plus A Tx 378 x Tx 430 in 1978.

The two year average shows that of the three phenological periods, the greatest temperature response occurred in GS-2, i.e., from panicle initiation to 50% bloom. GS-1 was lengthened by 3 days, while GS-2 was lengthened by 5 days by the cold night temperature. Of the three cultivars studied, the tropically adapted hybrid, A Tx 623 x Tx 430, was least sensitive to temperature, while the temperate hybrid, RS 671, was the most temperature sensitive.

There was no temperature effect on yield components of plants when temperature treatments were imposed from emergence to bloom, but temperature effects were apparent when treatments were imposed from emergence to maturity. In the latter case, number of seed per head were reduced by an average of 19% and 18% respectively, with an increase in night temperature from 14 to 22 C. Of the two cultivars receiving season-long temperature control, RS 671 showed greater reduction in seed per head under high temperature than A Tx 623 x Tx 430, but there was little difference between the two hybrids on seed weight per head.

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CHARACTERIZATION OF SORGHUM WITH RESISTANCE TO PREHARVEST GRAIN DETERIORATION
J. A. Glueck, L. W. Rooney, and D. T. Rosenow

ABSTRACT

The grain of several non-brown sorghum lines (SC 279-14E, SC 566-14, SC 748-14, and SC 630-11E and Martin) have been identified as resistant to preharvest microbial deterioration. Only one non-brown line (SC 630-11E) was identified as resistant to preharvest sprouting. More resistant lines have an intermediate to corneous endosperm and require less time to reach physiological maturity (the grain develops and dries rapidly). In addition, alterations in the cellular structure and organization in the hilar and stelar areas, more complete coverage of the seed by surface wax, and glume shape affect water uptake and movement in resistant grain.

Water enters the sorghum kernel through the pericarp primarily in the hilum and stelar areas of the kernel. The rate of water uptake and movement is affected by structural differences of the kernel. Lines or varieties with higher proportions of floury endosperm and/or a thick mesocarp appear to have increased rates of water movement into the kernel. Water uptake and movement in the kernel appears closely related to field grain deterioration resistance and processing properties of sorghum grain. Sorghum lines or cultivars with more rapid water uptake and movement seem more accessible to microbial attack and suffer more rapid loss of integrity when subjected to conditions favoring deterioration or germination.

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MANAGEMENT TACTICS FOR THE SORGHUM WEBWORM IN SORGHUM
J. R. Hobbs, G. L. Teetes, J. W. Johnson, and A. L. Wuensche

ABSTRACT

Damaging infestation levels of *Celama sorghiella* (Riley) were avoided by early planting of sorghum. Sorghum planted by April 1 and flowering before June 20 in the Coastal Bend of Texas, and sorghum planted by April 10 and flowering before July 1 in South-Central Texas escaped damaging infestations of sorghum webworm.

Sorghum panicle type (open vs. compact) ratings and sorghum webworm infestation levels were positively correlated ($r^2 = 0.8$). Data showed that as sorghum panicles increase in compactness, sorghum webworm densities also increase. There was no positive correlation between sorghum webworm infestation levels and seed endosperm type or panicle size. Also, no association was apparent between egg deposition and panicle type within 6 days of panicle exertion; webworm mortality was high by the 10th day after 50% flower.

Genoplasm resistant to sorghum webworm was difficult to identify, but differences in infestation levels indicated possible resistant sources.

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SORGHUM CULTIVARS RESISTANT TO THE SORGHUM MIDGE
J. W. Johnson, G. L. Teetes, B. R. Wiseman, and A. L. Wuensche

ABSTRACT

Sorghum [*Sorghum bicolor* (L.) Moench] cultivars from 20 countries were evaluated for resistance to the sorghum midge, *Contarinia sorghicola* (Conquillet). Cultivars evaluated were tropical sorghums that have been converted to types that flower in temperate areas by the substitution of the appropriate maturity genes. Cultivars were obtained from the TAES, USDA sorghum conversion program. The 210 items evaluated represented 38 of the "working groups" used by sorghum workers in India to classify the world sorghum collection. Midge damage ratings from three tests grown in two states in 1978 indicate that 10 cultivars are highly resistant (less than 30% seed loss) and 21 cultivars are moderately resistant (30 to 60% seed loss). Highly resistant lines had been collected from Sudan, Ethiopia, Uganda, India and Pakistan. Highly resistant types were found in six "working groups": Zerazera, Caudatum, Caudatum/Nigricans, Caffrorum/Darso, Durra and Durra/Nigricans. The ten highly resistant cultivars were derived from the following IS numbers: 12666, 3071, 12664, 2579, 12593, 8263, 8337, 12676, 7142 and 8231.

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THE SORGHUM MIDGE, WHAT'S NEW AND WHERE ARE WE GOING
Jerry W. Johnson

ABSTRACT

The use of plant resistance in midge management appears promising for the future. Genotypes with resistance have been identified in India, Brazil, and in the U.S. Studies have indicated that midge resistance is quantitatively inherited and not dominant. Since all lines that have been identified with high levels of midge resistance are restorer lines, the development of resistant B-lines is the most difficult breeding problem at this time. Eight midge resistant B-lines with moderate levels of resistance, B Tx 2754 through B Tx 2761, have been released. These lines should aid in the development of lines with high levels of midge resistance.

Evaluation of homozygous resistant, heterozygous resistant and susceptible hybrids in 1978 indicate that resistant hybrids will be damaged by extremely large midge populations. Homozygous resistant hybrids produced 1100 percent more grain than susceptible hybrids, 1380 and 120 pounds per acre, respectively. Indications are that the level of midge resistance presently available will be useful in pest management programs or in production situations where yield potential of the crop at flowering does not justify the additional expense of insecticide application. The cultivars derived from PI 383856 (AF28), IS 12666, IS 3071, IS 12664, IS 2579, IS 12593, IS 8363, IS 8337, IS 12676, IS 7142, and IS 8231 appear to have the highest level of resistance to midge.

Additional work is needed to improve the yield potential and disease resistance of midge resistant lines and to combine the highest levels of antibiosis and non-preference in improved parental lines.

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HYDROCYANIC ACID POTENTIAL IN SEVERAL GRAIN SORGHUM BREEDING LINES AS AFFECTED
BY N AND VARIABLE HARVESTS
G. G. McBee and F. R. Miller

ABSTRACT

Fifteen entries, mostly grain sorghum [*Sorghum bicolor* (L.) Moench] breeding lines were field grown under two rates of N application, 0 and 112 kg/ha for potential HCN (HCN-p) determinations. Harvests were scheduled for three stages of development: prior to boot, panicle exertion to prebloom, and after panicle removal. The youngest two leaves from ten plants/plot composed a sample and were analyzed for HCN-p by the CN^- electrode method. Significant variation in HCN-p existed among the various lines. All entries exceeded the "threshold of danger" level (200 ppm) at the first harvest. Four lines, TAM 428, SA 372, Tx 7078 and Tx 7000 were below 200 ppm at the second harvest and these plus Tx 2536, SCO 170-6 and Tx 398 were below the 200 ppm level at the final harvest. Tx 7000 was the lowest entry in HCN-p at all harvests whereas SCO 599-6 (Rio) was the highest. Added N increased HCN-p in all lines even for those inherently low in dhurrin. HCN-p tended to decrease with maturity of the plant, however, SCO 599-6 and SCO 056 contained levels above 200 ppm at the last harvest indicating a need to exercise caution in grazing stover of some varieties after harvest for grain.

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LYSINE PLATEAUS IN *SORGHUM BICOLOR* (L.) MOENCH GRAIN
Dan H. Meckenstock, F. R. Miller, and L. W. Rooney

ABSTRACT

The lysine concentration of five sorghum lines was evaluated by regressing percent lysine onto percent protein. An individual regression equation for each genotype was used to make comparisons among lines. The assumption of parallelism, i.e., equal slopes, allowed comparison of genotypes over a protein range. Effectively, the regression model resolved differences in the lysine concentration between corneous non-waxy, corneous waxy, and floury non-waxy genotypes. No differences were identified within the major genes controlling endosperm texture and starch type.

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UTILIZATION OF INTRODUCED GERMPLASM IN CROP IMPROVEMENT PROGRAMS
F. R. Miller

ABSTRACT

Utilization of germplasm through both public and private breeding programs has been large. Most breeders acknowledge from 25-95% of their current activities include exotic germplasm contributions for maize, oats, wheat, rice, cotton, soybeans, sunflowers, and sorghum. Sources of this material are from world, national or personal collections and cooperative breeders. Disease and insect resistance, stalk quality, yield stability, productivity, quality factors, alternate sources of cytoplasm are major identified traits that have been exploited. Crown-rust resistance from A. steriles, resistance to soybean cyst nematode, expansion of the cytoplasmic sterility system in sorghum, high oil in sunflower, etc. are other examples. Breeders feel that both major and minor traits still exist in untapped resources which will expand and increase the quality of all crops. The need for many as yet unneeded traits, unrecognized conditions and even unimagined improvement characteristics for which there is genetic variation will allow further use of domestic and alien germplasm resources. Most scientists recognize the major contributions of germplasm resources, but at the same time point with caution and urgency to the need for careful storage and preservation of naturally occurring genetic resources.

1979 Agronomy Abstracts. p. 116.

Research supported in part by the United States Agency for International Development through Contract AID/Ta-c-1384 entitled Development of Improved High Yielding Sorghum Cultivars with Disease and Insect Resistance.

SINK CHARACTERIZATION IN SORGHUM SEEDLINGS

R. J. Newton, D. A. Baltuskonis, D. H. Meckenstock, and F. R. Miller

ABSTRACT

Two sinks, sorghum (*Sorghum bicolor* Moench) seedling roots and shoots, were characterized while growing in dark at 25° in sand and/or paper towels. By utilizing radio-gas-chromatography it was established that sucrose was synthesized in the scutellum and was the primary sugar translocated to the growing shoots and roots. Sugar accumulation in sink regions correlated well with their disappearance in the source tissue, the endosperm. At peak levels of accumulation, glucose followed by fructose and then sucrose, were the primary sugars identified in both shoots and roots. Malate levels in the root were intermediate between sucrose and fructose levels. An unidentified carbohydrate, comprising 2-3% of the dry wt and nearly 50% of the total soluble carbohydrates, was found in the shoot. Levels of the unknown compound were highest in the growing portion of the shoot. Inhibition of shoot growth rate with water stress correlated with decreased levels of the unknown. This suggests that the unknown compound is intimately associated with growth processes.

1979. Agronomy Abstracts. p. 14.

Research supported in part by the United States Agency for International Development through Contract AID/Ta-c-1384 entitled Development of Improved High Yielding Sorghum Cultivars with Disease and Insect Resistance.

PRESENCE OF *COLLECTOTRICHUM GRAMINICOLA* FRUITING STRUCTURES ON SORGHUM SEEDS
M. A. Pastor-Corrales and R. A. Frederiksen

ABSTRACT

Previously undescribed symptoms of sorghum anthracnose were observed in experimental plots in Cuyuta, Guatemala, and Robstown, Texas. Fruiting structures of *Collectotrichum graminicola* (Cesati) Wilson, the causal agent of anthracnose of sorghum [*Sorghum bicolor* (L.) Moench] were found on sorghum seeds. Clusters of acervuli occurred as minute black structures growing superficially in concentric rings on the pericarp around the style area. Acervuli were also present on the surface of rachis branches, but not on the glumes. BTx 398, TX430, SC170, TP3, 77SC1, and Pioneer Brand 846 were among the lines and hybrids which showed the described symptoms. Anthracnose infected seeds were found on plants inoculated at flowering or soft dough stage of grain development in the Texas plots and on plants inoculated before flowering in Guatemala. Seeds were fixed and observed under the scanning electron microscope. These seeds showed mature acervuli crowded with short conidiophores bearing single falcate conidia and septate setae tapering at their tips.

1979. Proc. IX International Congress of Plant Protection. #537

Research supported in part by the United States Agency for International Development through Contract AID/Ta-c-1384 entitled Development of Improved High Yielding Sorghum Cultivars with Disease and Insect Resistance.

INTEGRATED CONTROL OF MAIZE AND SORGHUM DISEASES IN TROPICAL AND SUBTROPICAL AREAS

B. L. Renfro and R. A. Frederiksen

ABSTRACT

Several control methods are employed to minimize losses to maize and sorghum caused by diseases in tropical and sub-tropical regions. These are most often integrated by the farmer, the ultimate judge and practitioner. Host resistance and avoidance are most frequently applied and are the principal methods used in attempts to control the sorghum grain and head molds, maize ear rots, stalk rots, anthracnose, downy mildew, head smut and virus and spiroplasm incited diseases. Fungicidal seed treatment is often used to protect from seed decay, seedling blight, most smuts and, likely, for the future control of downy mildew. While generally uneconomical, other means of chemical control have occasionally been used to control insect vectors, foliar pathogens and bacterial stalk rot of maize. Storage rots are mainly controlled by avoiding grain moisture conditions above 12 percent. In the future multiple measures will need employment for successful disease control. These will need to be integrated with other pest control and management systems for economic reasons and because insects, nematodes, weeds, soil conditions, agronomic practices and host reaction have profound influences on disease development. Research must be flexible, cognizant of changes and, most critically, develop a better transfer system of technology.

1979. Proc. IX International Congress of Plant Protection. #415

Research supported in part by the United States Agency for International Development through Contract AID/Ta-c-1384 entitled Development of Improved High Yielding Sorghum Cultivars with Disease and Insect Resistance.

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DEVELOPMENT OF MULTIPLE DISEASE RESISTANT SORGHUM:
D. T. Rosenow and R. A. Frederiksen

ABSTRACT

Multiple disease resistant (MDR) sorghums have been developed in the TAES pathology-breeding program. Most sources of resistance were found in exotic germplasm recently converted to earlier-maturing types. Our initial selection scheme for sources of resistance in South Texas was by means of a MDR screening nursery. Further screening and selection for various diseases is done at Puerto Rico, Georgia and several locations in South and West Texas. The breeding procedure involves continual intercrossing of source lines and agronomically improved breeding lines with MDR. This multi-location screening of diverse breeding material for disease resistance across environments in natural occurring multi-disease nurseries has proven effective. The continual intercrossing of new sources and breeding lines even of early generation progeny rows has allowed us to select materials which possess an unusually high frequency of MDR. High levels of resistance to the following diseases exist in various combinations in these lines: Head smut (Sphacelotheca reiliana), downy mildew (Peronosclerospora sorghi), maize dwarf mosaic, anthracnose (Colletotrichum graminicola), rust (Puccinia purpurea), zonate leaf spot (Gloeocercospora sorghi), grey leaf spot (Cercospora sorghi), leaf blight (Exserohilum turcicum), Fusarium head blight (Fusarium spp.), charcoal rot (Macrophomina phaseolina), and grain mold (Curvularia, Fusarium and others). Examples of multiple disease resistant lines are TAM 428, SC 326-6, SC 170-6, SC 748-5, and SC 599-6. The original lines from which these were derived are IS 12610, IS 12661, IS 3758, and IS 17459.

1979. Proc. IX International Congress of Plant Protection. #579.

Research supported in part by the United States Agency for International Development through Contract AID/Ta-c-1384 entitled Development of Improved High Yielding Sorghum Cultivars with Disease and Insect Resistance.

ECONOMIC THRESHOLDS OF *HELIOTHIS* SPECIES IN SORGHUM
G. L. Teetes and B. R. Wiseman

ABSTRACT

Based on the research data available, and considering the factors discussed in this chapter, the economic threshold of *Heliothis* in sorghum would appear to be 1 to 2 larvae per panicle. The number of bushels of grain that must be saved to equal the cost of control is determined by dividing treatment cost by the value of a bushel of grain. If a market price of \$2.20 per bushel (bu) and a treatment cost of \$5.50 per acre are assumed, a saving of at least 2.5 bu of grain per acre would be necessary to offset the cost of treatment. If a grower produced 50 bu per acre at \$2.20 per bu, the per acre value would be \$110. A 6 percent loss (1 larva per panicle) and a 10 percent loss (2 larvae per panicle) would result in a monetary loss of \$6.60 and \$11.00 per acre, respectively, provided that all larval damage could be prevented by one treatment. The losses express the maximum potential damage attributable to a given level of infestation if it is assumed that the larval stage is completed by all individuals and that no environmental factors adversely affect the larvae.

Direct control tactics applicable to managing *Heliothis* in sorghum include growing open-panicle varieties, manipulating planting time, and the use of chemical controls based on the economic threshold level. Another possibility for control of *Heliothis* in sorghum would seem to lie in exploitation of the polyphagous feeding habit of these insects. Manipulation of the species by cultural, biological or chemical means in one crop host in the agroecosystem could mitigate infestations in other crops. Lopez and Teetes reporting on the relative similarity of sorghum and cotton in terms of species composition, noted a closely woven relationship, one that through

the season, progressively acquires greater intimacy. An uncharted opportunity for pest management seems to lie here.

1979. Southern Coop. Series Bull. 231:57-61

INSECT RESISTANCE IN SORGHUM
G. L. Teetes and J. W. Johnson

ABSTRACT

The state of the art of insect resistance in sorghum is discussed. Sorghums resistant to insect and mites are tabulated. Resistance has been reported in 17 U.S.A. sorghum pests. Details are given of the status and impact of resistant sorghums for management of chinch bug, greenbug, sorghum midge, sorghum webworm, spider mites, corn leaf aphid, and yellow sugarcane aphid.

1978. Proc. 33rd Annual Corn & Sorghum Res. Conf. 33:167-89.

Research supported in part by the United States Agency for International Development through Contract AID/Ta-c-1384 entitled Development of Improved High Yielding Sorghum Cultivars with Disease and Insect Resistance.

MECHANISMS OF SORGHUM MIDGE RESISTANT SORGHUMS
George L. Teetes, Allen L. Wuensche, and Jerry W. Johnson

ABSTRACT

Within the past decade, sources of sorghum midge, *Contarinia sorghicola* (Coquillett) resistance have been identified in temperate-type sorghum, *Sorghum bicolor* (L.) Moench, from the Texas Sorghum Conversion Program. In three years of study five lines (TAM 2566, TAM 428, SC 423, SGIRL-MR-1, and AF 28) were used to determine relative levels of resistance and mechanisms involved in the resistance exhibited by certain of these lines. High levels of midge resistance were exhibited by TAM 2566, and SGIRL-MR-1 in comparison to either SC 423, TAM 428 or the susceptible check, Tx 7000, in both yield and adult emergence studies. Results of experiments in which the development of sorghum midge in the spikelets of each of the five lines was observed, indicated that antibiosis was a major resistance mechanism in several lines, particularly TAM 2566. In addition, studies demonstrated that fewer female adults were captured on panicles at anthesis of several resistant sorghums than susceptible sorghums. Nonpreference was shown to be a mechanism involved in the resistance exhibited by TAM 2566. Tolerance did not appear to be an important resistance mechanism in any of the five lines tested.

1979. IX International Congress of Plant Protection

Research supported in part by the United States Agency for International Development through Contract AID/t-a-c 1384 entitled Development of Improved High Yielding Sorghum Cultivars with Disease and Insect Resistance.

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DAMAGE TO SORGHUM BY FOUR SPECIES OF SEED-FEEDING BUGS
G. L. Teetes and D. G. Hall, IV

ABSTRACT

Damage to sorghum seed by four species of bugs was assessed during 1978. Seed weight and percent germination generally decreased as numbers of rice stink bugs, southern green stink bugs, leaffooted bugs and conchuela stink bugs per panicle increased. Mean number of feeding punctures per seed and percent punctured seed generally increased as bug populations increased, especially at the highest infestation level. To some extent, weight of damaged seed decreased as the incidence of punctures increased. Damage to sorghum grain was largely due to direct feeding on seed by bugs, but indirect damage resulted from bugs feeding on panicle stems and rachis branches. Bugs were more injurious to grain in the milk and soft dough stages of development than to grain in the hard dough stage.

1979. Proc. 11th Biennial Grain Sorghum Res. and Util. Conf. 12-13.

Research supported in part by the United States Agency for International Development through Contract AID/Ta-c-1384 entitled Development of Improved High Yielding Sorghum Cultivars with Disease and Insect Resistance.

GROWTH ANALYSIS OF TEMPERATE AND TROPICALLY ADAPTED SORGHUM HYBRIDS AND LINES
G. L. Thomas and F. R. Miller

ABSTRACT

During 1978, 7 plantings were made at monthly intervals at College Station, Texas. Five sorghum genotypes were sown at each planting date. Two genotypes, RS610 and NB505, were considered temperately adapted (tea) while the other 3 genotypes, BTx623, RTx430, and ATx623 x RT430, were considered tropically adapted (tra). Green leaf area, number of green leaves and plant height were measured for each genotype for each planting date at the following phenological stages: 1) floral differentiation; 2) 50% anthesis; 3) black layer at the bottom of the head. Analysis of variance for all plantings where all measurements were made gave the following results. Significant ($\alpha = .05$) differences in total leaf area between the tra lines and other entries. For stage 1 and all plantings the tra genotypes were significantly taller. Stage 2 and all plantings the tra genotype had significantly more leaves than tea genotypes. For stage 3 the tra genotypes had significantly greater leaf areas and numbers than the tea genotypes. It would appear that the two tropically adapted lines and their hybrids have greater leaf producing potential than do the temperately adapted hybrids.

1979. Agronomy Abstracts. 71:111

Research supported in part by the United States Agency for International Development through Contract AID/Ta-c-1384 entitled Development of Improve High Yielding Sorghum Cultivars with Disease and Insect Resistance.

GROWTH AND SOIL WATER EXTRACTION BY DIFFERENT GRAIN SORGHUM AND MILLET CULTIVARS
C. W. Wendt, H. P. Harbert III, D. T. Rosenow, and J. W. Johnson

ABSTRACT

Eight grain sorghum cultivars were grown on an Amarillo loam soil at Lubbock, Texas 1976-1979. The soil profile was at "field capacity" prior to planting. No additional irrigation water was added after planting. Mobile shelters were used to prevent rain from falling on the plots. Parameters measured include soil water content, plant water potential, dry weight, leaf area, and sugar. Grain sorghum cultivars investigated include Tx 7000, SC 599-6, SC 56-14, 1790E, B 35, B Tx 378, A 35 x SC 599-6, and A 599 x SC 56-14. The amount of soil water extracted varied from 14.0 to 20.4 cm. There were generally more differences in soil water extraction between years than between cultivars in a particular year. However, there were major differences in extraction among cultivars in the time and location within the soil profile.

Soil water content changes indicate that depths greater than 210 cm should be investigated because major changes occurred at this depth.

Differences existed in leaf area index and dry weight among cultivars. The sugar content of the stalks and leaves were significantly different among cultivars. There was no relationship between the leaf and stalk sugars.

Yield data between cultivars were not consistent between years because of differences in planting date and infestation by Banks grass mite and midge.

Millet cultivars investigated included the Senegal population and RMP1(s)C1. The cultivars were found to be difficult to establish, poor extractors of soil water (9-11cm) and poor yielders of grain (537-575 kg/ha).

1979. Proc. 11th Biennial Grain Sorghum Res. and Util. Conf. p. 49.

Research supported in part by the United States Agency for International Development through Contract AID/Ta-c-1384 entitled Development of Improved High Yielding Sorghum Cultivars with Disease and Insect Resistance.

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FLORAL INITIATION IN SORGHUM HASTENED BY GIBBERELIC ACID AND FAR-RED LIGHT
E. A. Williams and P. W. Morgan

ABSTRACT

Floral initiation in sorghum was hastened by five minutes of far red light given at the beginning of the 12 hour night period plus gibberellic acid. The time of application of GA₃ was not critical, but the two treatments were more effective together than either alone. Flower initiation was hastened several days by these treatments.

1979. Planta. 145:269-272

Research supported in part by the United States Agency for International Development through Contract AID/Ta-c-1384 entitled Development of Improved High Yielding Sorghum Cultivars with Disease and Insect Resistance.

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DIFFERENTIAL RESPONSE OF SORGHUM CULTIVARS TO DROUGHT STRESS
C. A. Woodfin, D. T. Rosenow, L. E. Clark, and J. W. Johnson

ABSTRACT

Sorghum genotypes were evaluated for tolerance to drought stress under field conditions during the past two years. Notes were taken when plants were under drought stress at two stages; early in the season before flowering, and later in the season during the late grain development stage. Notes on leaf rolling, excessive leaf erectness, leaf bleaching, and leaf tip and margin burn were taken early in the season and combined into one drought stress rating, their presence being considered a poor response. During the late grain development stage, ratings on premature leaf and plant death were used to indicate an undesirable drought response. Early season stress susceptibility results in head blasting and delay in flowering. Premature plant death caused by late season stress often results in lodging and reduced seed size. Distinct differences in drought response among cultivars were found at both growth stages. There were distinct differences in drought reaction within cultivars at the two stages. No cultivars were found with high levels of tolerance at both growth stages.

1979. Agronomy Abstracts. p. 82.

Research supported in part by the United States Agency for International Development through Contract AID/Ta-c-1384 entitled Development of Improved High Yielding Sorghum Cultivars with Disease and Insect Resistance.

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NEWS RELEASES
AND
PUBLIC MEDIA ARTICLES

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NEWS RELEASES AND PUBLIC MEDIA ARTICLES

1. ICRISAT Hosts Five Workshops in Busy Winter. At ICRISAT, Jan/Feb/Mar 1979.
2. Will Sorghum Feed and Power the Future? Farm Journal, Feb. 1979
3. Sorghum Yield Barriers Beginning to Fall. Four Down, One to Go. Earlyriser, March 1979.
4. Sorghum Pathologists Meet. Phytopathology News, March 1979.
5. Kansas Hosts Utilization Meet. Grain Sorghum News, April 1979.
6. Looking into Sorghum's Future. Horizons, Department of Soil & Crop Sciences Newsletter, April 1979.
7. New Twin-Seeded Sorghums are Tested. Irrigation Age, May/June 1979.
8. Twin-Seeded Sorghums are Trend of the Future. Seedmen's Digest, May 1979.
9. Seed Treatment Will Stop Herbicide Injury. Southwest Farm Press, May 24, 1979.
10. Research Report - Brazilian Researcher Confers with A&M Scientists on Downy Mildew, TAES News, June 14, 1979.
11. Sorghums Pose Energy Hope. Beeville Bee-Picayune, Vol 93, No. 10, July 2, 1979.
12. Research Report - College Station. TAES News, July 16, 1979
13. Host Plant Resistance to be Explored at A&M Conference. TAES Newsletter to Station Staff, July, 1979.
14. Hydrocyanic Acid in Sorghum Lines. TAES, Brief Reports on Agricultural Research in Texas, Vol 5, No. 7, July 1979.
15. You Can Improve Your Sorghum Yield Record. The Farmer-Stockman, August 1979.
16. What the Scientist Sees. TAES, Brief Reports on Agricultural Research in Texas, Vol 5, No. 8, August 1979.
17. Crop Management in the South. Successful Farming in the South, September 1979.
18. Dr. Fred Miller Invited to Study Sorghums in China. TAES, Newsletter to Station Staff, No. 9, September 1979.

ICRISAT Hosts Five Workshops in Busy Winter

ICRISAT was host for five international workshops in a period of three months and 10 days in late 1978 and early 1979.

The busy winter program started with an International Workshop on Agroclimatological Research Needs of the Semi-Arid Tropics November 22-24. Twenty-five participants from 11 countries discussed techniques, methodologies, approaches, instrumentation and means of agroclimatological research.

ICRISAT and Texas A & M University jointly sponsored the International Workshop on Sorghum Diseases December 11-15 in Hyderabad. Fifty-six papers were presented from Asia, Africa, North America and South America on individual diseases of sorghum, including smuts, grain molds, downy mildew, head blights, and stalk rots, ergot, leaf diseases and virus diseases. Research priorities were recommended for each group of diseases.

A consultants' group discussion on Resistance to Soil-borne Diseases of Legumes was held by ICRISAT's Pulse Pathology section January 8-11 with 10 consultants attending from Australia, India, the Netherlands, UK and the USA. Visits to experimental fields stimulated in-depth discussions of ICRISAT's work in this area.

The International Workshop on Intercropping January 10-13 attracted some 35 delegates from 15 countries and highlighted the tremendous interest recently developed and the rapid improvements being made in experimental approaches to intercropping. At ICRISAT the work is based mainly on four intercrop combinations of the five ICRISAT crops: sorghum/pigeonpea, millet/groundnut, sorghum/chickpea, and sorghum/millet.

ICRISAT's Economics program was host for an International Workshop on Socioeconomic Constraints to Development of Semi-Arid Tropical Agriculture February 19-23. It was attended by 46 participants from 13 countries and 40 ICRISAT scientists. Papers and discussions emphasized the need to analyze labor bottlenecks in West Africa, to learn more about the goals of farmers throughout the SAT, to study in depth possible mechanisms for the improvement of agricultural produce markets, to extend to areas other than India the village level economic studies and research on farmers' attitudes to risk conducted here, and to assess the potential for development inherent in the transfer of already existing technology, such as irrigation tanks in India to countries with relatively lower levels of intensity in use of agricultural resources.

The International Workshop on Chickpea Improvement February 28 to March 2 drew 51 delegates from 14 countries and 31 ICRISAT scientists. Topics discussed included breeding strategies, including germplasm work and nursery trials; yield-improvement through kabuli-desi introgression; recent developments in chickpea agronomy and physiology; microbiology research in chickpeas; and plant protection. Two separate sessions were devoted to country reports, and special attention was given to the ICRISAT/ICARDA program.

Proceedings of these workshops are in various stages of preparation. Copies may be obtained by writing to Information Services, ICRISAT, 1-11-256, Begumpet, Hyderabad 500 016, A. P., India.



Director Swindale examines sorghum disease identification book with Co-Authors Williams (left), Frederiksen and Girard

Sorghum/Millet, Chickpea Disease Handbooks Published by ICRISAT

Two pocket-size handbooks, with clinical quality color photographs for identification of sorghum and pearl millet diseases and for wilt-like disorders of chickpea, were among ICRISAT publications in 1978.

The "Sorghum and Pearl Millet Disease Identification Handbook," published in cooperation with Texas A & M University, includes a disease-identification key and descriptions of the symptoms of 32 diseases. It has 59 full-color illustrations. The co-authors are R. J. Williams of ICRISAT, R. A. Frederiksen of Texas A & M, and J.-C. Girard of IRAT (France). Copies are available in English, French and Spanish.

"Diagnosis of some Wilt-like Disorders of Chickpea (*Cicer arietinum* L.)," is the result of an attempt to clarify the considerable confusion in diagnosing various chickpea disorders, particularly wilt and root rots. In the past, these disorders were frequently classified under the general heading of "wilt complex." ICRISAT pathologists have shown that the condition is actually a number of distinct diagnosable diseases.

This handbook describes the characteristic features of the main disorders of chickpea, and includes 37 diagnostic photographs. In addition to a disease-identification key, the handbook contains a summary of the chickpea wilt surveys conducted on the subcontinent of India. The authors are Y. L. Nene, M. P. Haware, and M. V. Reddy, pulse pathologists at ICRISAT.

The handbook is printed in English, and we are exploring possibility of supplements in Spanish and Arabic.

Single copies of both handbooks may be obtained free of charge by writing Head of Information Services, ICRISAT, 1-11-256 Begumpet, Hyderabad 500 016, A. P., India. Please specify by title in ordering.

Sorghum yield barriers beginning to fall. Four down, one to go

The year 1979 could be the year when traditional sorghum yield barriers begin to topple. It could be the beginning of a period when Southeastern, Mid-South and Southwestern sorghum yields start to catch up with the yields grown in the more favorable conditions of the High Plains.

Why? Because until recently, insects and diseases have kept the lid on sorghum performance in the Southern states. But downy mildew, anthracnose, head smut and greenbugs are now being whipped through improved genetic hybrid resistance. Only the midge remains as a major insect challenging sorghum producers—and work is well underway to build hybrids with resistance to that long-term sorghum pest.

In short, sorghum plant breeders can now put less emphasis on "putting out fires" and get down to the serious business of increasing genetic yielding ability.

"We have always placed paramount importance on yield," says Dr. Rodney Rogers, Funk's manager of sorghum research. "But yield potential has been limited seriously by the severe insect and disease problems existing in the South. So we have concentrated our efforts on solving these problems so that our hybrids could reach their full genetic yield potential."

Funk now has a comprehensive line-up of widely adapted hybrids that carry resistance to whatever problems a sorghum producer might have. Downy mildew and anthracnose have been two major disease problems, but now G-589, G-522DR and G-722DR grain sorghums, G-88F sorghum-sudan and G-102S silage

sorghum carry resistance to these two diseases of the Southeast. G-499GBR, G-642GBR and G-622GBR fill the need for greenbug resistant hybrids in areas where that pest has been a problem.

Dr. Norman Wilson, Funk sorghum breeder in the Southeast, has been working with Dr. Rogers on boosting hybrid yielding ability across the South. Dr. Wilson is testing hybrids at Funk locations in Greenville, Mississippi; Coolidge, Georgia; Corning, Arkansas; Lamar, Missouri; and Progresso, Taylor, Victoria and Lubbock, Texas.

He screens his sorghum hybrid candidates at these locations for a variety of factors and uses data compiled at many Funk Research Evaluation Plots (or REPs) located across the South and Southwest. The REP tests are made by farmers—under normal growing conditions. Testing done in the REPs is the final research stage before sorghum hybrids are produced in commercial quantities for public release.

While Drs. Rogers and Wilson will continue to bring insect and disease resistant hybrids onto the market, they have trained their guns on improved yield and have two hybrids in development that promise to be bull ringers.

The first is G-611, a new hybrid that was just numbered this winter. Formerly carrying the experimental number 2216, G-611 will be seen in limited commercial quantities for the first time this coming year. It is tropically adapted and carries the same disease resistance as G-722DR and G-522DR. It is also greenbug resistant!

But G-611 also pays off in the

SORGHUM YIELD BARRIERS BEGINNING TO FALL.

FOUR DOWN, ONE TO GO (continued)

yield column. Early tests show that it will yield as well as or better than G-522DR, a hybrid that consistently has placed high in yield trials. It set a new state sorghum yield record in Arkansas in 1978 with an irrigated yield of 11,588.3 lbs per acre.

A second hybrid likely will receive its "G" number in 1980. Carrying the experimental number 2194, it too provides wide resistance to diseases and insects. Early tests for 2194 indicate that its yield potential is equal or superior to existing hybrids of comparable maturity.

Both G-611 and the experimental hybrid 2194 are tropically adapted. Dr. Wilson feels that tropical adaptation is one research effort now paying big dividends in the yield column for Southern sorghum producers. Tropical adaptation allows many South Texas, Mid-South and Southeastern sorghum growers to achieve yields comparable to those grown in the High Plains.

"In fact, tropical adaptation has proven to be a real yield booster in all areas where night temperatures and humidity are high during the growing season," Dr. Wilson says.

"The advantage from tropical adaptation seems to be more a function of elevation and rainfall rather than latitude."

These new Southern-adapted sorghum hybrids with tropical adaptation show multigenic resistance to many different sorghum disease and insect problems and will withstand harvest-time rainfalls and other weathering problems better.

Hybrids carrying tropical adaptation have immediate usefulness in the Southern sorghum belt because this area is characterized by warm

night temperatures and high humidity during the crop growth and development stages.

Dr. Wilson developed the first commercial grain sorghum hybrids with tropical adaptation, a concept based on early work by Dr. Fred Miller of Texas A & M University, using World Sorghum Collection germplasm.

Sorghum midge still remains as the one nearly universal, and as yet unsolved, problem facing Southern producers. Drs. Rogers and Wilson expect to have at least a partial solution to that problem within a few years.

According to Dr. Rogers, sorghum midge is a minute orange fly which lays its eggs in the developing grain of the sorghum head. This egg hatches into a tiny maggot which feeds inside the kernel, causing head blasting and heavy yield losses.

The problem usually occurs in sorghum planted later than adjacent sorghum fields, the later plantings being subject to higher insect population levels.

"Dr. Wilson has been successful in selecting parent material with midge resistance," says Dr. Rogers. "We are making an intense effort to bring resistant hybrids on stream and will be evaluating some prototype hybrids in 1979."

"However," Dr. Rogers cautions, "when we do have midge resistance it will probably not be the complete solution. In other words, sorghum growers may still have to use an insecticide in combination with the resistant hybrid when midge populations are high."

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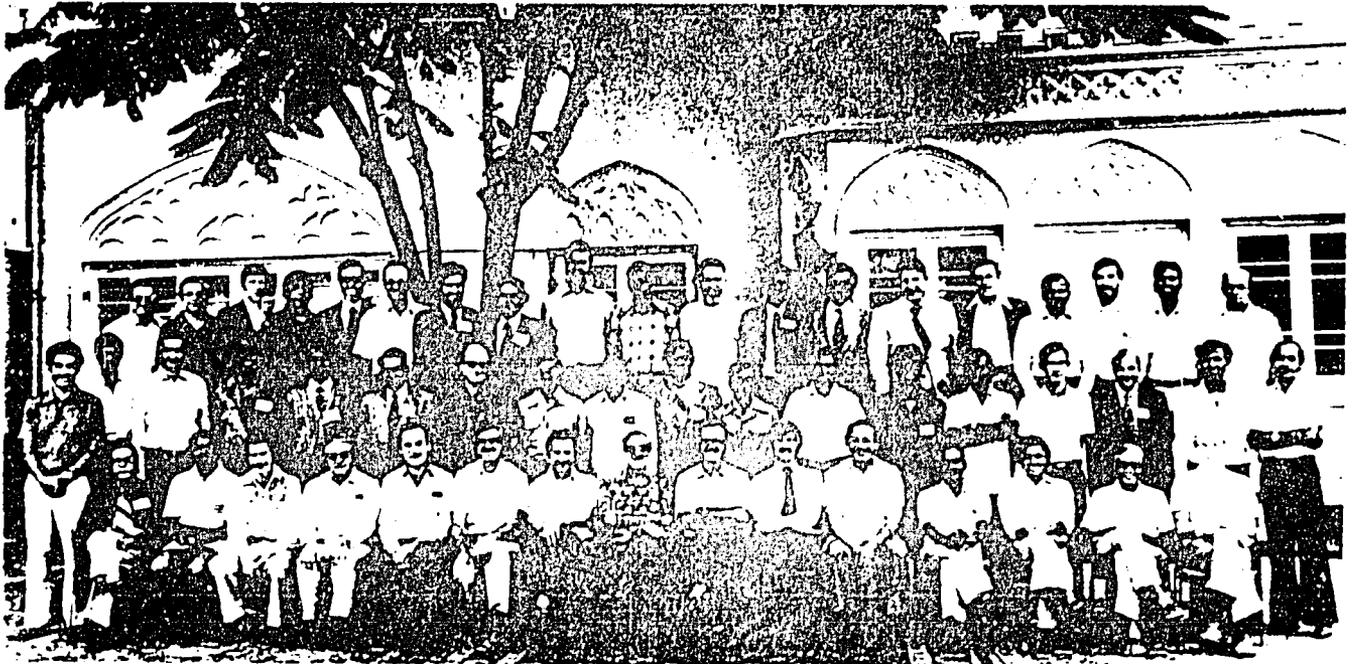
Sorghum Pathologists Meet

Pathologists from 20 countries met at Hyderabad, India, on December 11-15, 1978, to review the status of internationally important sorghum diseases. Organized by the International Crops Research Institute for the Semi-Arid Tropics and the Texas Agricultural Experiment Station of Texas A & M University, this was the first meeting of pathologists from North and South America, Australia, Africa, Asia, and Europe to establish internationally coordinated goals and

priorities.

Sessions on smuts, grain molds, virus diseases, downy mildew, leaf diseases, stalk rots, head blights, ergot, and utilization of resistance were held separately during the International Workshop on Sorghum Diseases.

Recommendations were drafted and will appear in the technical proceedings of the conference. The proceedings will reflect the current state of the art on sorghum pathology.



Kansas Hosts Utilization Meet

The Bi-Annual Grain Sorghum Utilization Conference was held in Wichita, Kansas on February 28-March 2. The Conference which has been held each year since 1957, was co-sponsored this year by Grain Sorghum Producers Association, Texas Grain Sorghum Producers Board, Kansas Grain Sorghum Growers Association, and Sorghum Improvement Conference of North America.

The three-day conference featured reports on research in entomology, cultural-management practices, plant breeding, physiology, chemical and nutritional improvement.

Arrangements for the conference were made by Kansas Grain Sorghum Board. Over 100 persons attended the Conference.



Sorghum specialists at the Bi-Annual Grain Sorghum Utilization Conference are Left to Right: Dr. Jerry D. Eastin, Professor, Department of Agronomy of University of Nebraska; Dr. R.A. Frederickson, Professor-Plant Pathology of Texas A. and M. University; and Dr. Richard Vanderlip, Professor-Agronomy of Kansas State University.

APRIL 1979



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OUTREACH

LOOKING INTO SORGHUM'S FUTURE

Just keeping track of all the projects Dr. Fred Miller of the Agronomy Field Lab has going would be a full time job for any average person. He is in charge of sorghum improvement for the southern two-thirds of Texas, along with providing tropically adapted types to countries such as Mexico, Brazil, India, Honduras, and Tanzania for evaluation.

On leave from TAMU, Dr. Miller spent five years in Puerto Rico in charge of developing a sorghum conversion program for the USDA. The program involved converting a tall, photoperiod sensitive African sorghum to a shorter sorghum, insensitive to photoperiod and that would develop a seed head in the Puerto Rican latitude. When Dr. Miller returned to College Station in 1970, Dr. M.E. Bloodworth challenged him to develop a sorghum adapted for South Texas. South Texas sorghum producers were attaining yields of only a third to one-half of farmers on the High Plains and further north.

Step one in the process was to look at the world collection of sorghums. Dr. Miller selected a tropical Ethiopian type to cross with a local type of greater yield potential. The hybrid was sent to Puerto Rico in the winter of 1971 and test planted. Results were good, and these sorghum types have been released and proven effective for use in South Texas.

The next question was, why did this plant yield well in South Texas and the types grown further north did not. An Australian graduate student, Geoff Thomas, made a computer analysis of a number of types and found that the major variable was the germination temperatures. Tropical varieties germinate at a lower temperature compared to temperate varieties. The opposite would thought to have been true, but this is believed to be a compensation mechanism for the shorter day lengths in southern or tropical climates. High Plains varieties grown in the south may not be able to achieve high productivity due to the fact that during the day their relatively high heat requirements are not fully met. This, plus high respiration losses during the hot nights of what little is produced may account for the low yields. At least this is a theory.

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HORIZONS (continued) Department of Soil & Crop Sciences NEWSLETTER

April 1979

A total of nine graduate students are presently working on sorghum improvement. Project topics range from practical agronomic aspects of growing sorghum and development of high lysine kernels to pathological and physiological studies. Armando Valdez, a doctoral student from Mexico, is determining if selection of a sorghum type can be made based only on temperature phenotype (of germination), when a low base temperature sorghum is crossed with a high base temperature type. This would allow for testing to be conducted at only a limited number of places instead of the numerous locations now used to account for varying climatic ranges. Substantial saving of time and money spent testing will particularly benefit the seed industry.

Another student is experimenting with an Ethiopian sorghum type which produces shrunken kernels of high lysine content, crossing it with a full kernel producing type with a lower lysine content. Where 1.9% of the protein of most sorghum is lysine, this hybrid will yield a lysine content of 2.2-2.3% of the total protein.

It has been theorized that sorghum is potentially capable of yielding 35,000 pounds per acre. This goal could be approached through work conducted with twin seeded heads and control of genetic indeterminacy of head size. Research is also beginning on the root system, as little is really known except that there are differences, probably related to the climate of evolution. In addition to the improvements specifically bred for, many of these new tropical types are proving better insect, drought, and disease resistant, and of good nutritional quality.

Seed company agronomists and breeders periodically visit the campus field lab and nursery to view developments and express problems experienced in their particular area. Dr. Miller's strong belief in keeping the door open to the public and maintaining a direct line of communication with the seed industry allows for a steady flow of interchanging ideas and concepts. This often stimulates the establishment of new research projects.

Cooperation between A&M and foreign sorghum producing nations is also strong. In fact, for the first time, A&M and a foreign country will jointly release a sorghum type. Probably within a year TAMU and Guatamala will release a tropically adapted sorghum type while Mexico and A&M will release a random mating population.

It is obvious that the concept of the "perfect" sorghum is an ever expanding idea, and will likely never be attained as someone always has an idea for improvement, especially Dr. Miller and his coworkers.

New twin-seeded sorghums are tested

The release of a twin-seeded hybrid sorghum by Northrup King for field testing may be the start of a new trend in sorghum production, according to Paul Menge, director of hybrid sorghum research at Northrup King.

"Twin-seeded sorghum has shown as much as a 50% yield advantage over conventional hybrids. However, we feel a 15 to 20% yield increase is a more reliable expectation."

Northrup King is releasing its first twin-seeded hybrids to growers for field

planting tests this year. The new twin-seeded hybrids could be available for widespread distribution in 1980.

Texas A&M scientists first recognized the economic potential of the twin-seed characteristic and have been the leader in developing breeding material. Their research agrees with NK's: a 15 to 20% yield increase could be typical, Northrup King officials said.

The difference is in the heads. Each floret contains two flowers instead of the traditional one. The result is two

sorghum seeds, explains sorghum plant breeder Menge.

While your first impression may be that yields should either be doubled or the seed size is smaller, neither is true.

"Twin flowering seems to reduce the number of florets per head. A typical sorghum head has around 2,500 seeds, twin-seeded types, 50% more," Menge explained. "The seed size depends most on the genetic material in the plant. We have some twin-seeded selections that have a large seed size. There is one difference though, the twin-seeded hybrids tend to produce a flatter seed."

The hybrids Northrup King has available for field testing and the hybrids Texas A&M are testing have the twin-seeded characteristic added only on the male side.

Northrup King has also developed a female line that has the twin-seeded characteristic. By having the twin-seeded characteristic on both male and female lines, Menge believes that nearly 100% of the florets will be more likely to set twin seeds.

Northrup King has found that the twin-seeded characteristic can be added to regular breeding lines, without changing the plant's other characteristics.

"We should be able to add the twin-seeded characteristic to our existing hybrids and expect them to maintain the characteristics that have made them popular with growers," concluded Menge. ▼

SEEDMEN'S DIGEST

May 1979

TWIN-SEEDED SORGHUMS ARE TREND OF THE FUTURE

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IA • May-June 1979

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Seed Treatment Will Stop Herbicide Injury

sorghum hybrids sold by the two seed firms for sales to farmers in 1980. For purposes of its own brand identification, Funk will market the safened seed under a trade name, Herbishield.

Final Testing Phase

The two new herbicides are in final phases of testing and registration with the Environmental Protection Agency (EPA) now.

The first new herbicide to be released and sold along with safened seed will be named, Bicep.

According to company officials, Bicep is a combination of Dual, a registered pre-plant herbicide for most annual grass and certain broadleaf weeds in corn and soybeans, plus AATrex. Dr. J. W. Peek of Greensboro, the chemical firm's manager of its biological research department said the two chemicals in combination will give farmers the broad-spectrum weed and grass control tool they need for successful sorghum production.

The second new herbicide, due out a year later, is to be named Milocep. It is a combination of Dual and Milogard.

The active ingredient in Dual herbicide is metolachlor.

Detoxify Metolachlor

Peek explained the seed safener serves to detoxify the metolachlor. Without first chemically safening the seed, Peek pointed out, using Dual (metolachlor) on sorghum would kill sorghum outright.

The label on Bicep, according to Peek, will cover the following annual grasses: barnyardgrass or watergrass, crabgrass, cupgrass, three foxtails - giant, green and yellow - goosegrass, yellow nutsedge fall panicum, signalgrass and witchgrass.

Broadleaf weeds covered under the anticipated registration includes carpetweed, cocklebur, lambsquarter, Florida pursley, ragweed, smartweed, velvetleaf, morningglory, pigweed, purslane and puncturevine.

Company officials say Bicep also gives partial control of both seedling Johnsongrass and sandburs.

Exactly how the safener detoxifies the herbicide's active ingredient is subject of further research, Peek said.

Broadens Options

Both Bicep and Milocep, said the company, are expected to be cleared for use by farmers as either a pre-plant incorporated herbicide or as a pre-emergence herbicide, broadening considerably farmers' choices in their pest plant control programs.

Peek told the seminar that since it was proving so difficult for his firm and others to find a good grass herbicide which was selective for sorghum, researchers at the firm's Basle, Switzerland, headquarters moved several years ago to find a herbicide antidote that would permit an existing grass herbicide to be used on sorghum.

What eventually has become Concep was discovered in 1974 and carried the experimental designation CGA-43039.

Chemical and seed firm officials said test marketing next year will involve use of Concep-treated Funk hybrid sorghum

seed and the initial herbicide, Bicep.

Registration of Bicep is expected to be completed this fall.

Exact areas of initial marketing are being chosen at this time.

Hybrids Selected

The initial hybrids to be Concep-safened and sold as Herbishield seed, according to the seed company, will be G-499GBR, G-550xx, G-522, G-522A, G-522DR, G-611, G-622GBR, and G-612GBR.

Some problems with seed germination have been encountered in cold, wet soils.

A Texas researcher who has evaluated the system is Dr. Allen Weise, weed control researcher at the Texas A&M Rural Experiment Station at Bushland, near Amarillo.

He said the method no doubt will be helpful to the state's sorghum producers. "This revolutionary idea will help farmers control grasses and weeds in sorghum that escape other herbicides," Weise said. "Nutsedge is a good example."

After a little more research with the treatment, we will know if Bicep and Milocep can be used

safely on sorghum growing in sandy soils. If it works, our growers will be able to control sandburs in sorghum for the first time.

"Controlling volunteer sorghum or shattercane in sorghum fields with the new herbicides is another possibility."

Weise said seed safeners are a viable approach to better grass control. He noted that several others are currently on the market. Stauffer Chemical Company's Eradicane is a combination of Eptam and a chemical seed safener, applied directly on the soil for weed and grass control in corn. The firm's Sutan Plus is a combination of Sutan plus a safener.

Gulf Chemical Company also has available such a product, called Protec.

Other major firms are known to be in pursuit of antidotes.

Noted Weise, "Safeners may be easier to find and less costly on manufacturers than new herbicides themselves, considering the many environmental factors which must be met and fulfilled before marketing can begin."

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THE TEXAS AGRICULTURAL EXPERIMENT STATION/Neville P. Clarke, Director
The Texas A&M University System/College Station, Texas/Telephone (AC-713) 845-2211

NEWS

6-14-79 RESEARCH REPORT

FOR RELEASE ON RECEIPT

BRAZILIAN RESEARCHER CONFERS WITH A&M SCIENTISTS ON DOWNY MILDEW

COLLEGE STATION--The Texas Agricultural Experiment Station's continuing research emphasis on downy mildew in corn and sorghum has attracted one of Brazil's top authorities on this fungus disease.

Dr. Nelson Gimenes Fernandes is a visiting professor at Texas A&M University during June and July, and his stay here is described as a good example of the mutually beneficial co-operation that can exist between nations.

Gimenes not only will study the Experiment Station's latest downy mildew (DM) findings, but he also will lecture on his own work and experiences to A&M graduate students.

The scientist, a plant pathologist at Paulista State University in Brazil, is working with Dr. Richard Frederiksen of the A&M Plant Sciences Department, and Dr. Jeweus Craig, a downy mildew specialist with the U.S. Department of Agriculture.

Gimenes says DM, when it occurs, is confined to about three states in Brazil. Fortunately, the malady has had little effect on corn, which is a far more important crop than sorghum in Brazil.

The disease did hit sorghum fairly hard about three years ago, but it has since been checked by the introduction of resistant varieties.

While at A&M, the Brazilian will concentrate on DM inoculation techniques in the greenhouse. Frederiksen and Craig will work with him on identifying resistance in corn and sorghum, and on determining the genetics and inheritances of that resistance.

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GIMENES--Add One

Downy mildew has never ravaged corn and sorghum crops in the United States, but it receives considerable research attention because it is potentially a real trouble-maker, Frederiksen says.

The disease has been kept under control, particularly in corn, through use of resistant varieties and certain cultural practices. Frederiksen calls this continuing preventive campaign "a great success story in agricultural science."

In some other countries, however, DM is a "crop limiting factor," meaning that it can devastate corn and sorghum production. Worldwide, it is one of the most serious diseases of corn.

The Texas Agricultural Experiment Station has completed its first phase of DM research and now has excellent sources of resistant plant material for sorghum and corn. Next on the program is to evaluate that resistance and to test it to any potentially new isolates of the DM pathogen.

In addition, Frederiksen says, this resistance can be combined with resistance to other pests such as insects--sorghum-damaging greenbugs, for example.

Gimenes, Frederiksen and Craig agree on the worth of the philosophy of working with scientists from other countries.

"Such an exchange of ideas provides a stimulus for us, and I think it certainly contributes to a better understanding among nations," Frederiksen said. "It just makes sense for scientists in different countries to communicate on their research."

Craig described the interchange of information among scientists as a major step in "building a framework for better worldwide plant disease control."

DM is an exotic disease introduced to the U.S., he said.

"In establishing this kind of cooperation with scientists from other nations, we are better able to investigate and learn about other diseases that might be introduced," Craig explained.



Beeville



Bee-Picayune

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Our 93rd Year

Beeville, Texas 78102, Monday, July 2, 1979

Two Sections—18 Pages

Sorghums Pose Energy Hope

Foreign Varieties May Yield Food, Gasohol

By GARY KENT

These days, with gasoline and diesel in such demand, the perfect farmer's crop would produce as much fuel as it yields edible fiber. Surely many farmers have dreamed of such a crop.

Well, Texas A&M sorghum researchers think they are on the verge of coming up with such a plant that is adaptable for growth in South Texas. Information concerning the research was actively sought when a group of over 100 sorghum research workers from all over the world visited Beeville recently as part of the 1979 Sorghum Workshop.

Like rice to the Orient, nutritious grain sorghum has provided sustenance for people throughout India and parts of Africa for centuries. Varieties of the plant, commonly known as milo, have been growing in Texas for over 100 years.

Many tropical varieties of sorghum grow a tall cane that is heavy with a sweet sugary pulp. The pulp from the cane can be distilled into alcohol which can be used as fuel.

The idea is not new. Some of the grain produced in the American Midwest today is being converted into alcohol and mixed with gasoline to make "gasohol." Gasohol is also the product being used to power an automobile at the A&M Research Station at Weslaco. The scientists are making alcohol out of sorghum. Instead of distilling useful grain to get their product they are using the rich pulp of the plant's stalk.

The Brazilians were among the first to use tall sorghum cane as a source of fuel. Because of shortage of oil in Brazil the nation has found it economical to produce a reported 20 to 30 percent of its fuel from the pulp of sorghum cane.

Although workers at the A&M Research Station in Beeville are not directly involved in gasohol experiments they are testing a number of sorghum varieties and hoping to help come up with a breed of the plant that will produce an abundant head of grain plus a thick, juicy stalk filled with distillable sugar.

in sorghum breeding and disease nurseries at the Beeville station and on the Otto Hertel Farm researchers have given special interest to grain sorghum hybrids which have been developed from converted tropical introductions. The new tropical introductions allow the hybrids to grow better in the hot, dry climate of South Texas.

The influence of the tropical varieties of sorghum has also aided in the development of a midge-resistant breed of the plant. Control of the midge disease is crucial because most milo has to be planted at a time when it will be subject to midge damage in order to meet seasonal timing requirements necessary in producing an optimum crop.

Extensive research involving many of the more than 12,000 varieties of sorghum in the world has been carried out since the early part of the century at stations throughout Texas and lately in Mexico and Puerto Rico. Recently developed members of the sorghum family, with dwarfed stalks for easier harvesting with a combine, have made milo a highly productive crop. Researchers are now hoping that a new variety of sorghum with a stalk of about six feet in height will soon be available.

BEEVILLE BEE-PICAYUNE

Beeville, Texas
Monday, July 2, 1979

SORGHUMS (continued)

Developing new sorghum hybrids requires a long and complicated process. Sorghum flowering is hastened by long nights, meaning the number of hours of darkness determine when the plant will mature. That makes the development of hybrids from both tropical and temperate varieties of sorghum very difficult. The time required for a sorghum plant to mature directly determines how tall the stalk will grow and how well the plant will produce grain.

The original intent of the researchers was, of course, to develop a variety of sorghum that is disease resistant. To accomplish this the scientists had to start by crossing resisting tropical varieties with temperate varieties in tropical locations. In 1961 work began in tropical Mexico and another project was approved between researchers in Texas and the U.S. Department of Agriculture station at Mayaguez, Puerto Rico, in 1963.

In the conversion process tropical varieties of sorghum are crossed with temperate varieties and the first generation is grown in the tropical climate. The second generation of the new variety is grown in the temperate zone. The result, after crossing and backcrossing the different varieties, is a sorghum plant that can function well in both climates and produce a maximum amount of grain and fiber. Hybrid varieties developed in Texas are being exported to other countries throughout the world.

Research in agriculture has put the United States ahead of the rest of the world in food production for many years. Disease resistant and early maturing crops are nothing new to farmers here. But the idea of producing fuel from stalks of grain sorghum, after the grain has been harvested, is something that has been brought about by the severe shortage of oil.

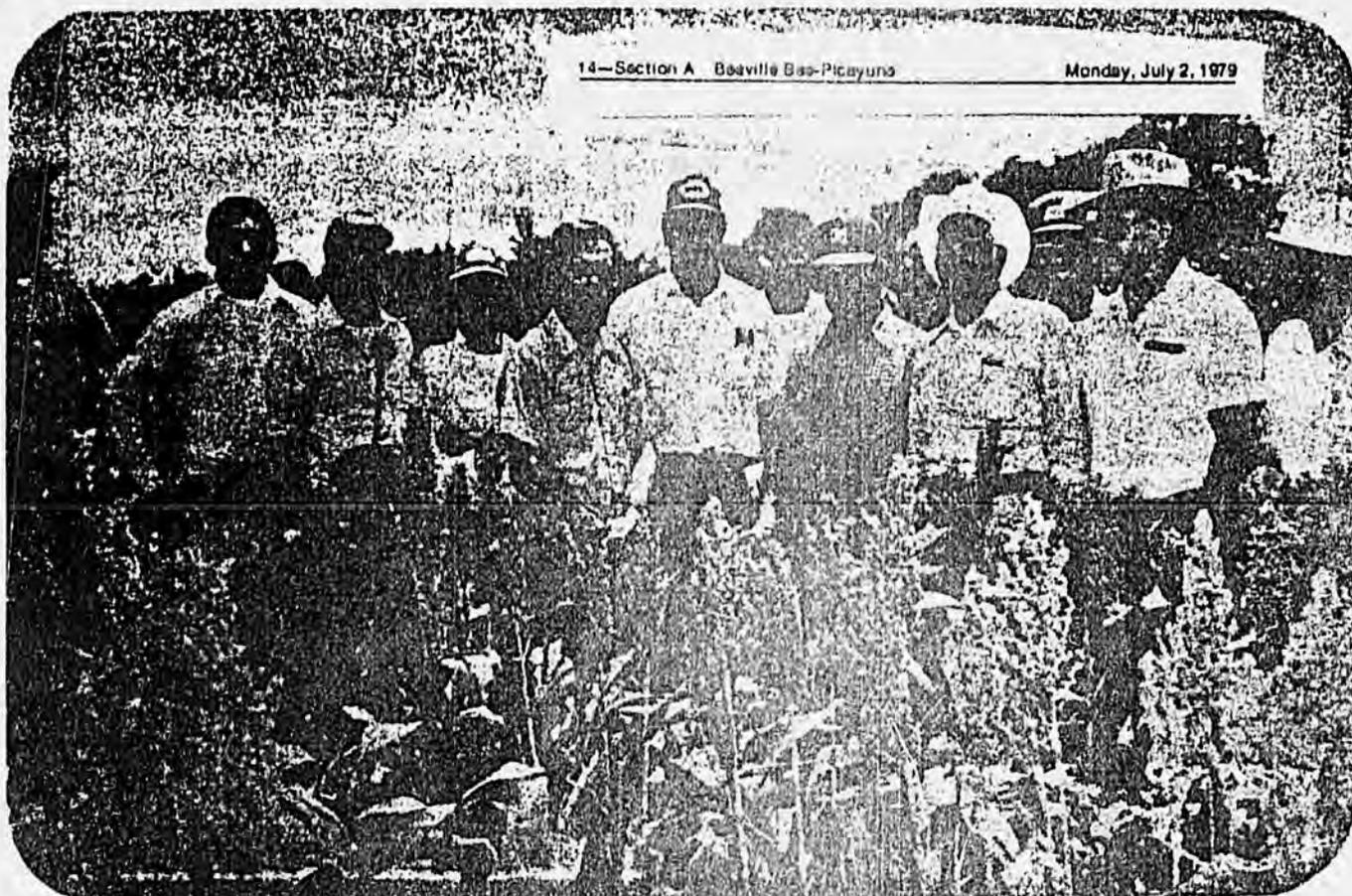
If the research proves successful and economical distillation methods can be developed, South Texas farmers may some day play an important part in the production of this country's fuel, while providing much of its grain at the same time.



Tarzan's Trail?

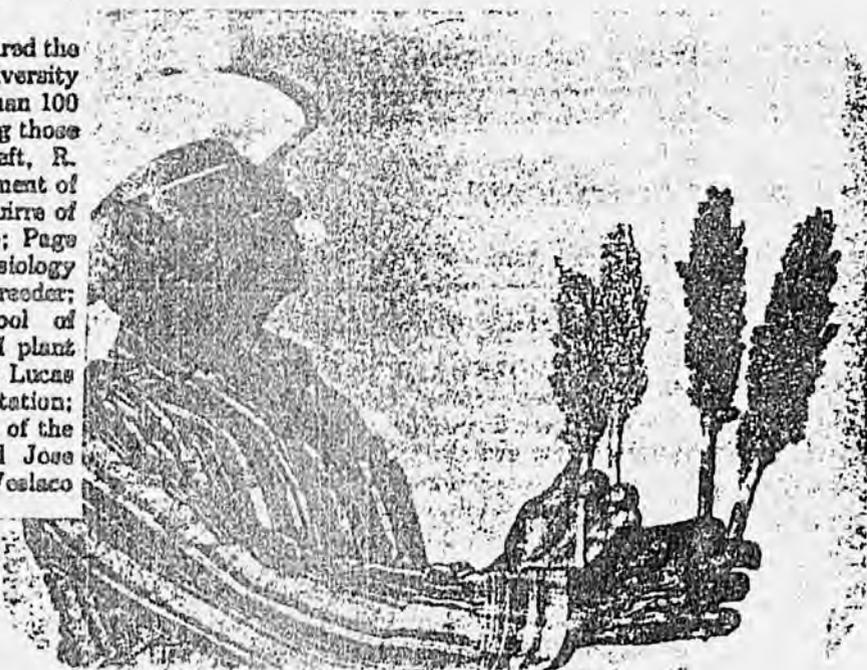
Lucas Reyes, left, and Dr. Fred Miller inspect the differences in short-statured, grain bearing sorghum plants and the taller, canelike tropical sorghum plants. Reyes, a research agronomist at the Corpus Christi Research and Extension Services Center, and Miller, a Texas A&M University sorghum plant breeder, were in Beeville inspecting test plants as part of the recent 1979 Sorghum Workshop, which drew over 100 sorghum research scientists from all over the world.

(Harrison Photography)



Worldwide Workshop

The recent 1979 Sorghum Workshop, which toured the sorghum test plots at the Texas A&M University Research Station in Beaville, attracted more than 100 sorghum experts from all over the world. Among those attending the workshop here were, from left, R. Fredericksen and J. Craig of the A&M Department of Plant Pathology; R. Giron and J. Ignacio Aguirre of the Rio Bravo Station in Tamaulipas, Mexico; Page Morgan, head of the Department of Plant Physiology at A&M; Darrell Rosenow, A&M plant breeder; Agustin Sanchez of the Monterrey School of Agriculture in Mexico; Dr. Fred Miller, A&M plant breeder; Jose Esparza of the Rio Bravo Station; Lucas Reyes of the Corpus Christi A&M Research Station; Nelson Jimenes of Brazil; Dr. Howard Joham of the Department of Plant Science at A&M, and Jose Amador, extension agronomist of the Weslaco Research Station.



Getting Ahead

Lucas Reyes of the Texas A&M Research Station at Corpus Christi is shown with examples of grain sorghum heads grown at the Beaville Experiment Station. On the left is an African hybrid which could possibly be adapted to South Texas production for human consumption. On the right is a common variety of grain sorghum grown in this area. Reyes noted that while the darker grain locally is marketed by weight and used primarily in livestock feed, the lighter-colored African species contains more nutrients and food value, which might serve to make it a more profitable export product in the future.

(Mike Bedwell Photo)



NEWS

THE TEXAS AGRICULTURAL EXPERIMENT STATION/Neville P. Clarke, Director

The Texas A&M University System/College Station, Texas Telephone (AC-713) 845-2211

7-16-79 RESEARCH REPORT

FOR RELEASE ON RECEIPT

COLLEGE STATION---A sudden surge of a disease variously known as grain mold and grain weathering is showing up in Coastal Bend sorghum, one of that region's most important cash crops.

The malady is believed to result from the unusually wet spring and is caused by parasitic fungi, plus natural deterioration by alternate drying and wetting.

Farmers whose crops have been affected so far are much disappointed because the overall season seemed to offer near-ideal conditions for bountiful sorghum production. The problem became apparent when they noticed grain weights weren't what they should be

"It's all a surprise to us," said Lucas Reyes, research scientist with the Texas A&M University Agricultural Research and Extension Center at Corpus Christi. "We learned about the problem when farmers began complaining about their bushel and truck weight being way down."

He described the Coastal Bend sorghum country as bounded by a line from Kingsville to Port Lavaca, inland to Austin, down through San Antonio and Cotulla, and back to Kingsville.

Although the acreage is down a little this year, farmers usually put about a million acres into the grain. That acreage, when disease doesn't strike, can gross an estimated \$90 million at current prices.

Reyes said the amount of acreage hit by the mold has not been determined yet, "but farmers are losing money."

A good average figure on sorghum production here is 56 pounds per bushel. But he said that has dropped to 52 to 54 pounds, and farmers have found that their truck weights are 10 to 20 percent lighter.

Figures on affected acreage should be available sometime next week, when the bulk of the crop will come out of the fields, Reyes said. And there may be some answers to other questions.

"If we can pin-point which new hybrids are causing most of the trouble, then maybe we can take steps to correct the situation in the future," he added.

He said mold-damaged grain is still usable, Small quantities can be blended with normal grain and fed to livestock.

Dr. Richard A. Frederiksen, plant pathologist with the Texas Agricultural Experiment Station, emphasized that the mold is not one that has drifted in from tropical climes. Instead, the fungi lurks in the soil all the time, waiting for the right conditions to strike.

Those favorable conditions occurred this spring, when frequent rains activated more molds than usual. Much of that moisture came as the sorghum was flowering, a time when some varieties apparently are more susceptible.

Frederiksen said grain mold may possibly be the most damaging sorghum disease world-wide, and it can "erode genetic progress" in plant breeding efforts.

He said the mold seems to come in three-year cycles, and that 1968 was a classic year.

Loral Castor, Experiment Station research assistant, said the mold causes reduction in kernel size and grain filling. Sorghum plant heads can look about normal and still have damage. Such damage may not show up until after harvest and the grain is weighed.

He said it is possible for sorghum varieties to have good weathering resistance and still be susceptible to mold fungi.

"This is because breeders sometimes base their weathering resistance findings on mature head conditions instead of the early flowering stage," the researcher explained.

On the positive side, Castor said there are sorghum lines vastly superior in resistance to these molds, both in early and late infection stages. The lines are on the way, and their mold resistance will eventually show up in new varieties.

The mold and weathering problem is being watched and monitored by a team of Experiment Station scientists. In addition to Frederiksen, Reyes and Castor, other members are Dr. Fred Miller and Dr. Lloyd Rooney of the A&M Soil and Crop Sciences Department, and Dr. Darrell Rosenow, based at the Texas A&M University Agricultural Research and Extension Center at Lubbock.

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THE TEXAS AGRICULTURAL EXPERIMENT STATION Neville P. Clarke, Director
Texas A&M University System | College Station, Texas

NEWSLETTER TO STATION STAFF

HOST PLANT RESISTANCE TO BE EXPLORED AT A&M CONFERENCE, JULY 22-AUG. 4

The most fundamental approach to resolving insect and plant disease problems--plant resistance--will be explored in a special conference July 22-Aug. 4 at Texas A&M University.

Participants in the Short Course on Host Plant Resistance, sponsored by A&M and the University of California AID Pest Management Project, will hear about 35 lecturers from entomology, plant breeding, plant pathology and related fields.

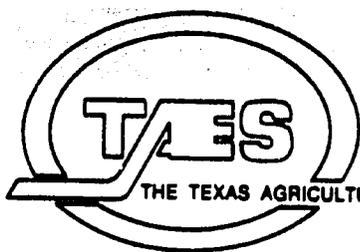
Dr. Marvin K. Harris, short course chairman, said the short course is significant in light of the large amounts of the world's food and fiber that are ruined each year by insects and plant diseases.

Short course lecturers have been drawn from Texas A&M, the U.S. Department of Agriculture, other universities, international institutes and scientific organizations. Harris said all major agricultural crops will be discussed, and sorghum, corn, cotton and peanuts will be seen in the field near College Station.

Short Course participation is limited to 35 persons, Harris said. Participants so far are entomologists, plant pathologists and plant breeders, and many are coming from Asia, Africa, Central and South America.

Harris said a proceedings of the formal presentations will be published after the short course.

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Brief Reports on

Agricultural Research in Texas

Vol.5, No.7
July 1979

HYDROCYANIC ACID IN SORGHUM LINES

Several factors influence the level of hydrocyanic acid (HCN-p) that may be present in sorghums. Major causes may be genetic; stage of growth; nitrogen fertilization; or stress, especially that due to moisture or frost. Fifteen sorghum lines, including thirteen grain sorghums, one sudangrass and one sweet sorghum, were grown under field conditions for studies on HCN-p potential. They were planted on a Shipler clay on land not fertilized the previous year and uniformly cropped with maize. Soil samples indicated levels of N, P₂O₅, and K₂O were less than 34, 114 and 970 lbs/acre respectively, for a depth of 6 inches. Findings of scientists with the Texas Agricultural Experiment Station include: 1) potential levels of HCN-p were increased by adding nitrogen, even in lines or varieties that are inherently low; 2) two entries were consistently low in HCN-p at all harvest dates and only exceeded the "threshold of danger" (200 parts per million) at the preboot stage; 3) as expected, HCN-p tended to decrease with maturity of the plant; 4) but, two of the entries still retained HCN-p in excess of 200ppm, indicating a need to exercise caution with some varieties when allowing cattle to graze stover after grain harvest. Source: George McBee, Fred Miller, College Station, Tel. 713/845-2541.

Agricultural Research in Texas

Vol.5, No.10
October 1979

MIDGE-RESISTANT SORGHUM CULTIVARS

Sorghum cultivars from 20 countries were evaluated for resistance to the sorghum midge by scientists with the Texas Agricultural Experiment Station (TAES). The 210 cultivars were obtained from the TAES-USDA sorghum conversion program. Midge damage ratings from three tests grown in two states in 1978 indicate that 10 cultivars are highly resistant (less than 30% seed loss) and 21 cultivars are moderately resistant (30 to 60% seed loss). Highly resistant lines had been collected from Sudan, Ethiopia, Uganda, India and Pakistan. These resistant lines are being improved for agronomic quality. Indications are these materials will serve as sources of resistance for commercial breeders. Source: J. W. Johnson, Lubbock, Tel. 806/746-6101; G. L. Teetes, A. L. Wuensche, College Station, Tel. 713/845-3411.

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NO MATTER what your sorghum yield will be this season, you can beat it. Probably by a considerable margin.

What doesn't just go for weedy, neglected cropland. It also applies to those "in-busling pampered fields" that have come from good seed, proper fertilization, weed control and adequate moisture.

That kind of challenge might not have meant so much in the last few years, but with a degree of optimism showing up in grain markets, better yield might be worth thinking about again.

That statement about improvement is not idle talk. Startlingly, Dr. Fred Miller, a sorghum breeder at Texas A&M, estimates that the grain sorghum plant has the potential un-

The grain sorghum plant has the potential under ideal conditions to yield up to 65,000 lb. of grain per acre.

der ideal conditions to yield up to 65,000 lb. of grain per acre.

Mind you, that's under ideal conditions. No one's done it yet. But the potential is there. And you can use some of the planning going into that figure in order to improve your own crop.

To do that, Extension agents advise farmers to follow some of the following guidelines:

system," which would include proper hybrid selection, land preparation and cultural practices such as crop rotation, proper fertilization including correct timing and placement of the fertilizer elements, proper planting, insect, disease and weed control.

"Through genetics," he says, "we've been able to come up with better hybrids than most would have ever believed possible. Higher yields,

stronger stalks, tropically or wide area adapted hybrids, twin seeded sorghum, disease and insect resistance."

Bremer says with some of the newer adapted sorghum hybrids, planting has become very critical. Especially important is adjusting plant populations to given soil and localized moisture conditions. Improper planting rates cut yields. And even

more important, poor, uneven stands invite weed problems and further yield cuts.

"We know from experience that crop rotation is very important for two main reasons. The first is to control sorghum diseases. And second, weeds can become problems when a continuous crop of sorghum is grown on a given tract of land year after year."

Bremer also cites research done at the Blackland Research Center at Temple that shows yields can be increased an average of 19 percent by narrowing rows from 40 to 30 in.

"We feel that certain tillage practices can be reduced by the judicious use of chemicals," he says. "We've continued to work over the field

Tropical Adapted Sorghums

TROPICALLY adapted sorghum hybrids hold lots of promise. If you're not already acquainted with them, here's a definition from Dr. Fred Miller of Texas A&M.

Tropical adaptation simply refers to a hybrid bred from at least one "tropical" parent line. It refers to the ability of a sorghum hybrid to maintain high yield potential under short day length and high night temperatures.

One way it differs from "normal" temperate hybrids is that temperate hybrids mature faster when moved to southern climates because of their genetic makeup. Shorter day lengths and long, hot nights cause rapid maturity in places like South Texas. Since the temperates mature faster when moved south, they don't set as many leaves, don't fill out heads as well as in northern zones, don't have the stalk quality they might normally have, and don't yield as well. This acceleration of the plant's maturity is the major reason southern sorghum yields have been traditionally lower than northern yields, according to Miller.

In contrast, tropically adapted hybrids grow well in areas where day length is short and nights are long and hot. In fact, he believes tropically adapted hybrids slow down their nighttime respiration rate, expending less photosynthate for plant maintenance and leaving more for grain.

A temperate hybrid might flower in 75 days at Lincoln, Neb., and flower at 50 days at Corpus Christi. On the other hand, a tropically adapted hybrid might flower in 70 days at Lincoln and 75 days at Corpus Christi.

New research indicates tropical hybrids have a lower base metabolic temperature. In laboratory tests at Texas A&M to measure temperature required for germination, tropical hybrids germinated at 46 to 48 degrees while temperate hybrids needed 50 to 51 degrees for germination.

Higher planting rates yields. Poor, uneven stands invite weed problems and further yield cuts.

shortage and higher fuel prices minimize tillage benefits. Corn is once again being considered as a production. However, tropical sorghum fields with heavy rotation can reduce the difference between crop profit, a break-even situation or even a loss. The plow isn't abandoned. There still is a definite place for it. But in many cases perhaps a reliable mechanical implement might be better than a more economical substitute."

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THE TEXAS AGRICULTURAL EXPERIMENT STATION

Agricultural Research in Texas

 Vol.5, No.8
 August 1979

WHAT THE SCIENTIST SEES

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Larvae of the sorghum midge, *Contarinia sorghicola* (Coquillett), are so small that one or more can complete development within a single sorghum spikelet, where they usually feed upon the developing plant ovary. Despite their small size, such larvae can, when present in large numbers, severely damage developing seeds and greatly reduce yields. Because sorghum midge larvae can cost Texas farmers millions of dollars annually in yield losses or chemical control measures, Texas Agricultural Experiment Station (TAES) scientists are seeking improved management strategies for the control of this pest.

In these new management strategies, plant resistance is expected to be a major component. TAES scientists studying the mechanisms involved in plant resistance believe that a better understanding of how the larvae feed is important. Light microscopes cannot provide the high magnification and fine detail required for study of the minute mouth-

parts. However, under the direction of Dr. George Teetes, associate professor of entomology, graduate research assistants Ron Petralia and Allen Wuensche have devised ways of using a scanning electron microscope (SEM) to provide magnifications of the larval head as much as 7500 times actual size. By means of SEM micrographs such as the one shown below (left), they have been able to identify and describe individual mouthparts. The results of their study indicate that midge larvae feed on plant fluids rather than by chewing plant tissue, and that extra-intestinal digestion probably aids in the production of sufficient quantities of fluid for larval development. In addition, the SEM micrographs have been used by Dr. Ann Sorensen to prepare the first detailed illustration of a larval sorghum midge head (right, below). (Source: Dr. G. L. Teetes, College Station, Tel. 713/845-3411.)



Plate 6

The electron micrograph shows the external morphology of the mouthparts of a late larval instar, including: labro-clypeal plate (lp), mandible (m), mandibular ridges (black arrows), mandibular lobe (black and white arrow), maxilla (mx), labrum (l), ligula (li), and sitophore (s).



Plate 8

This is a diagrammatic frontal view of the larval head and mouthparts, based on many SEM micrographs, X7500 (illustration by A. A. Sorensen).

in the South

Minimum tillage gives year-round forage. It may be the way to raise forages on grass sod in the South, say Louisiana State University agronomists Marvin Allen and Lee Mason. They're planting corn and forage sorghum in bermudagrass and bahiagrass sods after first suppressing grass growth with herbicides. Yields from tests have equalled those conventionally planted. They've also gotten good stands of ryegrass and clover the same way.

To suppress the grass for corn and sorghum, one qt/A of Roundup was broadcast prior to planting. Two qts/A of AAtrex was banded.

To establish clover and ryegrass, researchers applied 1 pt. of Paraquat. The dried up plant

residue was then burned before seeding the clover and rye with a minimum-till drill.

Ryegrass established on sod provides grazing from early December through May, when the bahiagrass starts providing forage again. Clover planted in October should be ready by March.

Nematodes cut grass yield. Yields of cool-season perennial forage grasses are severely reduced on light-textured soils when nematodes are present, advise Auburn University agronomists. When chemicals were applied to control the pests, second-year grass yields increased 50-90% during autumn and early winter periods.

Stubby root, stunt and lance nematodes reduced root weight of tall fescue 60%, orchardgrass 47%. Infested grass fields, the agronomists note, are highly susceptible to autumn drought. Infested stands lack competitive vigor and eventually become infested with weeds and warm-season perennial grasses.

Although the Alabama tests clearly show greater yields are achieved by treating for nematodes, the researchers caution chemical control measures are not practical at this time.

New lodge resistant soybean. If you're experiencing lodging problems with Lee 74 soybeans, hang on, help's on the way.

The Oklahoma experiment station has released "Sohoma," a new variety reputed to be lodge resistant and high yielding based on tests in both Oklahoma and western Arkansas.

Sahoma falls in maturity Group VI, about 2-3 days earlier than Lee 74. It's a bit larger seeded than most of the varieties being grown in the region, tests higher in both protein and oil. Plants are shorter than Lee 74, have average drought tolerance.

Alfalfa benefits from killing frost. Grazing or cutting alfalfa for hay soon after the first freeze-down not only stretches winter feed supplies, but more importantly reduces weevil damage next spring.

"Harvesting after a freeze this fall will destroy some of the weevils going into the winter and reduce the number of places for them to lay eggs," explains University of Kentucky entomologist Chris Christensen.

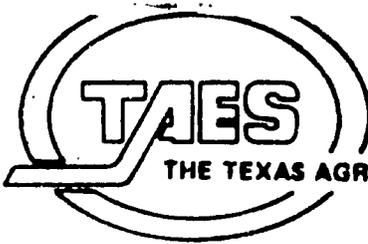
—By Charles E. Summers and Greg Lamb, Crops and Soils Editor

Tropical sorghums jump yields by 20%. And in some south Texas areas grain sorghum yields have doubled from tropically adapted varieties that were first introduced in 1974, explains Texas A&M sorghum breeder Fred Miller.

"In most of the world, sorghum is used as a human food and red color is a prejudice factor," he says. "That's why we're now breeding for white seeded sorghums which can be used for both human and animal consumption.

"By using best management practices and getting reasonable rainfall, some farmers are producing 5,000 lbs/A yields with white seeds, rather than the 3,000 lbs/A red seed average. Eventually, I'd like to see a total phase out of all red hybrids."

In the next few years, Miller expects more disease resistant and higher yielding white sorghums. Experimentally, Texas A&M now consistently produces white variety sorghums yielding 12,000 lbs/A.



THE TEXAS AGRICULTURAL EXPERIMENT STATION / Neville P. Clarke, Director

Texas A&M University System / College Station, Texas

September 1979

No.

DR. FRED MILLER INVITED TO STUDY SORGHUMS IN CHINA

Fred Miller, a leader in grain sorghum investigations, is one of seven U.S. scientists serving with an agricultural exchange team in the People's Republic of China for the next five weeks.

Miller, whose research with grain sorghums has received attention internationally, was invited to serve with the U.S. team, whose objective will be to study the acquisition of germplasm of soybeans, sorghum, millet and vegetables.

The U.S. recently entered into an agricultural exchange program with the People's Republic of China. The program involves reciprocal visits by scientists.

"Our team will visit production units where a broad range of germplasm of major crops is under evaluation, including soybeans, sorghums, millets and vegetables. We will be permitted to select materials of interest to the U.S. from PRC selections," Miller explained.

The U.S. team will visit the Academy of Agricultural Sciences and nearby production units near Peking, Kirin, Shanghai, Shenyang and other provinces.

"Our team will discuss the future exchange of germplasm and relate other crop information covered in this first exchange program. Once the team arrives in China, members will be guests of the Republic of China," Miller added.

Miller will be concerned with collection of data and germplasm on sorghums and soybeans.

Also concerned with soybeans will be Dr. W. R. Fehr of Iowa State University, Dr. K. Hinson of the Agricultural Experiment Station at the University of Florida, and Dr. K. R. Bromfield of the Plant Disease Laboratory at Frederick, Md.

Exploring possibilities for vegetable germplasm exchange will be Dr. E. Z. Wann, director of the U.S. Vegetable Breeding Laboratory at Charleston, S.C., and Dr. John Bouwkamp, of the University of Maryland. Dr. W. W. Hanna of the Coastal Plains Experiment Station at Tifton, Ga., will be concerned with collecting germplasm of millet.

Miller met other team members in Washington, D.C., Aug. 13 and attended a briefing session conducted by the U.S. Department of Agriculture. The team then left for Tokyo and continued on to Peking.

Miller returned to College Station, Sept. 17.



Dr. Fred Miller

Researcher Miller Invited To Study Sorghums in China

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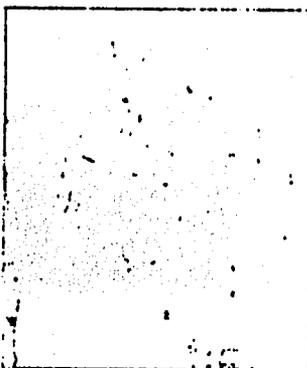
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Miller is scheduled to arrive back in College Station, Sept. 17.

FARM JOURNAL - Mid February 1979

Will sorghum feed and power the future? Despite many naysayers on the economics of producing fuel from grains, Frederick R. Miller, Texas A&M genetic engineer, thinks the answer may lie in changes that can be bred into the plant. The variety shown yielded about 7,000 lb. of



grain per acre and 37 tons of stover, he says. That stover yield would produce about 275 gal of methanol for use as alternative fuels. "The sorghums under development now could allow an entire plant to supply energy for man, animals and mechanical vehicles," he says.

MID-FEBRUARY 1979

THE FURROW

November/December 1979

BROADER ROLE FOR SORGHUM?

Researchers say there's potential for developing grain sorghum as a food crop

Sorghum, sorghum everywhere, but not a grain to eat—at least not for people in this country. It's no secret that among the major grain crops raised in the United States, none is used more exclusively for feed than grain sorghum is. Less than 1 percent finds its way into any type of product used for human consumption.

That doesn't necessarily have to be the case, however. Food scientists at several universities are using grain sorghum to make all sorts of tasty goodies, including bread, cookies, tortillas, noodles, a puffed cereal-like product, and a cheese-flavored snack that looks and tastes much like several made from corn that are now on the market.

Lloyd Rooney, a cereal chemist at Texas A & M University who's been working on sorghum quality for the past 12 years, says there's no question that sorghum is mainly a feed grain in the U.S., and will be for a long time to come. But he doesn't discount the possibility that some day it may become a food grain as well.

Used elsewhere. In many tropical areas—particularly in Asia and Africa—grain sorghum is a basic dietary staple for much of the population. In India, it's made into chapattis and rotis, which are similar to Mexican tortillas. Enjara and kissera, the basic breads of Ethiopia and the Sudan, are made from fermented grain sorghum flour.

Several African countries produce sorghum beer, which reportedly is less bitter than American brews but has a higher alcohol content. Other products made from sorghum include a cola-like soft drink, porridge, soups, baked goods prepared from a combination of sorghum and wheat flours, and even cosmetics.

Rooney says the red- and brown-seeded sorghums U.S. farmers grow aren't very satisfactory for these products. "Most of the sorghums raised overseas have nice white kernels, a bland flavor and better milling properties than the types we raise here," he explains.

New color. For a number of years, geneticist Fred Miller and others at Texas A & M have been working with some of the white-seeded types. Miller says most of the sorghums coming out of the Texas research program now are white-seeded. All have been analyzed for food grain quality and many have been selected in part for their human-food potential.

Miller is also working on some vegetable-type sorghums with sweet seeds that are harvested green and used much like garden peas. The grains are eaten in some parts of India and Africa, and Rooney says that when he took some to a Texas A & M faculty potluck, "people were coming back for seconds and asking what it was."

In addition to sorghums for food uses, the Texas scientists are developing types with more sugar in the stem. These, Miller says, could have real potential as raw material for tuel alcohol. "In Brazil," he explains, "some sweet sorghums have been found that yield more tuel per acre than sugarcane."

Another angle Miller is looking into is whether both grain yield and plant mass can be increased simultaneously in sorghum, or if one must be sacrificed to increase

Cereal chemist Lloyd Rooney displays food products made with grain sorghum, including a package of cereal sold in South Africa.

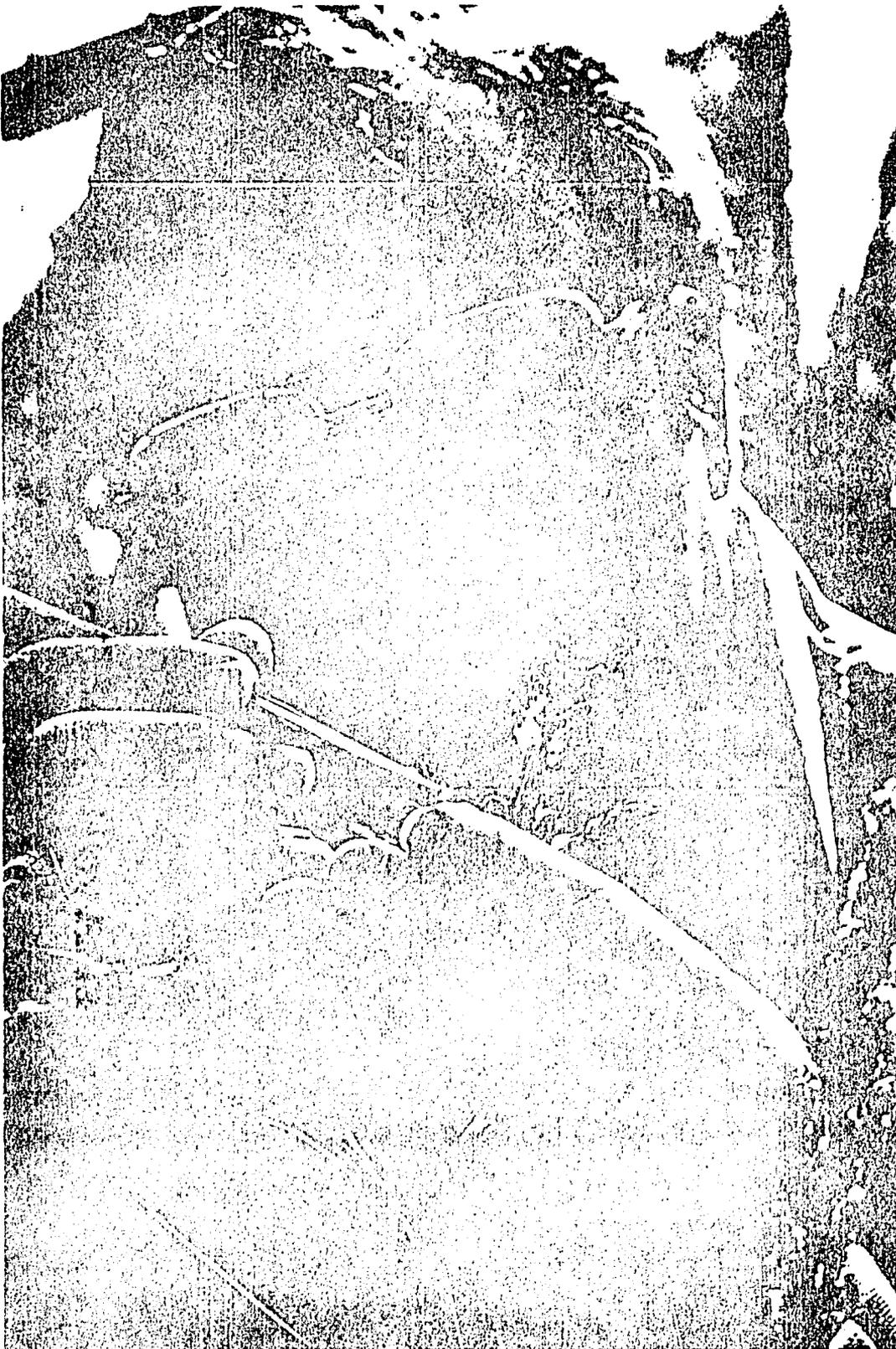


Mir Khan, a research scientist at Texas A & M University, checks loaves of bread made from a mixture of grain sorghum and wheat flours.



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Geneticist Fred Miller is developing sorghums that produce white, bland-flavored seed suitable for use in a variety of foods.



the other. He hopes eventually to develop selections that will produce large amounts of both grain and stover.

Scientists at other institutions are also looking at sorghum as a food crop. At Purdue University, for example, geneticist John Ax-tell is developing high-lysine varieties with twice the protein value of normal sorghums.

The federal government is getting into the act, too, by funding a major sorghum technology project to be conducted by researchers at eight universities.

Carl Hosenev, a cereal chemist at Kansas State University, explains that the main goals of the project are, first, to develop standards breeders can use to tailor new sorghums to specific food requirements and, second, to improve the primitive sorghum-processing methods now used in most developing nations.

Export potential. The sorghum technology project is primarily intended to help people in less developed countries get more out of the crop, but Hosenev says the work could benefit U.S. farmers as well. "I think sorghum has good export potential," he explains, "but in order to develop it, we'll have to pay much closer attention to what potential customers need."

Geneticist Fred Miller agrees. "Any sorghum that will work well in food products will also make a good feed grain, so there's really no reason not to develop and use food types," Miller says. "With sorghum being well established as a food grain in so much of the world, we should be able to export a great deal of it if suitable varieties are available and if American farmers will grow them."

Crops To Fuel

New Enthusiasm

Needs Caution

By Calvin Pigg, Editor

FORTH WORTH, Texas—Mushrooming enthusiasm over prospects for turning agricultural commodities into fuel energy can only be "cautiously justified" now.

Any major-scale conversion of biomass into alcohol fuels must come as part of a larger integrated feed, feed, fiber and fuel complex to be anywhere near economically worthwhile—at least for now, speakers reporting before Texas seedsmen generally agreed here recently.

Gasohol will play only a "trivial" role in solving the nation's energy dilemma, the 201 gathered members of the Texas Seed Trade Association were told.

However, among southwest-grown crops, corn, sorghum and sugarcane are leading candidates for fuel alcohol making.

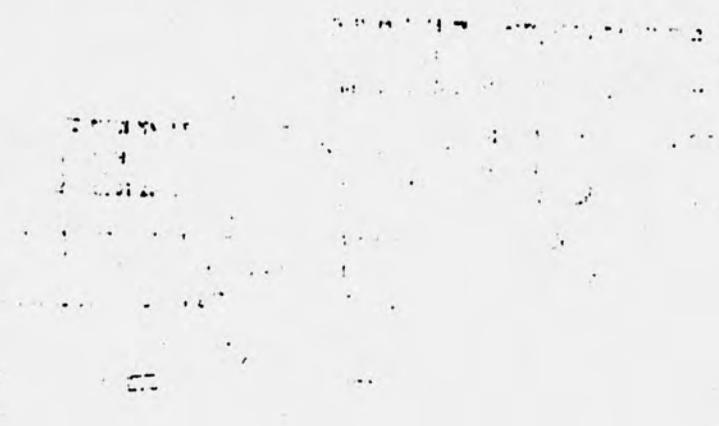
Dual-Purpose?

Noting few so-called farmers interest in an obvious "energy from farm crops" drive, state seedsmen considered at their 127 Hilton Inn meeting here whether their plant breeders should go to work "reconstituting" certain crops for dual-purpose roles.

Theme of the meeting was "Different Approaches to Alcohol Production in Texas."

Featuring top-flight experts on the energy field, the session focused on just how fragile the fuels from agricultural question actually is today.

In organization business matters, a South Texas, C. W. Worthen Jr., was named President. Worthen is with the Seed Company of Houston and is a prominent seed company executive. Also in the post...



Best Available Document



NEWLY ELECTED OFFICERS OF THE Texas Seed Trade Association: Warren Jr. of Houston, president; H. H. General, director of State and...

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Breeder Says Crops Easily Altered

Forages Important

"We also feel that in the very near future, forages for fuel-making are going to become more important," said Kresovich, noting new processing and conversion techniques.

He detailed two newly developed, cost-cutting conversion processes, one called the MIT process, devised at Massachusetts Institute of Technology and another popularly called the Chem System, developed by a private firm.

He said whereas older methods of making ethanol once required 150,000 Btus to produce, the figure with the newer procedures is no greater than 20,000 Btus.

"In the next year or so, I believe they'll have it down to only 5,000 Btus of energy—so we're not talking negative energy balance anymore," said Kresovich. Ethanol has an equivalent 80,000 Btus.

In a later interview, Kresovich told SOUTHWEST FARM PRESS that some of the current enthusiasm among land operators over the fuels potential, including investments a few are making, is only "cautiously justified."

"There is a large amount of potential in biomass energy in Texas. However, people must take caution in all the various aspects, not the least is safety on on-farm distillation of alcohol and the legal ramifications."

He said any successful alcohol system will need to consider use of all fermentation by-products for the fuels scheme to be profitable.

"Don't go overboard," he suggested.

"Energy systems for agriculture are multidisciplinary, you have to know about commodity production, chemical engineering and a good bit about economics."

Another speaker was Texas A&M sorghum breeder, Dr. Fred R. Miller of College Station.

Easily Altered

Dr. Miller said commercial grain sorghums can be altered fairly easily for an additional role as an energy crop. He envisioned a grain sorghum plant five to six feet tall giving high grain yields and having a large stem area providing increased sweetness (sugars) for conversion into fuels.

"Many new methods are available to plant breeders to genetically engineer specific types of plants," he said. He said a similar type of genetic rebuilding applies to corn, sugarcane, wheat, peanuts, soybeans, sunflowers and other high carbohydrate crops as well as to sorghum.

The plant breeder said sorghum might be reconstructed for fuel purposes easier than other commercial farm crops. "The main reason such reconstruction is possible, we believe, in sorghum," said Dr. Miller, "is that we know so much about sorghum through our work with the world collection of sorghums, where specific traits have been singled out and drawn into present day hybrids."

He said now with the fuel possibility, crops such as sorghum, could be altered to serve as multi-purpose crops such as for feed, food and for fuel.

He said building materials can be manufactured from cellulose fibers left from the sorghum plant.

Maximize Grain

"We need the grain to produce both food and feed from sorghums—we cannot change that part of our reconstructed plant," Dr. Miller told the seedsmen. "In fact, if we could produce more grain that would be to our advantage. Any excess grain over market needs for feed or food could go to produce alcohol."

He said the different need is for added sugar, or the amount of total fermentables, in the stem of plants as a source of energy for alcohol and as sugar for food.

The breeder's final prediction:

"We will see much more awareness of total plant utilization from our major agricultural crops as we move into this era of harvesting both food and fuel from our fields. This will bring the need to develop new plants with multiple uses rather than just sorghum or corn for grain." He called it a system of "more total utilization of green plant materials."

The Texas A&M expert said he is active in one research project to devise a high energy system with sorghum.

"From all we know about economics of farm fuel production today," concluded Dr. Miller, "producers are well advised to leave fuel making alone unless they can use all the by-products. He said dried distillers grain, a left-over from fermentation, is commonly utilized in cattle feedlots and on dairy farms in some areas.

A commercial seedsmen, Jim Lindsey of Plainview, president of Pioneer Hi-Bred International, Incorporated and president, Southern Seedsmen's Association, was next speaker on the program. He spoke on "The Seed Industry Challenge" to fuel crops development.

He challenged his fellow seed merchants to stay alert to all fuel crop possibilities, as a "new economics of energy" emerges.

Suspects Doubts

"I suspect that many people in this room harbor some doubts about the likelihood of farm crops becoming a serious source of energy—or that the production of fuel will be a serious market for farm crops," said Lindsey.

The seed official pointed out that the economy of the U.S. is based on energy and the cost of that energy will increase. "In other words," said Lindsey, "there'll be a new economics of energy. And, it's not just a matter of rewriting the old rules. The whole rule book has to be thrown out and a new one has to be written.

"If we feel we're sticking our neck out in getting serious about gasohol," continued the speaker.

"think of the guy who puts his money on the status quo today—he won't make it."

Lindsey told his fellow seed industry men that the "profitability" of gasohol, the 9-to-1 blend of gasoline and ethanol (alcohol), is a key part of the alternate fuels question.

Gasohol Advantage

"None of the other alternate energy sources can be moved around so easily—not nuclear, not solar, not really coal," stressed Lindsey. "Gasohol is the most easily available fuel for extending our fast-dwindling supply of motor fuels."

He said it will be exciting to see what the seed industry can contribute "to this critical energy in man's experience."

The three-day conference concluded with luncheon remarks by David E. Gushee, chief of environmental and natural resources policy for the Library of Congress in Washington, D.C.

In his assignment, Gushee provides congressional members with data on the nation's energy matters. He was less enthusiastic about gasohol serving the national interest. He said Congress has been reluctant to do much in "uneconomic energy areas" such as gasohol represents currently.

Congress Moving

Gushee's tact was that the U.S. Congress appears not to be moving forward on energy policy development but that actually it is. "Congress recognizes that many specific legislative actions on energy can be highly counterproductive to our citizens.

He told the luncheon audience that gasohol "in the national policy scene is actually a trivial matter."

"It is a trivial matter," he explained, "because as things stand today you must write off four cents a gallon in national highway tax funds in order for gasohol to compete in the open market place." He said for gasohol to be viable, it has to be subsidized by either direct or indirect means through the tax system.

"In the final analysis," said Gushee, "gasohol is more expensive the gasoline."

Cites Examples

He said Iowa and Nebraska are in trouble with their state highway maintenance programs due to diminishing funds for repair. Gasohol with a four-cent a gallon state tax exemption, he said, has penetrated 10 percent of the total motor fuels market in those states.

"As we view it, continued the Library of Congress official, "There is an opportunity for ethanol if you do it yourself, and consider it as a secondary enterprise to a farming operation." He said economic do not permit the consumer to fully charge his land and labor costs to fuel energy production.

LIBRARY OF CONGRESS energy
analyst David Gushee tells seedsmen
gasohol is a trivial part of national energy

question and that gasoline is still cheaper
than the alternative fuel.

"You cannot afford the cost of harvesting and stockpiling biomass materials for its energy purposes alone today," added Gushee. He said Congress is considering \$700 million in incentives to promote gasohol production but that the bill as originally proposed will be altered considerably.

Concluding, Gushee suggested that for gasohol to be feasible as a general motor fuel, prices for fuels must rise considerably or federal policy must be changed to subsidized gasohol and then to spread the increased costs to citizens through the taxing system.

During the TSTA business meeting, in addition to new officers chosen, three additional directors were designated to serve the organization. They are Delbert Hess of Plainview, Curtis Tally of Justin and Roy Holland of Loriane, Texas.

With headquarters in Waco, the TSTA's executive secretary is C. L. M. Nooy.

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major agricultural crops as we move into this era of harvesting both food and fuel from our land."

Richardson Seeds, Incorporated. He heads up Richardson Seeds, Incorporated.

"You have here in Texas systems that can produce both food and energy on the same land and at the same time," one speaker, Steven Kresovich of the Battelle Columbus (Ohio) Laboratories, told the gathering.

No Trade-Off

"There is no trade-off. We can get food and we can get energy from the same land," said Kresovich, who is agricultural coordinator for biomass production systems for the 1929-founded non-profit research organization, active in biomass energy development for the past five years.

Kresovich explained as an example that with either corn or sorghum grain, the carbohydrate to make the alcohol can be removed while protein for food is left intact in the kernel of grain.

"In the short term, ethanol (anhydrous ethanol) produced from starches and sugars through fermentation," continued Kresovich, "is the easiest and most cost-effective way to go. This could be done under a number of different skill levels," said the Battelle specialist. "Alcohol from biomass can be produced with farm skills, with co-operative skills or on a multi-million dollar refinery basis."

Kresovich said that with new methods it appears the conversion to alcohol can eventually be cost-effective at all three levels. He said, however, on-farm alcohol making via a modernized still device is highly continuing to the operator.

Carbon Materials

In the long-run, methanol (alcohol) will be produced commercially from all carbon containing materials, he said.

"When give me the biomass at the cheapest possible cost and the chemical engineers will do the rest," said Kresovich, adding: "Farmers can be fuel self-sufficient but the technology and marketing will determine the overall feasibility going public with massive fuel alcohol production."

Steering the U.S. into a "perspective" into its energy

situation, Kresovich said Texas farmers consume an equivalent 210,000 barrels of crude oil in their operation daily.

He said agriculture's energy requirement is expected to increase at a rate of 9 percent a year until 1985 and increase by 17.4 percent annual gain by the year 2000.

Fuels Not Same

As to the alternate fuels possibility, the Battelle energy coordinator reminded the seedsmen that methanol and ethanol are not the same, in terms of energy equivalents. Methanol has only one-half the Btu equivalents of gasoline. Ethanol, used in gasoline, contains two-thirds as much working energy as gasoline.

He said the U.S. Department of Energy has projections calling for methanol to be produced commercially by the year 2000 for 50 to 60 cents a gallon and ethanol for \$1 a gallon. "But we can't wait that long."

He said the only plant producing ethanol today with corn as a fermentation feedstock, located in Decatur, Illinois, is producing anhydrous ethanol for \$4.25 to \$4.50 per gallon today.

The sugar in biomass materials, such as in sugarcane, is fermented to make the alcohol.

Leading Crops

"When energy production through biomass in Texas is spoken of," explained Kresovich, "the three leading candidates are corn, sugarcane and sorghum, specifically sweet

sorghum." He said Battelle has conducted research on the latter two.

Much information has been brought out about "energy balance", whether you draw more net energy from the conversion than you put into the manufacturing process, Kresovich continued.

"A lot of critics say it takes more energy than you get out of the final product and that's true if you use information from 1951," he added.

"We have at hand today breakthroughs in technology that will give us a more positive energy balance." He said that as examples, with latest know-how, corn converted into alcohol fuel can give a 1.05-times net energy gain, a 2.43-times gain is possible through sugarcane and a two-times energy balance is found when sweet sorghum is converted.

Noting other considerations, he reminded his audience that "net energy balance" doesn't have anything to do with the cost of

making the conversion from raw material into any final fuel product.

Kresovich said with the latest conversion methods it is now possible to convert sweet sorghum or sorghum grain into alcohol for a "positive energy balance" at around \$1.10 to \$1.20 a gallon, "and hopefully this will come down some more."

He said one advantage in the fermentation of corn and sorghum is that a tremendous amount of new potential for ethanol as a food or as a motor fuel is readily available.

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Improvement of Grain Sorghum for Developing
Countries - Texas A&M University

Dr. M. Peterson, Chairman of the Subcommittee composed of Dr. Thorbecke, Dr. Whitney, and Mr. Wittnebert briefly described the project as follows. Both this project and the Nebraska project were discussed together before the presentation of motions.

This is a proposal to extend for three years, or until 1979, a sorghum breeding program which A.I.D. has partially supported since 1974. This is essentially a situation of A.I.D. buying into an already large and generally successful sorghum breeding program at Texas A&M. In 1963, the Texas Station and the USDA began a program aimed at converting the tropical sorghums of the World Sorghum Collection to temperate types. The sorghums originated in Africa in the zone south of the Sahara desert where wild types are still found. They are believed to have been selected for cultivation as early as 3000 B.C. and were grown in India, Syria, and southern Europe. Cultivation in the U.S. began with introductions in the 1850's.

These early introductions and those still grown in some areas of developing countries are very tall, late maturing, and photo-period sensitive. The U.S. breeding contribution has been the discovery and exploitation of dwarfing genes, greatly lessened photo-period sensitivity, and increased grain yield. This enabled the development of types which could be grown over a very wide latitude and "combine types" which were short enough to be harvested with a grain combine. Sorghums proved to be a host for a great variety of diseases and insects.

Plant breeders learned long ago that resistance to diseases is most likely to be found where the crop has been grown for thousands of years and where the greatest variability of genotypes exist.

The expanded program initiated in 1963 was to convert tall, late, photo-period tropical sorghums to short, early, photo-period less-sensitive types and test these against diseases and insects occurring in the U.S. by straight forward breeding procedures to make crosses and produce the segregating generations in tropical (Puerto Rico) and temperate locations. The conversion system from tropical to temperate zone sorghums could work both ways. Thus the highly desirable qualities of temperate zone sorghums could be converted to sorghums adapted to the tropics. The final goal is to develop dwarf or semi-dwarf hybrids that are agronomically desirable, insect resistant, multiple-disease resistant, photo-period insensitive, environmentally tolerant, and can be utilized immediately in breeding programs in developing countries.

The project has already released a significant number of improved lines. A.I.D. does not claim a great deal of credit for this, because much of this is the culmination of 10-12 years of program work. Issues include: First, a need for the inclusion of information on the identity and qualifications of individuals assigned to the projects. Second, none of the budget items are alarming with the exception of the overhead rate. Third, the role of Puerto Rico should be reviewed, and if this part of the system is discontinued, it is perceived to be no longer necessary, or will it be fulfilled by ICRISAT? Fourth, there should be some comment on the quality evaluation of sorghum for human

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food. The Purdue project has determined that the tannins in sorghums are detrimental to protein digestion. The results of the Purdue project need to be incorporated into the Texas project, if in fact the results are transferable. Although the Purdue project is not now up for review, the point is made that severe limitations are imposed on plant breeding efforts conducted in areas where the crop is not grown commercially. Grain sorghums in Indiana practically do not exist.

Turning now to the review of the Nebraska project without open discussion.

ANNUAL REPORT

ON

DEVELOPMENT OF IMPROVED HIGH-
YIELDING SORGHUM CULTIVARS

February 1977 - October 1979

REPORT NO 4

prepared by

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Contract AID/ta-c-1382

Submitted to:

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Washington, D. C.

REPORT SUMMARY

- A.
1. Project Title and Contract Number: Development of Improved High-Yielding Sorghum Cultivars. Project No. 931-17-130-575. Contract AID/ta-c-1382.
 2. Principal Investigator, Contractor and Mailing Address: Jerry D. Eastin, 205 Kiesselbach Crops Research Laboratory, University of Nebraska, Lincoln, Nebraska 68583.
 3. Contract Period (as amended): from February 1974 to October 1979.
 4. Period covered by Report: from February 1977 to October 1979.
 5. Total A.I.D. funding of contract to date: \$7767.44.
 6. Total expenditures and obligations through previous contract year: \$295,000 (estimate).

B. Narrative Summary of Accomplishments and Utilization. Grain sorghum is a major food source in many LDC's. Hence the yield per acre of a quality energy and protein source is critical. Climate in the LDC's of interest is usually characterized by environmental extremes (temperature and water) as is often the case in the Great Plains of the U.S.A. The twin problems of maximizing yield and, perhaps more important, of stabilizing yield over the years is critical. The combined disciplines of physiology, breeding, nutrition, entomology and pathology are being brought to bear on these problems.

Principal efforts during the current report period included the following: (1) continued evaluation of breeding methodology for use with random mating populations - S_1 progeny testing appears best at this point, (2) selected within several populations for absence of the testa (a quality consideration) - selections will be recombined in 1977, (2) incorporation of greenbug resistance, twin seeded character, and yellow endosperm into one population, (4) tested the influence of different cytoplasms on yield and found minimal effects, (5) found yields of F_6 lines selected from NP3R and their hybrids to be equal to or superior to good checks (line performance was particularly good), (6) found nontesta populations superior to testa populations for metabolizable energy, (7) expanded development of yellow endosperm populations, and evaluated genetic combining ability of selected lines, (8) evaluated recurrent selection procedures for grain protein, (9) determined three-way crosses to be equal to single crosses for R line testing with the advantage of greater seed production, (10) released a combine height line, N32, giving 2 dwarf hybrids of potential interest in LDC's when crossed to most combine height R lines, (11) released several superior parental lines from a general combining ability study, (12) determined that the minimum number of S_1 families needed for reliable population evaluation is on the order of 100, (13) reported base yield levels of several populations, (14) tested twin seeded versus single seeded S_1 progeny and noted

superiority in the normal for grain yield (15) determined soil bedding and conventional tillage methods to be equal in yield, (16) determined the value of soybeans in a soybean-sorghum rotation to be equivalent to 40 to 100 kg/ha suggesting more grain legume-sorghum rotation work is in order in LDC's, (17) found that mulches delayed plant growth but increased dry matter yields, (18) determined that N efficient plants are not necessarily the top yielders, (19) demonstrated a water gradient approach was a useful drought selection tool, (20) demonstrated water stress conditioning occurs in sorghum, (21) determined that a good deal of work is in order on temperature and water influences on metabolic efficiency, (22) designed, installed and began evaluation of a modified water gradient system for drought screening to reduce the land requirement, (23) demonstrated that water and high temperature stress usually increase root production, (24) showed water stress during panicle development reduced yield and photosynthesis, (25) demonstrated that only a small portion of the total root will keep plants functional, (26) demonstrated that a stress resistant hybrid required 31% less assimilate to support its root system compared to a normal hybrid during panicle development, (27) showed seed size to be a critical yield stability parameter in adverse environments, (28) demonstrated a 31C seed number and yield reduction in field sorghum when night temperature was elevated 5C above ambient for a week beginning at floret differentiation, (29) demonstrated a wide range in panicle respiration rates at different temperatures, (30) continued selection of adapted higher quality A lines for medium elevation areas, (31) analyzed growing degree unit requirements for each sorghum developmental stage, (32) completed development of several growth room and greenhouse controlled environment modules, (33) developed X-ray spectrographic analysis techniques for sorghum (34) screened for P uptake and utilization efficiency, and (35) developed an Al tolerance screening technique.

A. GENERAL BACKGROUND

Authorities generally agree that increasing world food needs created by rising populations are creating severe pressure on world food production capacity. Crop improvement progress will have to accelerate if production is to keep pace with demand, especially in developing nations.

Sorghum ranks fourth among cereals (behind wheat, rice and corn) as a human food source. Sorghum is particularly important in those developing nations where environmental stresses often severely limit crop growth one or more times during the season. Relatively little is known about how to effectively screen for desirable environmental stress reactions in sorghum.

The purpose of this project is to study the nature of environmental parameter (particularly stress) effects on essential physiological processes, related developmental processes and the resulting seed number and seed size components of yield. Environmental stresses of chief interest are temperature extremes and water deficits. A more recent related area of interest includes soil nutrient deficiencies or toxic excesses which bear on the efficiency of nutrient uptake and subsequent utilization by the crop.

Plant breeding programs traditionally have most often been carried out under relatively optimal conditions to assure full expression of a genotype's potential under the assumption that the top genotypes under good conditions will probably be the top under stress conditions. Reasonable evidence is available to suggest such is not necessarily always true. Therefore, effort

should be devoted to developing stress screening techniques for breeding programs.

Developing these screening techniques will require a good basic understanding of environmental stress effects on photosynthetic and respiratory mechanisms, synthetic processes associated with both, and translocation processes mediating metabolite transport interconnecting all those essential processes. The overall project objective is to better understand the nature of stress induced effects on essential physiological and developmental processes in order to (1) develop screening techniques for efficient genetic manipulation and (2) to improve cultural practices to conserve water while stabilizing and maximizing yields under some given set of environmental conditions. A more detailed breakdown of these broad objectives follow.

B. STATEMENT OF PROJECT OBJECTIVES AS STATED IN THE CONTRACT

Basically investigations are geared to understanding growth and development of the sorghum crop well enough to manipulate the crop toward fairly stable yields consistent with the environmental limitations or resources of a given production area. Sustained progress toward steadily increasing yield depends on (1) progress in current applied investigations bearing directly on improving cultural practices and genetic manipulation and (2) on basic studies on essential physiological processes which designed to develop new plant breeder screening techniques and cultural practices. Both types of investigations are included. Project objectives modified

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slightly from the original contract follow as taken from the Work Plan of Annual Report No. 3 (Febr. 11, 1976 to Febr. 12, 1977):

Objective 1. Incorporate yellow endosperm lines into a Nebraska-developed random mated population. Yellow endosperm lines are being incorporated into good yielding germplasm in current random mating populations because of 1) potentially higher food value, 2) extensive vigorous root systems which may function in a water stress avoidance capacity and 3) offer potential for gain in seed size.

Work pursuit.

1) S1 yellow endosperm population progeny have been selected and are being evaluated for nutritional quality. Special attention will be given B line populations.

2) Greenbug resistance is being incorporated into several populations.

Objective 2. Develop suitable selection techniques and breeding methods for use with random mating populations destined for the tropical environments. Grain yield, when grain number and size are not limiting, is a function of plant metabolic efficiency and length of the grain filling period. Particular emphasis will be placed on determining what the correlation is between length of the grain filling period and the yield of grain. A random mating population shall be selected and evaluated for variability in length of grain filling period.

Work pursuit.

1) One more year of tests will be done on the initial grain fill period selections made from NP3R in 1970. Hybrids of selected F6 lines will be used.

2) Additional head selections from a yellow R population were made in 1975

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for length of grain fill. Short and long fill heads will be recombined separately to generate both early and late random mating populations of yellow endosperm material. This relates closely with work in

Objective 1.

Objective 3. Evaluate the utility of soil bedding as a limited tillage practice for sorghum on upland soils initially and on wet soils later. Use of such practices on wet soils may produce better stands and growing conditions as well as reduce the number of tillage operations required and subsequently reduce expense. Hopefully, water loss will be lessened as the tillage is decreased. When soil is wet, sorghum planted on beds may have better root aeration. This objective was broadened last year and called cultural investigations.

Work pursuit.

- 1) A minimum till soil bedding experiment compared with conventional tillage in sorghum will be continued one or two years more. A similar soil bedding - conventional tillage experiment was initiated this year with corn and will be continued.
- 2) A larger sorghum experiment initiated after (1) above is in progress using additional minimum tillage approaches to minimize energy usage.
- 3) A crop rotation experiment was established in 1974 involving sorghum, soybeans, no-tillage and various rates of nitrogen. The objectives are to: (a) reduce fuel usage by direct planting after soybeans, through reducing heavy tillage every other year with soybeans-sorghum or every two years with soybean-soybean-sorghum; (b) conserve moisture by not disturbing the soil and controlling weeds with herbicides and (c) evaluate possible nitrogen fertilizer savings by determining the contribution of nitrogen from the soybean crop.

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- 4) Complete experiments on stand geometry (row spacing and population) and plant morphology (leaf type) as they influence light interception and yield.

Objective 4. Develop screening techniques applicable for stress tolerance and stress avoidance mechanism.

Work pursuit.

- 1) Use a heat test to evaluate germplasm from wide genetic diversity for heat and drought tolerance.
- 2) Grow genotypes under mineral stress conditions to evaluate germplasm for mineral uptake efficiencies and tolerances to excesses.
 - (a) Expand methodology to screen large numbers of plants in relatively short time periods in both soils and nutrient solutions under mineral stress conditions.
 - (b) Determine what plant properties and/or responses are responsible for high efficiency of mineral uptake and tolerance to mineral excesses.
 - (c) Mineral elements of particular interest include phosphorus, iron and zinc.
- 3) Continue work on root screening techniques.
- 4) Continue work on temperature adaptation screening.

Objective 5. Investigate survival mechanisms operative in environmentally stressed plants in the more marginal crop production areas of the tropics (both low and high altitudes). An attempt shall be made to develop additional screening methods.

Work pursuit.

- 1) Continue work on desiccation injury and associated leaf firing. The role of cuticular waxes and "bloom" needs further investigation.
- 2) Interactions between drought and high leaf temperature effects are being studied with particular emphasis on integrity of the photosynthetic apparatus as predicted from heat and drought screening tests.

- 3) A temperature-typing screening test is being evaluated in an effort to classify sorghums in terms of cool, temperate or tropical adaptation. For example, stress effects on the seed number component of yield are serious at the floret differentiation stage. Deleterious effects are compounded if cool adapted germplasm (high elevation tropical or temperate region) is placed in a temperate climate or if temperate material goes to a tropical low elevation climate. More quantitation on these effects is needed.

Objective 6. Develop screening methods for early season cool tolerance growth capability. Germplasm development shall be coordinated with CIMMYT.

Work pursuit.

- 1) The temperature-typing technique mentioned in Objective 4, item 4 and Objective 5, item 3 needs much additional evaluation as to its suitability for a broad screening technique across a broad temperature range.
- 2) Work during the summer of 1975 on correlating field emergence under cool temperatures and a new growth chamber cool germination screening technique is being tested further on a population basis. NP8BR and NP10BR selections have been selected, tested and are now in Puerto Rico for increase prior to additional testing here in 1976.
- 3) Field climatic evaluation of genotypes in relation to cool tolerance in continuing to see if both bloom date and length of grain fill under contrasting climates can be used as a temperature-typing screening technique.

Objective 7. Conduct analysis of energy requirements of sorghums for the developmental stages including the periods from planting to panicle initiation, panicle initiation to bloom, and bloom to physiological maturity.

Work pursuit

The initial phase of this will be concluded and results published.

Subsequent work will relate to Objective 6, item 3.

Objective 8. Publish results in appropriate journals and/or circulars.

Objective 9. Meet at Beeville, Texas in July with other cooperators.

Objective 10. This objective was not included in the original work plan as such and is more or less a supporting activity to Objective 4, items 1, 2 and 4; Objective 5, items 1, 2 and 3 and Objective 6, items 1 and 3. Specifically, work initiated will include (1) building large controlled climate rooms indoors. (2) Building outdoor rain shelters. (3) Work on designing night temperature control facilities outdoors under rain shelters will be initiated in order to study water x temperature stresss interactions.

Objective 11. Initiate investigations on genotype differences on nutrient uptake and utilization efficiency.

2. Procedures to be used are not changed too much. Activities place more emphasis on environmental control as outlined.
3. Significant factors relating to accomplishments. No serious problems are anticipated. Some consultation with Australian and New Zealand Scientists on comparative environmental control facilities would be very useful in the next 3 years.
4. Dissemination of results will follow established approaches.

D. ACCOMPLISHMENTS TO DATE.

1. Original objective statements are listed below and followed by a work pursuit statement for each objective.

Objective 1. Incorporate yellow endosperm lines into a Nebraska-developed random mated population. Yellow endosperm lines are being incorporated into good yielding germplasm in current random mating populations because of 1) potentially higher food value, 2) extensive vigorous root systems which may function in a water stress avoidance capacity and 3) offer potential for gain in seed size.

1) Kofoid, Maranville, and Ross. Grain quality as related to testa.

NP13R, a yellow-endosperm random mating population, and NP14B, a greenbug-resistant, normal endosperm random mating population, each were subdivided into 200 S_1 families with and without a testa layer. Data were collected 1976-1977 for various agronomic and quality traits.

While the two populations varied for certain agronomic traits as expected, the nontesta of both tended to have higher grain oil, less tannin, higher IVDMD, and higher metabolizable energy (ME) than the test types. While several adverse correlations existed among agronomic and quality traits, a selection index based on ME yield gave results similar to selection for agronomic traits alone but gave better correlated responses to the nutritional traits, especially protein, lysine as percent of sample, phosphorus, tannin, IVDMD, and ME. On the whole, the nontesta population of NP13R was no better from the quality standpoint than the nontesta population of NP14B.

2) Ross and Maranville. Grain quality population development.

Mass-selected S_1 's from NP12B and NP13B have been recombined in separate isolated plantings. The two populations have a high frequency of yellow endosperm and a low frequency of testa layer (low tannin). NP18B is another quality population that has been developed and contains high protein components from the Purdue program selected for combine height and early maturity. Additionally, NP20BR was formed from N7BR high protein components, NP17R (a population tracing to Purdue materials), and Nebraska high lysine crosses. S_1 's were selected from NP20BR in 1978 and evaluated in 1979 for metabolizable energy and metabolizable energy X yield, but the data have not been analyzed. Future recurrent selection in grain quality populations will not be based on grain protein alone because of that character's negative association with yield.

3) Otte and Ross. Genetic combining ability of yellow endosperm R-lines.

Nineteen yellow endosperm R-lines from North Platte and Lincoln and five other R-lines (SC 120, SC 133, Tx 414, Tx 415, and TX 430) were crossed to combine Kafir-60, Martin, and Wheatland male steriles; the F_1 hybrids were evaluated in 1977 and 1978. The yellow males generally gave hybrids that were tall (120-125 cm) as compared with the check hybrids (95-100 cm). Only a few yellow males produced hybrids equal to Tx 430 hybrids in yield, and those hybrids were among the tallest. Most of the experimental yellow R-lines were included in forming population NP12R. Now it would be of interest to see if

new lines can be extracted from NP12R as good as Tx 430. Tx 430 is a derivative of Tx 2536, and the latter was one of the most widely used R-lines in the United States.

4) Maranville, Kofoid, Ross. Selection techniques.

A procedure was developed to help estimate the feeding values of sorghums that contain tannin by utilizing the inhibition of methanol extracts on an alpha-amylase enzyme system. Correlation coefficients were calculated for several parameters from a population with a testa (tannin) and a population without a testa (no tannin). A strong negative correlation was noted between inhibition (% of control activity) and tannin content, and a strong positive correlation between inhibition and IVDMD values. The test is simple, rapid, and adapted to screening large numbers of samples in breeding programs.

Objective 2. Develop suitable selection techniques and breeding methods for use with random mating populations destined for the tropical environments. Grain yield, when grain number and size are not limiting, is a function of plant metabolic efficiency and length of the grain filling period. Particular emphasis will be placed on determining what the correlation is between length of the grain filling period and the yield of grain. A random mating population shall be selected and evaluated for variability in length of grain filling period.

1) Ross, Maranville, and Kofoid. Recurrent selection for grain protein.

The efficiency of mass selection for high and low grain protein in NP7BR was studied in 1975 and 1976 by testing 200 random S_1 progenies from each recombined population for each of two cycles. The results follow:

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<u>Population</u>	<u>Cycle</u>	<u>Protein %</u>	<u>Yield kg/ha</u>	<u>Protein Yield kg/ha</u>
High protein	C ₁	11.2	3400	381
High protein	C ₂	11.6	3310	384
Low protein	C ₁	10.7	3600	385
Low protein	C ₂	10.3	3660	377

As expected, a negative correlation between protein and yield existed, and each had a high correlated response with the other. While protein yield remained nearly constant in the above populations, selection for that trait might result in yield increases without corresponding decreases in protein.

2) Ross, Ritter, and Maranville. Sampling for grain protein.

Whole-plot threshed grain samples for protein using IRR were compared with composites of threshed mid-panicle branches of 5 to 7 heads in two hybrid and one line experiment in 1977. Differences were low for protein sampling methods (0.1, 0.5, and 0.4%) in the three experiments, but all were significantly different due to the large number of measurements (480, 400, and 80, respectively) and low standard errors. The method x entry interactions were significant, however, in only one experiment, and this may have been due to selfed plants arising in crosses with one female parent. For screening large amounts of breeding material, which in many cases is not threshed, sampling 5 to 7 heads was deemed adequate. If plots are threshed, the samples can easily be taken from the whole-plot grain.

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3) Ross and Kofoid. Evaluation of grain sorghum R-lines with a single-cross vs. inbred line testers.

Forty-two R-lines were crossed to Martin, Wheatland, and Martin-Wheatland F_1 male steriles, and the single crosses and three-way crosses were evaluated in a replicated yield trial in 1975 and 1976. The means of the three sets of hybrids differed significantly for height and bloom but not for yield, and the mean yield performance of the three-way crosses was not different from expectations based on single-cross data. The experiment did not indicate that epistasis was of any great consequence although more refined experimentation and more replications might have shown different results. Selection of the best 50% males, as might be done in a preliminary evaluation of breeding stocks, based on three-way cross performance had in common 15 of the best 21 Martin hybrids and 15 of the best 21 Wheatland hybrids. An advantage was noted in the amount of hybrid seed produced from hand crosses on the male-sterile F_1 's.

4) Ross and Kofoid. Identification of a Dw_1 tester stock.

All sorghum lines previously identified as carrying the Dw_1 height gene have possessed one or more other dominant height genes (Dw_2 , Dw_3 , or Dw_4). A combine-height partial conversion (SC 102-9) was sterilized in milo cytoplasm and was crossed to an array of height testers in 1976 and evaluated in 1977. The SC 102-9 lines was identified as $Dw_1Dw_1Dw_2Dw_2Dw_3Dw_3Dw_4Dw_4$, the first recorded instance of such a genotype. The line has been released as N32 and could be useful in both grain and forage hybrid production:

In countries where 2-dwarf hybrids are desired, seed production of grain sorghums could be accomplished easily by combining the N32 seed parent where the male parent (also combine height) would carry the Dw_2 or Dw_3 gene.

5) Ritter, Ross, and Sullivan. Combining ability in grain sorghum F_1 hybrids.

Two sets of hybrids (10 females X 10 males and 4 females X 30 males) were grown in 1976 and 1977. Variances of GCA effects were larger than those for SCA effects for all traits in both experiments, ranging from 1.1 to 15.1. The results indicated the relative importance of additive over nonadditive genetic effects and imply that recurrent selection procedures applied to sorghum should be successful. Several superior parental lines were identified in the study.

6) Ross and Kofoid. Effect of non-milo cytoplasm.

In 1970 and 1971 southern corn leaf blight devastated corn production because of the disease's association with a particular male-sterile cytoplasm used in hybrid production. Sorghum is equally vulnerable since 97% of the U.S. grain sorghum hybrids have the same cytoplasm which traces to milo. KS34A through KS39A are male-sterile sorghums with cytoplasm other than milo, primarily from introduced noncultivated grass sorghums. While certain test crosses indicated that some of the cytoplasm may have been different, combined 1976 and 1977 data revealed no adverse or beneficial effects on yield or other agronomic performance of crosses where the KS male steriles were used in single crosses and modified backcrosses; nor were there any genotype x year interactions. The commercial hybrid sorghum seed industry should convert widely used male-sterile stocks into several or all of the KS lines as precautionary measures.

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7) Ross. Recurrent selection for grain yield.

The C_0 , C_1 , and C_2 recombined populations from NP3R and NP5R were tested in 1977 and 1978 with the following results (kg/ha):

<u>Cycle</u>	<u>NP3R (HS)</u>	<u>NP3R (FS)</u>	<u>NP3R (S_1)</u>	<u>NP5R (S_1)</u>
0	4050	4050	4050	4720
1	4920	4800	5090	4780
2	4610	4440	4740	4710

The yield reduction in NP3R from the C_1 to the C_2 was due to selection pressure necessary to attain acceptable height and maturity. Although no apparent yield differences existed among the three cycles of NP5R, tall and late types were selected against so that each successive cycle was improved agronomically.

Evaluation of 200 families each from three types of selection from three cycles (C_0 , C_1 , and C_2) in NP3R have been completed. Simultaneous testing of half-sib, full-sib, and S_1 families allows a breakdown of the total genetic variance into additive, dominance, and additive* as appropriate. These data for yield follow:

<u>Family</u>	<u>Variance</u>	<u>C_0</u>	<u>C_0</u>	<u>C_0</u>
HS	Total	12.73	16.57	12.48
	Additive	50.93	66.28	49.92
FS	Total	43.03	20.59	22.95
	Dominance	70.28	-50.20	-8.04
S_1	Total	39.92	30.08	20.46
	Additive*	22.35	42.63	22.47

While dominance variance disappeared during full-sib testing, additive variance in half-sibs and the additive-type (additive*)

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variance in S_1 's remained adequate so that further progress in breeding for yield is likely. In practice the negatively computed values for full-sibs can be assumed to be zero or have very small positive value. In either event, dominance variance appears to have little or no role in explaining the variance of yield in a sorghum random mating population.

8) Ross, Kindler, Kofoid, and Hookstra. Evaluation of RPlR half-sib families for resistance to the European corn borer.

In a test of 100 half-sib families from RPlR, a greenbug-resistant population, artificially infested with second-brood European corn borer egg masses, yield was reduced 20% in 1977 and 7% in 1978 below the same families where the insects were chemically controlled. Kernel weight was reduced significantly, and potential head and stalk breakage was 40 to 50 times greater in the infested treatment. Stalk rots developed as a severe secondary problem due to borer entry and feeding and contributed to lodging.

Heritabilities were calculated for several pertinent traits in both the infested and controlled groups. These values (h^2) are listed below:

<u>Trait</u>	<u>Infested</u>	<u>Controlled</u>
Seed weight	0.80	0.68
Broken stalks	0.56	0.24
Broken heads	0.32	0.08
Total grain yield	0.60	0.57
Combined grain yield	0.64	0.56

Although the amount of head breakage was the trait of most apparent difference in the two groups, heritability was relatively low in the infested families. This was probably due to non-random actual in-

hostation by the insect larvae even though every plant was hand infested on the lower surface of the upper leaves. C_1 families were evaluated in 1980, but data are not analyzed.

9) Ross. Sample size (number of families) for evaluating S_1 progenies.

Two hundred S_1 families each from NP12B and NP18B were evaluated in 1977 and 1978 for yield, grain protein, and other characters. A block-in-replications design was used where sets of entries per population equalled 20 which remained together in each replication. The total analysis of variance for each trait in each population was partitioned into smaller experiments of 2, 4, 6, and 8 blocks (40, 80, 120, and 160 entries, respectively) and compared with 10 blocks (200 entries). Five, two, one, and one respective subexperiments resulted without duplication of entries. The family variances for yield only and standard errors are listed below:

<u>No. of blocks</u>	<u>No. of families</u>	<u>NP12B</u>	<u>NP18B</u>
2 (high)	40	28.43 \pm 10.40	40.71 \pm 12.84
2 (low)	40	10.73 \pm 7.02	13.29 \pm 5.56
4	80	19.62 \pm 6.28	33.22 \pm 7.24
4	80	17.01 \pm 5.68	34.73 \pm 7.98
6	120	18.65 \pm 4.99	32.95 \pm 6.26
8	160	18.32 \pm 4.26	33.98 \pm 5.42
10	200	20.34 \pm 4.00	.84 \pm 4.38

While two blocks (40 entries) failed to give consistent estimates of the family yield variance components, four blocks (80 entries) generally gave about the same estimates as 10 blocks (200 entries) in both populations. Standard errors progressively decreased with larger sample sizes. The standard errors, of course, relate directly to the probable success of recurrent selection practices. If one accepts standard one-fourth as large as the variance component, then 8 blocks or 160 entries would be necessary to sample NP12B. With NP18B considerably less numbers (4 blocks or 80 entries) produced this level of precision. The variances and standard errors of other traits should also be considered as well as family X environment variances. In any event, 100 families would appear to be an absolute minimum number while 150 or 160 would be more comforting numbers.

10) Ross and Hookstra. Yield of base populations.

Five random-mating populations and hybrids RS 626 and RS 671 have been yield tested for 8 years, 1972-79, while 18 populations and the same checks have been tested 4 years, 1976-79. Their yields (kg/ha) are summarized as follows:

<u>Population</u>	<u>1972-79</u>	<u>1976-79</u>
NP1BR	4140	4340
NP2B	4350	4620
NP3R	4790	4930
NP4BR	--	4880
NP5R	4800	5170
NP6B	--	5310
NP7BR	4320	5180
NP8BR		5480
NP9BR		4580
NP10BR		5480
NP11BR		5450
NP12B		4480
NP13R		5040
NP14B (RP2B)		5000
NP15R (RP1R)		4880
NP16BR		5190
NP17BR		5170
NP18B		4860
RS 626	5290	5520
RS 671	5540	6130

Populations, like hybrids and lines, differ in their yielding ability. A few have remarkably good records in the short-term period, and NP3R and NP5R have averaged 90% of the yield of RS 626 over the 8-year period. After one or two more seasons, a complete analysis of variance will be made, and population and population X years variance components will be determined. Stability parameters also will be calculated. Physical limitations preclude recurrent selection studies with all of the populations, but the above offers a wealth of germplasm for such pursuits.

11) Ross and Hookstra. Effect of year of selection on S_1 progeny performance.

A tenet of population improvement requires random family selection.

While simple in theory, randomness in practice is more difficult to obtain.

Two hundred S_1 families were taken from the same base population (NP16BR)

in 1976, 1977, and 1978 and were evaluated in 1979 and 1980. The mean results from 1979 follow:

<u>Year of families</u>	<u>Yield kg/ha</u>	<u>Bloom days</u>	<u>Height cm</u>
1976	5089	69	109
1977	5351	70	117
1978	5371	68	104

The difference in yield of the 1976 families is unexplainable except for the fact that different people were involved in the random selection of families each year. Most individuals inadvertently tend to take the better heads from a population, but some may try to avoid an upward bias and instead create a downward bias by taking more than the usual number of poor plants. In any event, the data illustrate the care that must be taken to accomplish randomness of selection. The difference in heights can be explained by a drought period in 1977 that did not allow plant heights to be properly expressed. As a result, supposedly normal-height plants became taller under the better growing conditions of 1979.

12) Hookstra and Ross. Male-sterile F_1 's as hybrid seed parents.

Nine A and B lines were intercrossed to give 36 male-sterile F_1 and were compared to the 9 A-lines in a replicated experiment in 1978 and 1979 designed to study hybrid seed production. The pollinator rows were a composite of 100 different grain sorghum hybrids. The F_1 's averaged 5248 kg/ha of seed versus 3504 for the A-lines, an increase of 48%. Seeds per head were also 51% more for the F_1 's while threshing percentage and seed germination were slightly in favor of the F_1 's. Genetic variances were generally lower for the F_1 's as expected.

In general F_1 's involving Wheatland, Combine Kafir-white Sourless, and Combine Kafir-Ellis with other lines produced the highest yielding seed parents. A few other lines like KS4, KS23, and WD 4 were involved in specific F_1 's that were superior. Seed costs of three-way hybrids should be materially less than for single crosses, and variability in the hybrid can be minimized if the proper F_1 seed parents are used. Three-way crosses should be feasible for developing nations that are ready to embark upon a hybrid seed production program.

13) Finley and Ross. Evaluation of the twin-seed character.

NP19BR, the source material, was developed from NP13R (yellow endosperm) NP2B (greenbug resistant), 19 experimental twin-seeded lines having a common Indian durra twin source, and 10 twins from the Texas-USDA conversion program, some of which may be duplicates. After two random matings, 160 normal and 160 twin-seeded S_1 's were randomly selected in 1977 and evaluated in three replications in 1978 and 1979 for agronomic and grain quality characters.

The normal S_1 progeny rows were higher yielding than the twin-seeded S_1 progenies and averaged 6645 versus 4754 kg/ha. Sterility seemed to be associated with the twin-seed material since seeds per head were not materially different from the normals (1918 for the twins versus 1943 for normals). Moreover, twin seededness resulted in decreased seed weight (1.97 grams/100 versus 2.34 for the normals) and was a contributor to decreased yield. While this experiment seems to discourage the use of the twin-seed character, it is possible that some of the different twin-seeded sources are controlled by different genes and that they could be expressed differently in different genetic and/or cytoplasmic backgrounds.

Objective 3. Evaluate the utility of soil bedding as a limited tillage practice for sorghum on upland soils initially and on wet soils later. Use of such practices on wet soils may produce better stands and growing conditions as well as reduce the number of tillage operations required and subsequently reduce expense. Hopefully, water loss will be lessened as the tillage is decreased. When soil is wet, sorghum planted on beds may have better root aeration. This objective was broadened last year and called cultural investigations.

- 1) Eastin. Comparative results between normal and minimum tillage with the soil bedding approach again showed no yield differences in 1979 and 1980. The system does reduce the number of tillage operations. Possibly it might have some utility in mechanized agriculture schemes such as those in Sudan. Its utility there might be more in stand establishment during intermittent dry periods. The bedder operates by elevating dry soil away from an underlying moist layer. Since planter units follow the exposed moist area seedling establishment is usually quick and adequate.
- 2) Clegg. Light interception vs plant type experiments was expanded to include not only the normal and erect leaved hybrids but also tall hybrids. Six genotypes consisting of non-erect leaved (RS 626 and RS 671), erect leaved (Martin x SC33 and wheatland x SC33) and tall (Funks Hal752 and Ha4060) were planted in row spacings of .38, .76 and 1.14 meters at a plant population of approximately 172,000 plants/ha. Light interception was measured during a period from one hour before and after solar noon using a 1 meter rod filtered for the 400-700 mu band.

The combined heights for the tall hybrids were 200, 197, and 196 cm; for the non-erect hybrids 106, 110, and 115 cm; and for the

erect hybrids 111, 111, and 115 cm for the .38, .76 and 1.15 m row spacings respectively. Light interception by the hybrids in the .38 and .76 m row spacings were very much the same regardless of plant type. In the 1.14 m row spacing the non-erect types intercepted less light than the tall or erect types. No difference in light interception by the erect leaved and tall types was observed, thus, erect leaved types which have a stiff mid-rib maintained a canopy which was similar to the tall types. The leaves of non-erect hybrids tended to droop as the plants aged allowing more light through the canopy. Height and stiff mid-rib erect types maintained a more complete canopy. However, going to a narrower row spacing negated the height and erect leaf habit affects.

Yields of the hybrids were not related to light interception. This occurred because other environmental variables were more influential such as water and heat stress.

3) Clegg. Lodging in sorghum: Changes in sugars in sorghum stem tissue following anthesis.

Lodging is a problem in most grain crops. Structural stalk strength or the ability to maintain healthy stalks enable grain sorghum to resist lodging. However, sorghum is a perennial and because of this habit once the grain is filled the stalks become storage organs. Thus, the questions arise as to the sugar levels at first freeze and are they at a level which could support fermentation and other microbial activity resulting in tissue breakdown? This would weaken the stem and increase its susceptibility to breakage.

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Eight sorghum genotypes were planted and harvested at weekly intervals beginning a week after anthesis. The peduncle node, basal node segments and sheaths were separated, dried and analyzed for sugars. The peduncle node was of special interest because it is the last node to develop and is a primary site of stem breakage.

In general the basal nodal sections were the highest in sugars, followed by the peduncle nodes and then the sheaths. The sugars in the various parts changed with each successive harvest. The reducing sugars decreased with harvest from approximately 84 mg/g dry wt to a low of 20 mg/g dry wt at the last harvest. Non-reducing sugars were high by the second harvest, 184 mg/g dry wt, attained a low of 37 mg/g dry wt. by the last harvest. All the genotypes responded similarly over harvests. Differences were observed in levels of sugars between genotypes.

The concentration of sugars at the final harvest would indicate that by the first killing freeze there would be a concentration of sugars (180 mg/g dry wt) available for fermentation and microbial activity to degrade and weaken the stem.

- 4) Clegg. Grain sorghum grown in a continuous cropping system responded to increased nitrogen applications by increasing grain yields. A similar positive but much lower yield response to applied nitrogen occurred when grain sorghum was grown after soybeans. The yield level of the control (zero N) was significantly higher following soybeans rather than sorghum. In 1975, the yield response of grain sorghum following soybeans in rotation was equivalent to approximately 50 kg/ha N. In 1978 and 1979 the nitrogen equivalent was approximately 100 kg/ha N. The yields of grain sorghum were reduced when 170 kg/ha

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was applied to the crop when rotated with the soybeans. This may suggest that there was residual N available for the soybean crop following sorghum and a reduced need for nodulation of the roots. Thus, there would be less fixed N for the following sorghum crop.

Nitrogen tended to shorten the number of days the sorghum required to bloom. However, only in 1975 was the difference significant.

The effectiveness of the release of residual nitrogen from the soybean root system is closely related to the environment. If there is adequate moisture, presumably there is the microbiological activity to release the nitrogen and make it available for the following crop. At our location under good conditions the nitrogen equivalent was around 100 kg/ha.

Measurements of leaf chlorophyll content and photosynthesis were made in a preliminary study. Higher chlorophyll content and photosynthesis rates were related positively to higher nitrogen levels regardless of whether the nitrogen source was from applied nitrogen or residual nitrogen after soybeans.

5) Maranville and Clark. Limited tillage.

A study was conducted to measure nutrient uptake in plants grown under 0, 2200, 4400, and 8800 kg/ha mulch. Moisture was not limiting and dry matter production as well as total mineral uptake and concentrations were determined at several growth stages. Mulches altered growth by delaying plant maturity and by increased dry-matter yields. Nutrient uptake increased with increased dry matter production although elemental concentrations declined. Hybrid reaction to mineral uptake was dependent on specific environment created by the mulch. Results indicated that

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management considerations for soil fertility under mulch conditions should be made on the basis of expected dry-matter yields for the specific environment.

Objective 4. Develop screening techniques applicable for stress tolerance and stress avoidance mechanism.

Maranville, Clark and Ross. Nitrogen efficiency in sorghum genotypes.

'Nitrogen efficiency' was determined on 12 hybrids based on the following definitions: 1) total dry matter production per total N uptake; 2) total grain production per total N uptake; and 3) total grain production per total N uptake times superior N partitioning to the grain. The results showed that hybrids having 'wheatland' female or 'SC33-9' male parentage exerted the strongest influence on either grain or stover yields, but were not particularly N efficient. The high yield level influence exerted by these two parents also resulted in higher total N uptake but again they appeared inferior for desirable N partitioning to the grain. Hybrids containing 'CK60' female or 'SC119-9' male parentage were the most N efficient by definition No. 3. These hybrids were intermediate grain yielders.

- 2) C.Y. Sullivan, J.M Bennett, D.P. Garrity, and R.E. Maurer. Screening for heat and desiccation tolerance. Stress resistance of selected sorghum genotypes was further tested on sandy soils of western Nebraska on a sprinkler irrigation gradient. Selections from population NP9BR were included which were screened for seedling drought resistance and which had higher stress stability on the irrigation gradient in 1977. There was no significant correlation between seedling drought resistance and yield stability with increasing drought stress. Forty-eight advanced yield hybrids and eight selected yellow hybrid were also grown on the gradient. Yield reductions from the wet to dry side ranged from 15 to

74%. The irrigation gradient was shown to be an effective method of selecting for yield stability to drought stress.

- 3) C.Y. Sullivan, J.M. Bennett, D.P. Garrity, and R.E. Maurer. Investigate physiological aspects of limited irrigation including stress conditioning.

A comparison of sorghum and corn to limited irrigation by an irrigation gradient was done a second year. The data is incomplete, but it has been concluded that when starting with a full soil water profile an application of less than 30 cm. water applied favors sorghum yields.

Conditioning to stress, increased osmotic potentials and maintenance of turgor potentials was apparent. Stress conditioning was also apparent in hydroponically grown plants by monitoring photosynthesis of pre-conditioned and control plants. The conditioned response was also apparent in rates of recovery of photosynthesis from stress with differences in genotypes.

- 4) Eastin. Growth efficiency measurements were made on dark seedling growth (g new growth per g seed weight lost) and revealed differential genotype responses to different combinations of temperature and water levels. While no other exactly equivalent data are in the literature, the results are somewhat at odds with limited data available. Therefore, experiments will be repeated.

- 5) Clegg. Lodging in sorghum: Some post-freeze effects on stalk breakage of grain sorghum.

Lodging is an important factor influencing the harvestability of a grain sorghum crop. Stalk lodging usually is not a great problem if the plant remains alive. However, any factor causing death or injury of the plant such as disease, insects, drought, freezing, etc., will increase the incidence of lodging. The growing season for sorghum in Nebraska

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is usually terminated by a killing freeze. Therefore, knowledge of biochemical and physical changes which occur in stalks after freezing should aid in our understanding of factors connected with stalk strength.

Seven grain sorghum hybrids were planted and after emergence thinned to a population of 170,000 plants/ha. The hybrids ranged in maturity from early to late. Plant samples were harvested before and after the first killing freeze. Plants harvested before the first freeze were separated into two samples. One sample was placed into a freezer for two hours and the other sample remained at room temperature. Sub-samples from frozen and nonfrozen samples were broken using an Instron to determine the maximum breaking moment (M) and modulus of elasticity (E) and then dried for sugar analysis. The remaining sub-samples were placed in respiration containers and carbon dioxide loss was measured. Temperatures around the containers alternated between 30 C for 16 hours and 10 C for 8 hours. Carbon dioxide was measured at 10:00 a.m. each day when the temperature was 30 C. After seven days the samples were removed from the container and processed as above. The plants harvested post-freeze were broken and the samples dried for sugar analysis.

Considerable variability occurred in the data, therefore, all the data over hybrids were combined. Sugar decreased in both frozen and nonfrozen stalks after storage with little difference between treatments. Daily rate of CO₂ loss was higher for the frozen than for the nonfrozen stalks. Initially after freezing there was an increase in elasticity as indicated by a decrease in E. Because of this

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change in flexibility, there was an increase in M of the frozen-treated stalks. After storage E and M did not differ between treatments.

Measuring sugars pre-freeze and after freezing showed that sugars in the stalks increased until the plant was killed by freezing. This increase was probably a response to lower temperatures. After freezing there was a linear decrease in sugars. Breakage of sorghum stalks showed an increase in E and a decrease in M with time after the killing freeze. This indicated the stalks were becoming brittle, probably from drying and required less force to break the stalk. Breaking time was also shortened. Sugar measurement does not appear to be a viable screening technique for lodging resistance.

- 6) Eastin. A modified water gradient system was set up in Garden City, KS, for drought stress screening. See Objective 10.
- 7) Clegg. Grain sorghum responses to temperature and moisture.

Root growth pattern studies of grain sorghum in relation to soil temperature and field moisture levels showed that the rooting depth was influenced more by soil temperature than soil moisture. With respect to moisture, as moisture availability decreased, root dry weight increased. Presently experiments are being conducted to evaluate root growth and branching in relation to temperature and moisture using a temperature gradient sand table.

Studies were undertaken to examine the physiological nature of tolerance (CT) in grain sorghum using the hybrids NB 505, RS 671, RS 691 and Ms83x189. Ms83x189 and NB. 505 were the cool tolerant hybrids. Dry matter accumulation and leaf elongation rates were significantly reduced for all hybrids used by 5 degree C night temperature. Contrary

to our hypothesis, the CT hybrid Ms83x189 was affected more by the 5 degree C night temperature than the non-CT hybrids. This was based on comparison to a control 15 degree C night temperature. However, if the night temperature response curves of CT and non-CT hybrids are similar in shape but do not have the same optimum, then 15 degree C would not give a fair comparison point and temperature response curves for each hybrid should be determined.

Objective 5. Investigate survival mechanisms operative in environmentally stressed plants in the more marginal crop production areas of the tropics (both low and high altitudes). An attempt shall be made to develop additional screening methods.

- 1) Bennett and Sullivan. Effects of drought and heat stress on photosynthesis and growth.

Sorghum was grown to maturity in hydroponic cultures with water stress controlled by additions of polyethylene glycol. As previously found, a root media stress of -6.5 bars during panicle development reduced yields 40 to 50%. A differential response was found to stress at bloom. If stress occurred during early bloom, photosynthesis was severely inhibited and yields reduced about 30%. If the stress occurred during late bloom neither photosynthesis nor yields were significantly reduced.

- 2) Sullivan. Investigate characteristics of root systems as related to stress resistance.

It was shown that sorghums could grow well with only a small portion of their total root systems in contact with available water. Plants were grown for up to 22 days with only their root tips in contact with nutrient solutions. Root tips in contact with water representing only 3% of the

total dry weight of the roots supported the plants and produced 30% of control yields. However, when about 10% of the root dry weight was held in contact with available water during bloom through grain filling, yield was reduced only 35% compared to controls. Water use per unit leaf area was reduced by 37 to 53% by three genotypes during a period of 17 days when only 10 to 20 cm of root tips were in contact with nutrient solutions. Total dry matter production was reduced only 10%.

- 3) Rice and Eastin. Root growth and respiration characteristics of drought resistant and normal sorghum hybrids (one each) were studied in nutrient solutions. Slightly less root growth generally occurred in the drought resistant hybrid. Test conditions used were control, -5.0 bars and -7.5 bars water stress all at 25, 30, 35, and 40C. On the average, the normal hybrid roots respired 31% faster (on a per g dry wt basis) than the stress resistant hybrid during the period the panicle was developing suggesting that the roots provided a 31% larger sink competitive to the developing panicle. Generally this causes a greater reduction in seed number and grain yield. Minimal root energy requirement during critical growth stages may represent a desirable stress resistance mechanism.
- 4) Francis. Field experiments were conducted in 1978 and 1979 in seven locations to evaluate genotype by environment interactions in relation to cultural and moisture variables. Treatments included different plant densities, planting dates, tillage practices and irrigation levels. Genetic materials included 56 hybrids, lines and random mating populations. There were more than 50 environments included in the study. Data collected included early plant vigor and growth, time of flowering and physiological maturity, plant height and leaf number, tiller number per plant, and yield and components of yield. Preliminary analyses indicate a broad range of

adaptation of some commercial hybrids. There is an over-riding effect of maturity on performance in the range of environments used, and this obscures other effects of specific morphological traits. Hybrids were most stable in the best environments, while random mating populations were superior in some of the worst environments in the study, indicating why farmers may cling to their traditional varieties in some areas.

Stability analyses of yield and the evaluation of yield components suggest that seed size may be a critical factor in final yield compensation during the grain fill period. Further studies are under way to determine capacity for grain fill in several commercial hybrids, and how this is influenced by density, tiller removal, and moisture. The interaction of seed number and seed size may be critical in final determination of yield and stability.

- 5) Ogunlela and Eastin. The effects of night temperature on sorghum yield were evaluated in 1978 by controlling night temperature at a field 5C and 10C above ambient during panicle development. Panicle development was divided into five 6-day periods. The most sensitive period was 12 days after panicle initiation when 6 days of ambient +5C reduced yields 31% (44% seed number reduction) and ambient + 10C reduced yields 61% (72% seed number reduction). Ambient + 5C is not a severe stress. Obviously sorghum is quite sensitive to adverse environmental parameters at that stage. Yield reductions closely parallel seed number reductions. However, seed size increases partially affect the seed number losses.
- 6) Gerik and Eastin. Since previous experiments by Eastin and Ogunlela showed that temperature is an important seed number and yield determinant, experiments were conducted to check variation in plant reaction (respiration rate as a measure of metabolic pace) to temperature. Two populations were field screened for panicle respiration rates at different temperatures

(by choosing different times of day) using the field portable CO₂ measuring system developed by Sullivan and Clegg. Stated differently, the rationale was that temperature controls metabolic pace which relates to the maximum seed number potential realized in the crop. Respiration rates were measured hoping they would be a reasonable indicator of metabolic pace at different temperatures. Respiration rates were taken (below) from 100 heads in each of two random mating populations to check for variability in respiration response. Knowledge of variability is essential in order to tell whether or not one could reasonably be able to select for differential temperatures common to divergent environments.

<u>Variable</u>	<u>Units</u>	<u>Range of Variability</u>		
		<u>X</u>	<u>Min.</u>	<u>Max.</u>
<u>TP₄ - RO⁻ sub -4 population</u>				
Panicle Respiration	mg CO ₂ g ⁻¹ hr ⁻¹	1.78	.31	5.22
Grain fill	days	31.4	20	41
Yield	g grain panicle ⁻¹	74.5	19.5	167.0
Seed number	panicle ⁻¹	2948	961	6356
<u>TP 11 Population</u>				
Panicle Respiration	mg CO ₂ g ⁻¹ hr ⁻¹	2.10	.14	12.4
Grain fill	days	29.8	20	39
Yield	g grain panicle ⁻¹	73.8	23.5	71.4
Seed number	panicle ⁻¹	3170	737	7366

The respiration values are averaged over 3 temperatures. Obviously a great deal of variability exists in respiration response to temperature. Selection for temperature response should be possible. Unpublished work from our laboratory shows respiration in plants at PI increases 12 to 14% for each degree C over the range from 12 to 27C (measured at 5C intervals). If metabolic pace relates to metabolic

efficiency and it is that heavily influenced by temperature, perhaps more care in selecting the right metabolic pace (temperature response) for a given environment would be useful.

- 7) Mahalakshmi and Eastin. Mahalakshmi first, and Gerik later, demonstrated that increases in panicle respiration in response to temperature proceed at about half the pace of increases in respiration of plant vegetative matter. Mahalakshmi further tested the influence of temperature on starch synthetase activity in RS 671 and RS 626 sorghum grains at 10, 15, 20, 25 and 30 days after anthesis. Seasonal mean starch synthetase activities ($\mu\text{moles glucose incorporated g fresh weight}^{-1}\text{hr}^{-1}$) follow.

<u>Hybrid</u>	<u>Temperature (C)</u>		
	<u>32</u>	<u>37</u>	<u>42</u>
RS 671	8.14	8.42	9.22
RS 626	7.97	8.08	8.68

Enzyme response from developing grain is modest as is respiration response. This may relate to the fairly stable grain fill rate within a genotype across a range of environments as observed by several workers. Perhaps starch synthetase activity relates to control grain fill rates in sorghum.

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Objective 6. Develop screening methods for early season cool tolerance growth capability. Germplasm development shall be coordinated with CIMMYT

1) Clegg and Eastin. Cold tolerant lines.

Certain crop growing areas have day temperatures which are adequate for growing grain sorghum, but night temperatures become minimal. At Sidney, Nebraska, 145 lines with two years selection from this site were planted for yield evaluation. Yields from these lines ranged from 1600 kg/ha to 4380 kg/ha. Twenty lines yielded 3500 kg/ha and over. Maturity ranged from 56 to 80 days to 50% bloom. Over half (54%) of the lines were in the 56 to 62 day range. Yield was not greatly influenced by maturity. Only the very early 56 to 57 days or those later than 76 days to 50% bloom yielded significantly less. The seasonal temperatures at Sidney for 1977 were much higher than normal and lessened the influence of maturity.

Yield levels obtained with this selection of lines would indicate that progress can be made in obtaining genetic material for this type of environment and add yield stability.

2) Francis. Nursery work was expanded in Sidney to seek out lines with acceptable grain quality and good agronomic potential under cool conditions and a short growing season. Introductions from CIMMYT were compared with materials selected in Western Nebraska over the past several years. Selections are observed as head rows in Sidney and in Mead (longer season) in each field cycle. Genotypes with a long fill period and efficient grain production for short season conditions will be available for broader testing on an international scale within a few cycles.

An isolation block of B population material was grown in 1978 and 1979 in Sidney, and a number of sterile and fertile heads selected for further testing. This will be grown in isolation as head rows in the next season, and further selections made for earliness and grain quality in Western Nebraska. Promising individual families will be chosen for recombination, and the best advanced selections converted to A lines. It is expected that hybrids could be produced for testing the adaptation and yield potential of female lines within two cycles of selection.

3) Eastin, Clegg, Mendoza. Screening methods for cool tolerance germination and growth.

Mendoza reported earlier on a tray method of testing for cool germination ability. Seeds were perched on the edge of an upright piece of thick blotter paper with additional longer paper sheets placed on the outside of the thick support blotter paper in order to hold seeds in place. The seeds, sandwiched between the outer sheets of paper which extended slightly above the blotter paper on which the seed rests, can be viewed directly as they germinate. Seed sandwiches rest upright in a water tray.

Mendoza's test was extended by first taking germination notes and then allowing the plants to grow 4 to 5 weeks at which time length of roots and shoots were measured. In two preliminary trials, average root and shoot lengths were estimated from 30 seeds selected at random from S_1 heads from different populations. Root growth was of particular interest since soil mulching in semiarid environments to improve catching and retaining water also means cooler soils. Plant development from individual S_1 heads are then removed, transplanted and grown to maturity

usually in the field. Plans are to recombine seed lots from the different growth habits, permit them to random mate for at least 3 seasons and eventually test contracting populations for yield under much (ecofallow) systems. Simultaneously individual plant selections from the original S_1 heads will be retested each generation and the best contrasting types will be carried to the F_4 and later generations for testing under ecofallow.

4) Clegg. An index for field evaluation of cool tolerance in grain sorghum.

An index was developed to identify cool tolerance in grain sorghum genotypes. This index was based on growing degree units to 50% bloom calculated for each genotype at two locations where temperatures (28 C, day, 14 C, night) were considered typical for sorghum growth (Mead), and another location where day temperatures (27 C) were typical but night temperatures (8 C) were lower (Sidney or Scottsbluff).

Growing degree units (GDU's) to 50% bloom were calculated according to the equation

$$GDU = \sum_{l=1}^n \left(\frac{\text{Max.} + \text{Min.}}{2} - BT \right)$$

where l is the day planted, n is the day of 50% bloom and BT is the base temperature (15.5) at which minimal growth occurs. The selection index (I_s) was calculated according to the equation:

$$I_s = (GDU_{mg} / GDU_{mx}^-) - (GDU_{sg} / GDU_{sx}^-)$$

where GDU_{mg} and GDU_{sg} refer to growing degree units to 50% bloom for the same genotypes at Mead and Sidney or Mead and Scottsbluff respectively.

GDU_{mx}^- and GDU_{sx}^- refer to the location means of all genotypes at Mead

and Sidney or Scottsbluff respectively. The location means provided a useful method of removing variations due to environment. Positive index values for a genotype classified it as cool tolerant.

In this study, the lines identified as cool tolerant using this index were lines that had been labeled cool tolerant either by source or laboratory tests and were generally one of the parents of the cool tolerant hybrids used in this study. Of interest, CK 60 and Tx 414, two lines used as parents for commercial hybrids had positive index values. Possibly because of this cool tolerance, their hybrids have wide adaptability.

Genotypes selected at Mead had a mean value less than a base population of check hybrids and parent lines labeled cool tolerant, whereas, genotypes selected at Sidney had a mean value which was greater. Also, lines originating from northern USA and Canada had positive values. In both cases, these results would support the cool tolerant index for a method of identifying cool tolerant material.

Objective 7. Conduct analysis of energy requirements of sorghum for the developmental stages including the periods from planting to panicle initiation, panicle initiation to bloom, and bloom to physiological maturity.

- 1) Eastin. Growing degree unit (GDU) and solar thermal unit (STU) requirements for grain sorghum. Data for 2 years are summarized in Table 1. Solar thermal units are Langleys x GDU's.

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Table 1. Growing degree units (GDU) at base 15 C and solar thermal units (STU) for respective growth stages in 20 sorghum hybrids.

Genotype	GDU			STU x 10 ²		
	GS ₁	GS ₂	GS ₃	GS ₁	GS ₂	GS ₃
820 ¹ ₂	301	280	261	1695	1471	1161
E59 ²	254	304	279	1404	1643	1272
828 ¹ ₂	300	281	263	1678	1488	1170
E57 ²	251	286	297	1394	1525	1386
Nb 691	278	291	278	1560	1541	1238
C42C ²	244	274	314	1377	1433	1487
833 ¹	281	276	284	1572	1459	1297
Nb 635	234	292	308	1322	1529	1454
Nb 634	237	293	305	1328	1543	1433
Acco R109	243	299	294	1353	1591	1366
C42Y ²	237	277	313	1342	1445	1503
RS 626	224	276	298	1287	1434	1450
Exel 808	249	302	285	1390	1609	1314
RS 671	250	295	286	1393	1575	1329
266 ³	227	298	305	1291	1554	1447
222 ³	262	285	286	1471	1510	1330
845 ¹ ₂	251	293	290	1414	1544	1347
F64 ²	298	274	269	1670	1450	1209
RS 625	212	291	290	1219	1515	1416
233 ³	203	279	310	1174	1450	1531
Ave	263	287	290	1425	1513	1352
Ave/day	6.68	8.06	6.37	36.17	42.50	25.70

1 Pioneer
2 Dekalb
3 Northrup King

Knowledge that generally from 200 to 300 GDU's (15C base) or about 1400 STU are required to complete the vegetative (GS₁), panicle development (GS₂) and grain fill (GS₃) periods should be useful in evaluating where grain sorghum production might be feasible. Minimal data required would be estimates of or actual maximum and minimum daily or weekly temperatures. Also some estimate of appropriate maturity classes for different areas should be possible.

Objective 8. See Section D.

Objective 9. Not applicable

Objective 10. Continue work on developing both indoor and outdoor environmental control equipment.

- 1) Eastin, Clegg, Maranville, Clark, Sullivan. Development of controlled environment facilities. Four AID purchased growth rooms 9'W x 9'L x 7.5' H were installed in the new Plant Science Complex. Also state funds were used to construct a new headhouse-greenhouse complex and install an additional 13 growth rooms (4 from AR, SEA). Growth rooms are housed in the headhouse: Excellent "field stature" millet and sorghum crops have been grown in the AID growth rooms. The millet crop was grown by Mr. David Andrews, head of the ICRISAT millet program. The rooms are especially valuable for water x temperature stress interaction studies.

An excellent, simple temperature control device was designed and built for field investigation of above ambient temperature. A control was developed which permits controlling night temperature in a 5' x 6' x 4' movable field tent at a fixed temperature differential above ambient (see Ogunlela's results). The devices will be used next to test for water x temperature interactions in drier fields at Garden City, KS.

Two linear move irrigation systems were purchased and set up to do drought screening and water stress evaluation work at Garden City, KS, and Sidney, NE. The KS unit was operated in 1980. The NE unit was delivered in May and will be operated in 1981.

The above three system developments were very time consuming. However, the facilities are all functioning to expectations and will contribute heavily to our Title XII sorghum and millet effort.

Objective 11. Initiate investigations on genotype differences on nutrient uptake and utilization efficiency.

- 1) D. Knudsen, R. B. Clark, J. L. Denning and P. A. Pier. Use of energy dispersive X-ray spectrography for plant analyses.

Energy dispersive X-ray fluorescence is proving to be an effective technique for multi-element analyses of plant tissue samples. It can effectively assay elements above the molecular weight of Mg (24) including S, Cl and Br. Once the parameters and programs have been selected or developed, operation is relatively simple and easy. However, the programs and parameter selection can be quite complex, but critical for quantitative work.

Best overall results have been obtained using 500 mg x 31 mm pellets or 100 mg x 13 mm. The smaller pellet is used for samples with small amounts of material. Somewhat better intensities for low-Z (Mg, P, Al, Si, S, Cl, K, Ca) elements are obtained with thinner samples, but lower intensities then occur for mid-Z (Zn, Cu, Mn, Fe, Mo, Cr, Co, Ni, As, Se, Br, Rb, Sr) elements which are usually in low or trace quantities. Samples pelleted with 1 g of boric acid backing in Al cups provided good intensities for the low-Z elements, but much lower intensities were obtained for the mid-Z elements. This technique might be superior for low-Z elements when 100 mg or less of sample material is available. Samples must be ground to pass 40 mesh in order to form a stable pellet.

National Bureau of Standards plant material has been used initially to prepare standard curves. This has provided satisfactory standard curves for some of the elements. Cellulose (filter paper pulp) was

found satisfactory for preparing additional standards for elements above molecular weight of Cl. Materials of known concentration will be needed and used in addition to NBS standards in order to obtain concentration ranges needed for standardization. A technique for spiking cellulose or samples is being developed to obtain additional materials for standardization of all desired elements.

Programs and parameter selections have been developed to determine 25 elements automatically and simultaneously on the same sample. This has been done for twenty 31mm pellets in about 2.5 hours or twenty-five 13mm pellets in 3.2 hours. Current quantitative detection limits (3 x standard deviation of background) for some of the elements are given in Table 1. Except for Mo and Co, these lower detection limits for the essential elements for plant growth are well below the concentrations normally found in healthy or deficient tissues. This program consists of three passes at the sample, each with different excitation parameters. Further refinement is expected to improve some of the detection limits and to decrease time of analysis. Software will be incorporated into the system to make matrix corrections when necessary.

Over 500 samples have been analyzed with the above programs. In addition, at least 400 standards and other samples have been run in order to develop standard curves, sample preparation techniques, and appropriate excitation parameters. Considerable analyses by atomic absorption and other analytical methods have been performed to characterize standard materials.

Table 1. Detection limits of some mineral elements in plant samples by energy dispersive X-ray spectrography.†

<u>Macronutrients</u>		<u>Micronutrients</u>		<u>Nonessential Elements</u>	
<u>Element</u>	<u>Detection Limit</u>	<u>Element</u>	<u>Detection Limit</u>	<u>Element</u>	<u>Detection Limit</u>
	ug/g		ug/g		ug/g
P	30	Mn	8	Al	340
K	27	Fe	5	Si	100
Ca	23	Cu	2.2	Cr	6
Mg	410	Zn	2.5	Ni	2
				As	1.0
S	21	Mo	9	Se	1.2
Cl	65	Co	3	Pb	2.5
				Hg	2.3
				Br	3
				Rb	3
				Sr	5

† Assuming three times the standard deviation of background.

- 2) R.B. Clark, J.W. Maranville, and H. J. Gorz. Phosphorus efficiency of sorghum grown with limited phosphorus.

Sorghum (Sorghum bicolor L. Moench) genotypes showed marked differences for uptake, accumulation, and distribution of P. When grown with limited P, the genotypes differed in dry-matter yield, mg dry-matter produced/mg P, P concentration and content, and accumulation of P in roots, lower and upper leaves. Roots showed greater differences than leaves for these parameters. Phosphorus content had the widest ranges among genotypes and mg dry matter produced/mg P had the narrowest ranges. The ratio of P in the upper leaves to that in the lower leaves ranged from 2 to 46. Phosphorus top/root ratios ranged from 0.8 to 2.3, indicating that in some genotypes, roots retained more P than did leaves. These differences may be important in adaptation of plants to low P conditions and should help explain differences among sorghum genotypes for efficiency of P uptake and utilization.

- 3) R.B. Clark, H.J. Gorz, and F.A. Haskins. Effects of mineral elements on hydrocyanic acid potential in sorghum.

Sorghum (Sorghum bicolor L. Moench, cv. 'Early Hegari') seedlings were grown in nutrient solutions to determine the effects of varied levels of mineral elements on hydrocyanic acid potential (HCN-p). Maximum growth of seedlings occurred at ion levels of 0.3 to 30 meq and seedlings grown at ion levels below or above this were abnormal. HCN-p was influenced by mineral elements only when seedlings were grown for at least two days in treatment solutions. Individual salts had the following effects: KCl decreased HCN-p; NH_4Cl , NH_4NO_3 , $\text{NH}_4\text{H}_2\text{PO}_4$,

K_2SO_4 , and $Ca(H_2PO_4)_2$ increased HCN-p; and KNO_3 , $CaCl_2$, $Ca(NO_3)_2$, $CaSO_4$, $MgCl_2$, $Mg(NO_3)_2$, and $MgSO_4$ had no effect on HCN-p. Factorial experiments were set up to compare the cations across a common set of anions (Cl^- , NO_3^- , $H_2PO_4^-$, and SO_4^{2-}) and the various anions of the common cations (K^+ or NH_4^+) to determine the individual effects of ions on HCN-p. HCN-p decreased when KCl and NH_4Cl or KNO_3 and NH_4NO_3 were combined in the medium. HCN-p increased when KH_2PO_4 and $NH_4H_2PO_4$ were added singly or in combination. HCN-p also increased when $(NH_4)SO_4$ was added at high levels of K^+ but not at low levels.

4) P.R. Furlani and R. B. Clark. Screening sorghum for aluminum tolerance in nutrient solution.

Sorghum (Sorghum bicolor L. Moench) genotypes were grown in nutrient solutions to evaluate their tolerance to Al toxicity. Seedlings were grown 10 days in treatment solutions with large numbers of plants (up to 125)/container of 6.3l (50 ml/plant). The optimum level of elements for good sorghum growth and wide differential responses to Al were (mg/l): 2.0 P, 300 Ca, 40 Mg, and 4.0 Al. Phosphorus added at 4.0 mg/l or higher caused relatively severe 'red-speckling' or leaves of many genotypes and at 1.0 mg/l P deficiencies appeared. When Al was added at 2.0 mg/l toxicity symptoms were nil and when added above 6.0 mg/l toxicity symptoms were very severe on all genotypes. Severity of Al toxicity symptoms were reduced when Ca was increased from 150 to 300 mg/l, but Ca was detrimental to root growth at 600 mg/l. Magnesium added at levels higher than 90 mg/l was detrimental to root growth on most genotypes. Severity of Al toxicity symptoms were increased as the temperature increased. The relative tolerance of many sorghum genotypes to Al will be discussed.

- 5) Angela M. Furlani, R.B. Clark, and C.Y. Sullivan. Properties of a phosphorus-induced 'red-speckling' on sorghum leaves.

Sorghum (Sorghum bicolor L. Moench) seedlings showed varied amounts of 'red-speckling' when grown with different levels of P in solution. A relatively susceptible genotype Martin and a relatively resistant genotype N9040 were used to characterize some of the chemical and physiological properties of this P-induced disorder. 'Red-speckling' appeared on Martin leaves at P levels as low as 0.2 mg/l and were rather severe at P levels of 0.5 mg P/l. No 'red-speckling' appeared on N9040 leaves at these P levels. The amount of 'red-speckling' on seedling leaves also depended on the type of P compound used: phenyl phosphate \geq potassium nonbasic phosphate \geq ethylammonium phosphate \geq glycerophosphate \geq calcium tribasic phosphate \geq aluminum phosphate \geq ferric phosphate = calcium pyrophosphate. Leaves which had greater amounts of 'red-speckling' also had higher leaf concentrations of P, lower leaf surface areas, and lower photosynthesis rates.

- 6) G. Franca, R.B. Clark and J.W. Maranville. Differential nitrogen uptake, distribution and use by sorghum genotypes.

Fifty-four sorghum genotypes were grown with 10 mg N/plant in nutrient solutions to determine differences for N uptake, use, and distribution. Differences among genotypes for dry matter yields, N concentrations and contents, dry matter produced per unit N, and top N/root N ratios were determined. Dry matter yields among genotypes ranged from 0.70 to 1.75 g/6 plants (a 2.5- fold difference) with a Tx3934 x GH8-17 genotype yielding the highest and an SC110 x SC120 genotype yielding the lowest. The same Tx3934 x GH8-17 genotype also produced the highest dry matter per unit N (41.5 g dry wt/gN) compared to an

SC150 x GH8-17 genotype which produced the least dry matter per unit N (29.3 g dry wt/gN); a 29% reduction. An SC150-6 x SC150-9 genotype translocated less of its total N from roots to tops (top N/root N = 1.49) compared to an SC300 x SC52 genotype (top N/root N = 2.69) which translocated more of its total N to the tops. Using the definition that N efficiency is the highest dry matter produced per unit N, a Tx3934 x GH8-17 genotype was the most N efficient and an SC150 x GH8-17 genotype was the most N inefficient genotype. Wide differences among genotypes were also noted for N concentrations and contents, for root and top dry weights, and for top/root ratios of dry weights. Considerable differences appeared to exist among sorghum genotypes for N uptake and distribution as seedlings. Selected genotypes from those tested are being studied for other N uptake, distribution, and use properties with age and N level in both nutrient solution and in soil experiments.

2. Interpretation of data and supporting evidence:

The objective and work pursuit statements under each numbered objective in C above are generally interpretive statements. However, the following selected statements will add emphasis to certain areas.

Objective 1. Yellow endosperm lines, low tannin and nutritional value. 1) Adverse correlations were found in two populations for agronomic and quality traits, but progress for higher nutritional value was demonstrated while retaining desirable agronomic traits. 2) Grain quality populations featuring low testa (tannin) frequency, high yellow endosperm, high protein, high lysine and high metabolizable energy have been formed. 3) A simple, rapid test using alpha amylase was developed to estimate feeding value of sorghums containing tannin. The test is adaptable to breeding programs.

Objective 2. Breeding techniques. 1) Recurrent selection for protein demonstrated, unfortunately, a typical negative yield-protein correlation. 3) Use of single cross steriles made seed production for R line testing easier with no yield reductions compared to use of inbred line steriles. 4) Two-dwarf sorghums (tall) are desired in some LDC's where both grain and forage are needed. An unusual combination of dwarfing genes discovered in SC 102-9 was used to make a sterile combine height parent, which when crossed to selected combine height males, gives high grain yielding tall hybrids. Tall hybrids from crossing tall lines are very difficult to produce whereas this production scheme is normal making the potential advantages of hybrids easier to realize in LDC's where tall hybrids are desired. 9) Family sizes of 100 S_1 progenies from populations were determined to be minimal for yield and protein testing.

Objective 3. Limited tillage and cropping systems practices. 1) Soil bedding did not influence sorghum yields compared to conventional tillage. Fewer field trips were necessary using the bedding approach which might be of some value in mechanization schemes (i.e. the Sudan). No additional bedding work is planned. 2) Mulching associated with certain kinds of minimum tillage delayed plant growth (cooler soil) and increased yields. Fertility management would need to be adjusted on the basis of yield level. 3) Soybean-sorghum rotations give the effect of 50-100 kg/ha N equivalent on sorghum following sorghum. The value of grain legumes in LDC cropping systems merits attention.

Objective 4. Stress tolerance and avoidance mechanisms. 1) Efficient N partitioning to grain did not correlate well with high yields. 2) No significant correlation between seedling drought resistance and field yield stability was

noted. Therefore the seedling test was not deemed useful. The field water gradient technique, however, was shown to be an effective drought screening technique. 3) Drought stress conditioning was shown to benefit photosynthesis and stabilize yields under limited water. 6) A modified water gradient system was assembled to be tested for field screening for drought resistance. The system uses half the land and seed required for a normal water gradient system. 7) Heat and limited water were generally shown to increase root growth which leaves a lower proportion of limited assimilates available for grain production.

Objective 5. Plant reaction to stress and survival mechanisms in stressed plants.

1) Stress during panicle development and right at bloom are quite detrimental to photosynthesis (ca 30% reduction) and yield (40-50% loss). 2) Relatively small proportions of roots exposed to moist soil or nutrient solutions supported good plant growth. 3) A drought resistant hybrid used 31% less assimilate to support its roots than did a normal hybrid under both normal and stress conditions. The lower root assimilate requirement is probably partly responsible for the higher seed number and yield of the resistant hybrid under stress. Much research is needed on root characteristics based on results in 2 and 3. 4) Based on extensive genotype x environment interaction work, the seed size component of yield appears to be an important yield stabilizing factor in harsh environments. 5) Modest night temperature elevations (5C above ambient) during the differentiation and early development of pistil and stamen primordia (6 day period) reduced yields 31% because of a 44% seed number reduction. No suitable screening technique for screening for stability during this period is available. 6) Field screening for temperature effect on grain respiration rate is possible and genetic variability for variability in respiration rate at the same temperature is appreciable. Therefore, it should be possible to fit genotypes to environments

varying widely in temperature profiles. 7) Grain starch synthetase activity is not influenced much by temperature. This may or may not be related to fairly stable and nearly linear grain fill rates noted over a range of environments.

Objective 6. Cool temperature growth. 1) William Amen selected about 200 lines out of 7000 seed lots grown by Elmer Johnson at Chapingo, Mexico. Many were found to be suitable for Sidney, Nebraska, as tested subsequently by Clegg and Eastin. Other materials gathered by P. Nordquist were also included. A reasonably wide range of dwarf materials have been gathered which fit moderate altitude (4400') semiarid, short-season climates like Sidney, NE. 2) Two Nebraska populations planted at Sidney were observed to have sufficient earliness to be used for selection at Sidney. Francis has been selecting and forming B line populations suited to that area which will be available for international testing in the future. 3) Mendoza earlier developed a cool temperature germination screening test where germination progress could be observed at any time. The method has been extended to observe root-shoot ratios up to a month in age. Good root growth is of interest since that appears to be a problem under cool soil conditions resulting from ecofallow forming where mulches are left on the soil. Mulching should be of interest in some high elevation, semiarid tropical locations. Desirable plants are being selected from S₁ heads for field testing under ecofallow.

Objective 7. Growing degree units for sorghum. Twenty hybrids spanning a range of maturities were evaluated for growing degree days required to complete the vegetative, panicle development and grain fill stages. The likelihood of adapting sorghum to new locations or successfully implementing shifts in maturity at a location can be estimated if reasonable maximum and minimum temperatures are available.

Objective 10. Controlled environment facility development. A unique, simple temperature controlling device for regulating field temperature at night in small tents was developed. Yield results from temperature variation effects conform well to growth room results confirming the value of the growth rooms developed to supplement field efforts. A sizeable growth room-greenhouse complex has been put into operation to help investigate temperature x water interaction effects. Much basic knowledge needs to be gained regarding stress mechanisms in hopes of improving the rate of crop improvement through breeder selection techniques.

One general breeder screening technique is a modified (shortened water gradient system which was tested just one season and appears to be excellent.

Objective 11. Mineral nutrient uptake and utilization. 1) An excellent energy dispersive X-ray spectrograph was acquired (station funds) and calibrated for plant material analyses. Primary use is for nutrient uptake and utilization efficiencies. Much work was required for calibration but now sample analyses are more rapid with less technical labor required than for inductively coupled plasma spectrograph analyses. 2) Marked differences have been shown amongst genotypes for uptake, accumulation and distribution of P. 3) The influence of different anion and cation concentration on HCN production were tested. Phosphates generally increased HCN while chloride and nitrate decreased HCN. 4) Sorghum lines were screened for Al toxicity. Genotypes vary substantially in tolerance levels and can be separated through the screening technique developed. A number of tropical soils require Al tolerant genotypes for successful culture in lieu of phosphorus fertilizer which generally is not readily available or is too costly. 6) A nutrient solution screening technique was set up for differential N uptake and utilization. Additional effort is needed regarding screening potential.

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3. Research design. The research design has generally been adequate. Research techniques in some of the areas being emphasized require rather regular modification. This is in the area of quantitating the plants reaction to different combinations of environmental stresses and attempting to find out what the mechanisms of resisting stresses are. Understanding mechanisms sometimes permits developing breeder screening techniques. Success with the water gradient system for drought screening led to development of the abbreviated system using half the land requiring only half the seed for the normal system. AID cooperation in this is greatly appreciated.
- E. Dissemination and Utilization of Results.
1. See appendix for bibliographic list.
 2. Use of findings.
 - a. The normal water gradient screening techniques is being used in Yuma, AZ and at ICRISAT. It may be tried soon in Egypt under Title XII efforts. The linear move abbreviated systems are now at Garden City, KS, and Sidney, NE. Apparently one is being planned at Lubbock, TX.
 - b. The heat tolerance test has been or is being used in several places. Overseas sites include IRRI and Taiwan.
 - c. The cool tolerance test is being used in Mexico.
 - d. Screening for aluminum tolerance is being done in the U.S. and Brazil. Three Brazilian students were or are being trained.
 - e. A student from Botswana was involved in research on nitrogen contribution of a grain legume to sorghum and is now beginning his Ph.D. training in Botswana applying that approach to local N problems.

- f. Invited participation in an International Workshop on "Stress Syndromes and Crop Production" sponsored by the Canadian Society of Plant Physiologists and the International Association of Plant Physiologists.
 - g. Participated in Educational TV program informing Midwesterners of the US AID - Nebraska program and the mutual benefits for everyone.
 - h. Participated in University of Nebraska Media Days promoting international involvement of Nebraska Scientists. A continuing effort proceeds on explaining the mutual benefits derived for Nebraskans and LDC's through international work.
 - i. We have hosted many individuals and groups from LDC's, SAFGRAD and DC's regarding sorghum work at Nebraska.
 - j. Nebraskans have participated actively in the planning and work effort required to get the Title XII sorghum-millet CRSP going while it overlapped with the current contract.
 - k. Presented a paper on Tropical Adaptation at a 1979 International Workshop at Corpus Christi, Tx.
3. One way to expand use of research results is to evaluate more closely doing a portion of a graduate student's thesis research in his native country or other developing country. The case of Mr. Lucas Gakale serves as an example. He worked here on an MS degree and gained experience in grain legume-cereal rotations. Now he has returned to Botswana to begin work on his Ph.D. research using crops specifically adapted to that area of the world. While this arrangement sounds ideal, certain facts have

to be recognized. The arrangement is expensive. Graduate colleges can not accept this kind of research arrangement without (1) the supervisor visiting the research area once or twice and (2) another adequately trained local scientist being there for regular consultation with the student. We are attempting to do more of this under the sorghum-millet Title XII CRSP. Expansion of the approach will require extra input from AID and other world development agencies if the approach is desired.

4. Since this is a terminal report there is no future work plan. However, the basic effort under this contract is being continued under the sorghum-millet CRSP.

F. Statement of Expenditures and Obligations and Contractor Resources.

Since the contract number was changed both budget-cost sets are included.

Contract ta-c-1382

	<u>Budget</u>	<u>Cost to 2/77</u>	<u>Costs 2/77 to compl.</u>	<u>Final</u>
Salaries & Wages	101,757	109,542.64		
Fringe benefits	8,832	6,985.92		
Equip. & mat.	79,389	105,557.90		
Travel & transp.	6,940	4,466.29		
Other D.C.	18,605	12,361.12		
Ind. C.	79,477	56,086.13		
Totals	295,000	295,000.00		

Contract ta-c-138^

Salaries & Wages	161,000	153,161.40
Fringe benefits	6,640	8,284.56
Equip. & mat.	142,970	157,819.10
Travel & transp.	17,000	23,737.60
Other D.C.	72,080	60,628.57
Ind. C	<u>82,050</u>	<u>78,108.83</u>
Total	481,740	481,740.00

Combined totals

Salaries & Wages	262,757	262,704.04
Fringe benefits	15,472	15,270.48
Equip. & Mat.	229,299	263,377.00
Travel & Trans.	23,940	28,203.89
Other D.C.	90,685	72,989.63
Ind. C.	<u>161,527</u>	<u>134,194.96</u>
Total	776,740	776,740.00

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Two significant events during progress of the project bear comment. First we were able to establish two good semiarid nursery areas for drought-temperature field screening. A long season-dry area nursery was established at Garden City, KS, in cooperation with Kansas State University. This involved several delays due to getting a research well permit in an oversubscribed groundwater area. Patience on the part of Drs. R. Jackson, J. Yohe and several of the contract staff in helping us get that project completed is greatly appreciated. A short season-dry area (cool night) nursery was established at Sidney, Nebraska. Combined with other nurseries at North Platte and Lincoln, this gives us a wide range of screening-testing areas to evaluate germ plasm and study mechanisms of plant adjustment to adverse water and temperature variables.

Second, we have been able to design and build a variety of environmental facilities to further help in quantitating plant reaction to stresses and help in developing breeder screening techniques. AID fund input into this effort constitutes a very small portion of a total package but, in our case, it was a very significant input.

G. Work Plan and Budget Forecast for Coming Year.

This is a termination report.

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SORGHUM SEED SHIPPED OVERSEAS THROUGH USDA PLANT GERMPLASM QUARANTINE CENTER

	<u>Country</u>	<u>No. of Samples</u>
<u>1977</u>	Albania	4
	Argentina	4
	Australia	12
	Djibouti	4
	Egypt	40
	Hungary	23
	India	32
	Japan	103
	Mexico	146
	Philippines	4
	Rumania	6
	Turkey	28
	Switzerland	3
	United Kingdom	<u>24</u>
	TOTAL	436
<u>1978</u>	Brazil	345
	France	47
	Hungary	76
	Mexico	17
	Niger	14
	South Africa	30
	Thailand	12
	Uruguay	1
	Venezuela	2
	Zaire	<u>7</u>
	TOTAL	551
<u>1979</u>	Argentina	166
	France	24
	Gambia	36
	India	6
	Indonesia	34
	Japan	14
	Philippines	9
	Rodesia	2
	Venezuela	<u>10</u>
	TOTAL	299

Development of Improved Sorghum -
Physiology of Yield and Stress Reaction -
University of Nebraska

Dr. M. Peterson, Chairman of the Subcommittee composed of Drs. Ehrenreich, Falcon, and Whitney, briefly summarized the proposal as follows.

This proposal is for an extension for three years of a program first funded by A.I.D. in 1974. In November 1972, A.I.D. presented the RAC with a project on sorghum improvement involving a consortium of three universities: Puerto Rico to work on tropical types, Texas A&M to work on disease and insect resistance, and Nebraska on the physiology of grain yield. These three institutions, together with Purdue which was doing work on protein quality, were to be an integrated team on sorghum research. The three university consortium was divided into separate projects in 1974. The present Nebraska project became operative in February 1974 building on much earlier work; hence the annual report for 1975 is shown as Annual Report No. 9. Issues include:

- 1) Is this the best way to organize an international sorghum research effort?
- 2) Are the objectives as outlined important and attainable?
- 3) What is the quality and quantity of past performance and future promise?

This does not appear to be a very tidy package. The historically factored parts of the program need to be brought back together to produce adapted varieties. Regarding objectives, 2 appear to be

out of place with the other 4. Objectives 1,2, and 5 seem to be worthy and compatible. An examination of the adaptation of sorghum to stress situations is important because the areas where sorghums are grown are always stressed, mainly by drought. However, stress from heat, cold, nutritional deficiencies, and pests are equally important.

The primary concern of this reviewer is that the data generated from this study of basic physiological processes may be superficial. For example, the experiments designed to test for heat-hardening of nitrate reductase measure only the ability of the enzyme to withstand elevated temperatures in a cell-free system. It would appear that a more representative interpretation of the effect of heat-hardening on nitrate reductase could be obtained by measuring enzyme activity under elevated temperatures in an in vivo system. Technology developed by P. Filner, Michigan State University; E. Jaworski, Monsanto Chemical Company; and J. Radin, U.S.D.A., Tempe, Arizona would be applicable.

The effect of moisture stress (R. H. Hageman, University of Illinois) and low temperatures (G. Brown, University of Missouri) on protein synthesis in higher plants is well documented. Perhaps a study of this type would be beneficial to the current program. Dr. Eric Davies, Life Sciences Department, University of Nebraska, has made many outstanding contributions to the understanding of protein synthesis in plants. His experience might be useful to this project. It is surprising to find that Dr. N.J. Rosenberg, Engineering, University of Nebraska, a highly competent meteorologist is not involved in this project. He would be a valuable addition.

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The differential ability of genotypes to utilize essential nutrients is noted. The evaluation of populations for differential responses to mineral elements might be enhanced by a study of the plasma membrane and associated uptake mechanisms in genotypes in question. Technology developed by T. Hodges and co-workers at Purdue University might be helpful.

We recognize that the data generated to date represent only the initial effort in the program. Subsequent reports will deal with physiological parameters in greater depth. The questions generated and the conclusions reached are obviously valid. However, it is felt that a more basic approach to the proposed physiological studies would significantly improve the project.

Objective 3 was a study of heritable difference in mineral uptake. If this can be done utilizing breeding materials developed for a sorghum breeding program outside of this project, it is probably worthwhile. As a sorghum breeding and genetic study within this project, it is not well balanced since it is simply one single breeding objective among many possible objectives.

Objective 5 does not fit a project on the physiology of stress. It is concerned with developing breeding techniques with emphasis on recurrent selection for objectives which are identical to the Texas A&M project. Inclusion of this objective has the appearance of an expediency arising out of a compromise. In addition to this difficulty, it is interesting to know that Doggett, the sorghum breeder at ICRI SAT, last year had a 50 acre winter nursery and an 80 acre summer nursery for the identical purpose.

In spite of these problems, Dr. M. Peterson was in favor of the project. It would be a far better project if Nebraska concentrated on sorghum physiology, brought in some added talent they are known to have, and follow a more sophisticated approach to sorghum physiology.

Dr. Thorbecke stated that he had little to add to the subcommittee report. Texas had little to say about achievements under the period of A.I.D. support. Contributions to macro-economic targets, such as yield and DC conditions, should be stated. He wanted a better statement of impact.

Mr. Wittnabert accepted the subcommittee report with a positive reaction. He urged more reference to A.I.D. plans for support following the project.

Dr. Ehrenreich assessed the progress, communications, and probability of success as good. Possibly the project is over ambitious; there is general need for a decrease in the breadth of work with greater concentration on the physiological methods. Objectives 4 and 5 on disease resistance should be tied into the Texas A&M project. Objective 6 is vital, but it should be more specific. He suggested stress on system simulation modelling as a goal, moving from an analogue approach to stochastic methods. More cooperation among the four projects is desirable, as well as, an international symposium at the proper time.

Dr. Falcon was strongly supportive, complementing the project statements. However, the total funding requirement is not clear, and there is lack of specific information as just what aspects A.I.D. is supporting.

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Dr. Whitney was gratified to see the specificity of the Texas A&M objectives, although he was troubled by the lack of A.I.D. role specificity. How and by whom will the Puerto Rico testing be checked? The budget increase is apparently related to the plan for the international nurseries and the three new staff positions. The scope of the Nebraska proposal seems like a lifetime undertaking leading to a U.S. Sorghum Center. What will be useful, and when, for the developing countries? The work appears to be more beneficial to the developed countries. The cultural methods appear to be very complex; can these be transferred to DC's? This is a good design, and a competent staff, but he doubted that they could meet the stated objectives in the proposed three year period.

Mr. Ludington commented that there was no reference to the food aspects. If insects won't eat the product, perhaps people won't either.

Dr. Earl Leng, TA/AGR, stated that he was pleased to look at the whole program. It had never operated as a consortium. However, the several contractors are now working well together. Meetings were held at Lincoln a little over a year ago concerning help to the International Centers, ALAD and ICRISAT. Dr. Frederick is now in Senegal. Greater integration may be appropriate under Title XII, although this is not clear at this time. These are large and complex programs with some possible conflicts from the individual project point of view. However, A.I.D. supported parts can be interleaved in many areas, although this is difficult to specify, and we may have to judge

in terms of the outcomes. He commented on the several questions raised as follows: Food utilization: This was a matter of first importance at the Cereal Chemist Meeting in Vienna in May 1976. Nebraska objectives reflect the interests of individual staff members. Breeding methods face difficulty of general agreement; Nebraska and Texas do use different methods, but they do interact. One symposium has been held at Mayaguez, Puerto Rico, and more are planned. International trials are likely to be conducted through ICRISAT as the home for such work and as a best way to distribute germ plasm. Travel is important in support of an international program, and possibly Texas should be encouraged to do more. Review of the program will be planned; but basic work should go forward until appropriate alternate mechanisms become available.

Modelling is important, but should be considered separately from this project. Dwarfing is a function of a four-gene system which can produce many variants; an intermediate length is likely to be the best for overseas areas. Buying-in to ongoing projects by A.I.D. provides support for overseas travel and participation which would normally otherwise simply not be available, and this is a valuable asset.

Dr. D. Peterson pointed out that there tends to be confusion over the several possible objectives of the concept of modelling. We need to identify what we are after, since models may be sensitive only to the limited purposes for which they were designed. Three classes of model objectives commonly recognized include: information retrieval, crop productivity processes, and structural model of an efficient plan.

Dr. Moss stated that this appears to be an excellent project and worthy of support. He hoped that the time does not come when A.I.D. support goes only to the International Centers. There is need for the continued support to U.S. Universities.

Evaluation requires adequate information on the qualifications of the personnel involved. RAC might perform the role of assisting to identify and fill the gaps in needed expertise.

Motion: re: Texas A&M project

Note: Because of the interrelationship of sorghum research projects, this and the subsequent (Nebraska) project proposal have been discussed together.

That the project extension for three years at the requested funding be approved.

It is recommended (not a condition of the motion) that field review of this and two related A.I.D. financed sorghum research projects be held next summer by one review team in the context of work done on this problem by International Research Centers and other entities. RAC representation on that review is suggested.

Moved by M. Peterson; seconded by Whitney.

Vote: Unanimous approval

Motion: re: Nebraska project

That the project extension for three years at the requested funding be approved.

It was the general consensus of RAC that the physiological studies could be strengthened by techniques developed by various other research laboratories and that additional scientific talent at Nebraska as well as advice from physiologists elsewhere would be useful in these studies.

The same recommendation was made as on the previous project regarding the conduct of a field review of all related sorghum research projects by the same team.

Moved by M. Peterson; seconded by Whitney.

Vote: Unanimous approval.

Improvement of Grain Yield and Quality of Pearl
Millet for Semiarid Areas - Kansas State University

Dr. Peterson, the RAC subcommittee chairman reported to the RAC as follows:

Like the sorghums, Pearl Millet exists in two forms: one adapted to forage production which is tall and leafy and selected for green forage purposes and the other selected to produce grain from the seeds for use as human food. When one reads the proposal several general questions arise that can be discussed under the following headings:

1) Importance of Pearl Millet Improvement

Although pearl millet is by far the most important millet, there are a number of other genera and species known as millets. Of the world production of all millets estimated at 21.6 million metric tons, about 12.3 are produced in the Far East, and 5.3 in Africa leaving only insignificant amounts for the remainder of the world. That geographic distribution is in a belt just to the north of the equator and is characterized by limited rainfall occurring during 2 to 7 months of the year, with very limited irrigation possibilities. The people of India, Africa, and elsewhere who depend on millets for food are among the world's poorest. Sorghums are grown in areas generally too dry to support corn. Similarly, millets are grown where poor soils and drought limit sorghum production.

Pearl millet, aside from being a crop almost without alternatives in these extremely dry areas, is also a crop with substantial nutritional virtues. Protein content is 3-4 percent higher than grain sorghum. With AID's commitment to helping the world's poorest, pearl millet is a good choice of crop to work towards this goal.

2) Prospects for Improvement and how long will it take.

Goals of the proposed research are to (1) to produce higher yielding synthetic varieties and to investigate the possibilities of developing hybrids, (2) to characterize certain desirable traits such as seedling vigor, drought resistance, and herbicide susceptibility, (3) to determine nutritional quality, and, (4) to determine storage and rancidity problems.

Kansas State in cooperation with USDA began research on pearl millet as a grain crop in Kansas in 1971. The work plan proposed by KSU is exceedingly detailed as to precise actions to be taken each year. Step by step they have shown the procedures for population improvement, determining hybrid possibilities, studying moisture stress of different genetic lines, determining genetic variability in digestibility and nutrient composition, and studying factors that contribute to losses in storage and rancidity.

It appears that the project has a high probability of reaching most of its objectives. Among crops that have benefitted little

from research, initial progress is usually fairly rapid and predictable. Production of improved synthetic varieties of unselected populations is almost certain to be successful.

RAC should expect that this project will not reach its potential in 3 years. A reasonable expectation would be a 3-year or possibly a 5-year extension. Plant breeding takes time and as old goals are achieved, new ones take their place.

3) Qualifications of the Contractor

Kansas State University has the staff to conduct a successful breeding and quality evaluation program. Their agronomy program has a long record for successful plant breeding. They have a department of Grain Science that specializes in quality evaluation and of Food Science interested in human nutrition.

4) Duplication of effort with ICRISAT

Kansas State has more than 6 years of experience and a considerable backlog of material to build on. They have a specialized staff, facilities and departments that cannot be duplicated elsewhere. ICRISAT cannot possibly do all the work in the world on millet. In addition, monopolies in research are not desirable because they sometimes lead to limited approaches. The two organizations can exchange materials and information. However, we would expect the outreach program to be handled by ICRISAT. Overall we are supportive of the project.

Dr. Ludington agreed with the subcommittee chairman's report but wished to stress to the staff that this project has numerous rather major objectives and to be cautious of forming too many permutations in the research design. He also cautioned the investigators about focusing too closely on the nutrition values in millet breads. He would hope that KSU would attempt to develop strains directly applicable to both India and Africa.

Dr. Moss indicated that he was generally positive about the project but wished to raise 3 additional questions. The Bio-data supplied indicated that the investigators were agronomists with little plant breeding experience. Who wrote and will carry out the plant breeding portions of the project? Since millet already has a higher lysine content than corn, why is this component being stressed in the research design. The project paper leads one to believe that there are really 2 independent projects going on, one at Fort Hays and the other at Manhattan. Will these stations compete or cooperate?

Dr. Swanson favored the project but wished clarification from the staff on several points not addressed previously by RAC members. He wished to know what were the prospects for more precision in determining what genetic traits are desirable? Are there any marketing implications to increased millet production? Will the training of foreign nationals occur naturally or is training programmed into this project?

Dr. Montgomery asked if the end product of this project was to create an industry to provide the farmers with seed or would the farmer keep sufficient seed from his own harvest?

Dr. Long (AA/TA) asked RAC to address whether KSU and ICRISAT was the right combination to give optimum competition and cooperation. Also, why is the goal to develop a dwarf variety of millet considering the by-products of the plant may be extremely important to the local poor farmer?

Dr. Earl Leng (TA/AGR) represented the AID staff in addressing RAC comments and questions. He provided the RAC with a short Genesis of the project. This project initially came to the Agency over a year ago. We were redirecting our program priorities and requested that they submit a new proposal which would include coordination with ICRISAT. The present document indicates this direction and we are well satisfied with it, however, we will watch carefully how this working relationship develops.

Pearl millet may be important in very poor soil areas in the U.S. someday, but not in the near future. Prospects for progress are good but obviously we cannot go from start to finish in 3 years. A great deal of work has been done in India but they ran into ergot problems. Considering the tall versus dwarf plant types, what was meant by dwarf was a medium sized plant that would give good yield yet be manageable to work with since the tall strains can be over 10 feet high.

Eventually a seed industry would be necessary and this is important because farmers do not save seed as well as a seed company would. It is however not possible to develop a seed industry in 3 to 5 years.

Dr. Long added for the RAC's information that the sorghum-millet package is being developed and looks promising for cooperative work with a U.S. consortium. A lot of things need to be worked out yet but plans are working out well so far.

Dr. Lang continued addressing RAC's comments by indicating that the Agency wishes to keep the focus of this project narrow. The staff is as well anxious to see adaptation of products developed from this research adapted to India and Africa. Lysine content of pearl millet is relatively high compared to other plants but the content varies greatly throughout the world. We will watch the breeding experiments for any loss of protein or change in amino acid balance. Training is part outreach and part programmed into the project. It is difficult to build into the program and currently no funds are earmarked specifically for a training component. Prior to reading the RAC motion, Dr. Peterson asked the AID staff if ICRISAT was going to develop a global nursery and whether staff was comfortable that a major effort of the project would be to develop a high yielding synthetic variety?

With an affirmative answer from the staff on both questions,

Dr. Peterson read the motion:

Motion: That the project be approved as proposed, with the recommendation: (1) that a major initial effort be directed toward production of high yielding synthetic varieties; (2) that there be free exchange of breeding materials and information between KSU and ICRISAT; and (3) that the field outreach aspects be primarily the responsibility of ICRISAT.

Dr. Luddington seconded the motion. The motion was approved by a unanimous vote of the RAC.

THE DEVELOPMENT POTENTIAL OF NEW LANDS SETTLEMENT IN THE TROPICS
AND SUBTROPICS: A GLOBAL STATE-OF-THE-ART EVALUATION
WITH SPECIFIC EMPHASIS ON POLICY IMPLICATIONS

EXECUTIVE SUMMARY

Thayer Scudder

Institute for Development Anthropology
and
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October 1981

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EXECUTIVE SUMMARY

I. GOALS

This global evaluation of new lands settlements in the tropics and subtropics has had two major goals throughout. The first was to provide a "state-of-the-art" analysis of new lands settlement as a development option for settler, farm laborer and nonfarm families; national development agencies; and international donors. The second was to demonstrate the development implications of current knowledge in order to provide information which could be used for improving settlement design, implementation, management, and evaluation.

II. NEW LANDS SETTLEMENT DEFINED

A. INTRODUCTION

New lands settlement is defined as the spontaneous and sponsored settlement of areas which are largely uncultivated at the time of their occupation. It includes what has been referred to in the literature as "colonization" (especially in Latin America and in Indonesia prior to independence), "resettlement," and "transmigration." All these terms emphasize the settlement of land by people rather than land reclamation or land preparation as such.

Aided by international development assistance, many national governments have attempted to plan and implement sponsored settlements as one of a variety of mechanisms to realize various economic, social, and political goals. Generally speaking, sponsored settlers are selected from established communities according to a relatively narrow set of age and other criteria and then are required to follow a closely supervised program of agricultural development for the production of annual and perennial crops. To date, returns have been disappointing, while costs per settler family have increased steadily. According to the World Bank's Agricultural Land Settlement (1978b:16), "Typically, evaluation of settlement projects three to five years after the start of implementation shows economic rates of return at least 50 percent below those in project appraisal documents." (Van Raay and Hilhorst [1981:7] make the same general point.) Even in the more "successful" cases multiplier effects have not been impressive. Unfortunately few evaluations are carried out over longer time intervals so that planners tend to be unaware of those cases in which major multiplier effects have occurred and, of course, they also tend to be unaware of the nature of these effects.

Governments have also exaggerated the capacity of new lands settlements to absorb population surpluses. According to the World Bank's Agricultural Land Settlement, massive government sponsored settlement in Indonesia over a twenty-year period has absorbed only about 5 percent of the population increase in Java during the same time period, while Kenya's major settlement program over a ten-year period has only absorbed approximately 10 percent of the population increase. In Latin America, Nelson reports that new lands settlement has absorbed only 2 percent of the rural population increase (1973:198). This is the general situation.

It would be a mistake, however, to conclude that new lands settlement has insufficient potential to warrant government investment with or without international finance. A major conclusion of this evaluation has been that while planning expectations tend to be too high in regard to the rapidity with which early returns can be expected, they are too low in regard to possible long-term benefits. Throughout the tropics and the subtropics, the majority of settlers are small scale operators. This is a major benefit of settlement projects for low income rural populations. Whereas rural elites are apt to co-opt the benefits of rural development in old lands, in new lands settlements the overwhelming majority of settlers are low income to start with.

Though the term "settler" will be used time and again, it is essential to emphasize from the start that this term refers to the settler family as a production and social unit. Though this may seem to be so obvious as to not require special attention, national and international planners alike all too often write about the settler as if he was a single male, with the term "farmer" used time and again. Not only does this usage emphasize farm activities for the male head of household while neglecting the farmwife and the children, but it also emphasizes the agricultural component to the neglect of the off-farm component of the farming system, the recruitment of settlers with nonfarm skills, and the development of community diversification.

In the definition of new lands settlement, the phrase "largely uncultivated" is important since most new lands are in fact occupied by others (hereafter called the hosts) at the time of settlement or, if currently unoccupied, are almost always the subject to rights of customary use and tenure by the hosts.

In most cases, population densities tend to be relatively low. For this reason, and because the hosts also tend to have relatively low social status and little regional (let alone national) influence and power, their lands are frequently taken away without adequate compensation during the settlement process. And even if they do not lose most of their lands, rarely is a systematic attempt made to incorporate the hosts within the settlement design, hence increasing the chances of host/settler conflict.

The distinction between spontaneous and sponsored settlers refers to whether or not the settlers are self-recruited or respond to the recruitment initiative of a sponsoring agency. It has nothing to do with the reasons or motivation for leaving current residence for a settlement area.

Though government administrators with settlement experience often remain skeptical, evidence from different parts of the world suggests that generally speaking spontaneous settlers make better farmers in less time and at a lower financial cost than do government sponsored settlers. The comparison here is with pioneer settlers, that is, those who arrive during the first time phase (hereafter called the pioneer phase) of the settlement process. A range of explanatory factors appear to be involved. There is considerable evidence for example that spontaneous settlers have access to more resources than do the majority of government sponsored settlers. Most government sponsored settlers are poor. They are more apt to be landless laborers or sharecroppers than spontaneous settlers whose resources often place them above the lowest 20 percent of the sending population in terms of income. Under such circumstances, it makes sense to combine both types of settlers in the settlement process rather than favoring one type to the exclusion of the other. Indeed, the evidence suggest that without government assistance spontaneous settlement alone cannot generate a process of integrated area development.

B. TYPES OF NEW LANDS SETTLEMENT

In classifying settlement types, emphasis is placed on both the type of settler and on the nature of the involvement of the sponsoring agency or agencies. Four types are separated out for purposes of analysis, although several different types may in fact be represented in a single settlement. These are the following:

1. Spontaneous settlement with very little government or other assistance.
2. Spontaneous settlement facilitated by government and other agencies.
3. Voluntary settlement sponsored by government and other agencies.
4. Compulsory settlement sponsored primarily by government agencies.

1. Spontaneous Settlement with Very Little Government or Other Assistance

In this era of project and program planning and of national development plans, we tend to forget that inhabited portions of the world have been largely populated by spontaneous settlement. Even today the majority of settlers moving into the last frontiers of the humid tropics are primarily spontaneous, whether to locales in South America, the equatorial belt of Africa, Nepal, Indonesia, or the Philippines.

2. Spontaneous Settlement Facilitated by Government and Other Agencies

To date this type of settlement has been comparatively rare. While the evidence is impressive that spontaneous settlers time and again make better farmers nonetheless there are major disadvantages associated with spontaneous settlement. Three reasons in particular have been stressed. These are the low yields and environmental degradation associated with spontaneous settlement and the tendency of spontaneous settlers to displace the host population.

Though serious, these criticisms need to be placed in historical perspective. While systems of bush fallow or shifting cultivation (as practiced by many spontaneous settlers) have lower biological productivity than primary forests, they also provide the cultivators with the highest yields per unit of labor within the limits imposed by their current technology.

In relating to this influx of spontaneous settlers, governments have tended to emphasize one of three responses. These are (1) to condemn the process and vilify the settlers, (2) to ignore or even encourage such movement but with no provision of assistance to individual settler families, and (3) to facilitate the process of settlement. The first two responses have been historically dominant, although there appears to be a growing awareness of the need for the third.

Without assistance, most spontaneous settlers continue to be primarily subsistence farmers. Furthermore, research indicates that unassisted spontaneous settlement is not even an effective mechanism for land redistribution since over the years the vulnerability of small holders causes increasing proportions to sell out to both rural and national elites.

Accordingly a major recommendation of this study is that governments should assist spontaneous settlers if higher returns (at lower financial costs) from new lands settlement are to be achieved in the future. Assistance can take a variety of forms. All-weather

access roads and potable water supplies are crucial, as is credit and some sort of mechanism to provide the settler with secure use rights to the land in question. Access roads and potable water may be best provided through a site and service approach such as has been so successful in a number of low income urban communities in parts of Africa and Latin America.

An especially attractive approach would be for government to establish communities of sponsored settlers around which spontaneous settlers would be encouraged to take up residence. The communities of sponsored settlers initially could serve as local service centers for both sponsored and spontaneous settlers alike. They might also be used to demonstrate appropriate farming systems and other research based programs concerned with community and settlement development.

3. Voluntary Settlement Sponsored by Government and Other Agencies

Sponsored settlement has been emphasized throughout the global evaluation. Proportionately the importance of government sponsored voluntary settlement has been increasing in recent decades in comparison to settlements sponsored by commercial firms and religious organizations.

4. Compulsory Settlement Sponsored Primarily by Government Agencies

Compulsory settlement is rarely carried out for the good of the people concerned, aside from occasional instances of removal as part of a disease control program. Rather it is a by-product of larger scale events in which the future settlers find themselves embroiled.

Because it represents such an extreme example, settlement based on compulsory relocation throws into relief a number of problems which to a lesser extent characterize all types of new lands settlements. These have been studied in considerable detail in connection with dam relocation in the tropics and sub-tropics. The results of such studies have been very useful in improving our understanding of settler responses to settlement, of settlement stages, and of a wide range of issues associated with each stage.

C. THE MAGNITUDE OF CONTEMPORARY SETTLEMENT

Both spontaneous and government sponsored settlement have increased since the end of World War II. While the World Bank's 1978 Issues Paper notes that there are no reliable global estimates on the amount of new land settled during this time period, it also states that "there is no doubt that the extension of cropped area has been a major source of agricultural growth in large parts of Latin America and Africa and, to a lesser extent, Asia" (p 20), with most of the increase being rainfed cultivation.

Looking to the future, the best general summary of the situation is contained in the World Bank 1978 Issues Paper which draws heavily on FAO data. According to those data, "cultivated land in 1970 constituted about 57 percent of the world's total potentially arable land" (pp. 20-21), with over 40 percent of the estimated reserves in Latin America (459 million hectares), followed by tropical Africa with between 15 and 20 percent. Though Asia contains only about 5 percent of the global reserves, approximately 50 million hectares are nonetheless involved. While the above totals are impressive, there is generally a tendency to overestimate the agricultural potential of arable lands in the tropics and especially in the humid tropics. Perhaps over half of the above totals would be better kept in silviculture in the humid tropics and in pasturelands in the semi-arid areas and more arid savannas.

The largest areas of under-utilized potentially arable lands are in the humid tropics where approximately 75 percent of settlement is spontaneous. Presumably, at present rates of settlement much of the remaining land in the humid tropics will be occupied during the next twenty years. The same applies for the savanna environments in Africa, which are the most extensive in the world.

D. CURRENT JUSTIFICATION FOR GOVERNMENT INVOLVEMENT IN NEW LANDS SETTLEMENT

I believe that there is a greater role for government involvement than I and other critics had previously realized. The authors of the Institute for Social Studies Advisory Service (the Hague) 1981 Draft Discussion Paper, Land Settlement and Regional Development in the Tropics: Results, Prospects and Options, have reached a similar conclusion. Noting persistent inter- and intra-regional imbalances in many countries, their summary stated that "the contribution of government-sponsored land settlement to a reduction of these imbalances could be more significant than tends to be the case at present" (van Raay and Hilhorst, 1981:ii). What they mean is that there is tremendous room for improvement provided certain lessons from the past are learned and translated into new approaches to design, implementation, management, and evaluation. As another lesson from

experience, the authors of the ISS Advisory Service report also propose that "land settlement may be an attractive alternative to the further intensification of agricultural production in already settled area [sic], especially if low-cost solutions of land settlement can be developed."

III. METHODOLOGICAL DESIGN

As a comparative and longitudinal evaluation of new lands settlements, the research on which this report is based consisted of three major components. These were (1) a global evaluation of the literature on over one hundred sponsored and spontaneous settlement areas in thirty-five countries plus Micronesia and Melanesia; (2) field studies in Egypt, Nepal, Sri Lanka and Sudan by grantees funded through the global evaluation of specific settlements which have been in existence for a minimum of ten years; and (3) site visits by myself, with and without consultants, to a number of settlement areas in nine countries in Africa, the Middle East and Asia. A special effort was made to select settlements which have been in existence for at least a generation and which were considered relatively successful by administrators and scholars.

IV. SETTLEMENT SUCCESS DEFINED

Successful settlements are those that stimulate an ongoing process of integrated area development. Essential to this definition are linkages between rural and urban sectors, with agricultural development stimulating the emergence of a hierarchy of service centers as well as manufacturing and industrial development within the region. The word "ongoing" means that the development process must be sustained at least into the second generation.

This definition of settlement success as a mechanism for initiating a process of integrated area development is so important that it requires further emphasis. According to the World Bank's 1978 Issues Paper on Agricultural Land Settlement, "future settlement activities should be viewed within a comprehensive development framework which recognizes the need for careful use of all resources in the project area" (page 8). Moreover, a wide range of considerations "lead to the conclusion that settlement must be planned within an integrated regional framework which includes development of related agro-industrial and service sectors" (p. 40). In a 1978 ILO Working Paper on Employment and Income Generation in New Settlement Projects, Weitz and his colleagues (1978) conclude that successful settlement projects "must be multisectoral. Agriculture does not develop itself. It requires a complex institutional system to support it, market its products, and provide inputs, credit and professional

advice. The full capacity of employment generation in new settlement projects beyond a certain size cannot be realized unless there is a simultaneous growth of agriculture and industry. The term 'simultaneous' implies an intrinsic link between the two sectors. . . . In other words, even though a project is based mainly on agriculture, it should include as an integral part of its plan, the establishment of industries" (p. 5). Furthermore, "from the evidence brought so far it seems clear that integrated planning will bring about the best results from new settlement activities" (p. 65).

In their 1981 Draft Report on Land Settlement and Regional Development in the Tropics: Results, Prospects and Options for the Advisory Service of the Institute of Social Studies (the Hague, Netherlands), van Raay and Hilhorst come out even stronger for regional planning and integrated area development. In their opinion, without explicit linkages "between land settlement and area development, there is the real danger that minimal conditions for attaining a measure of viability cannot be met" (p. 55). And, "if there is one lesson to be learned from past failures, it is the fact that rural development is best served by a specific locational matrix of urban activities and functions. It is not the proximity vis-a-vis main metropolitan centres that matters most but rather the proximity in respects of urban and rural centres in the region" (p. 66).

As these recent statements indicate, the current consensus of those who have completed comparative studies of new lands settlements is that regional planning and integrated area development (including both agricultural areas, rural towns and regional towns) are essential for the development of successful settlement projects. Since the financial costs per settler family are high, averaging, for example, \$8,650.00 per settler family for World Bank-assisted projects between fiscal years 1962-75, major government funding is unlikely to be cost effective unless settlement is associated with major multiplier effects. While these are not frequently associated with government sponsored settlement projects and are rarely if ever associated with spontaneous settlement, area development has been attained in a small number of cases. Furthermore, the evidence is suggestive that it could have been attained in a still larger number if more attention had been paid to certain basic issues associated with the settlement and development process. These are outlined into the sections that follow.

A. SCALE

For new lands settlement to stimulate a process of integrated area development -- with a simultaneous evolution of agriculture, services, and industry -- settler families must number in the thousands rather than the hundreds. Few multiplier effects can be expected from the smaller settlements in terms of nonfarm production

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and employment. As Weitz and his colleagues state, "Obviously, the benefits of industry cannot be gained if the project is very small; a minimum volume of agricultural raw materials is required to create a market for perishable foodstuffs" (1978:6).

B. THE SPACIAL LAYOUT OF SETTLER COMMUNITIES AND THEIR RELATIONSHIP TO RURAL TOWNS

While there are obvious benefits to the settler of a homestead pattern where the family is surrounded by its fields, in terms of employment generation, the provision of services, and the facilitation of area development a nucleated settlement pattern has the advantage in most cases.

Summarizing various sources the World Bank concludes: "The interests of both settlers and their children are best served in most instances by larger, nucleated settlement. . . . The benefits are of several types -- greater employment opportunities, higher service standards, reduced infrastructure costs, reduced migration to large urban centers, and more balanced regional growth" (1978b:40).

There is overwhelming agreement that settlement projects "must be based on a hierarchy of communities" (Weitz et al, 1978:70), with settler communities linked to, in increasing order of magnitude, rural service centers and rural and regional towns. A major failing of settlement planning throughout the tropics and subtropics is the lack of attention paid to rural towns as opposed to smaller rural service centers with their cooperatives, schools, clinics and other service facilities but with virtually no industrial capacity. In over 100 cases examined during the global evaluation, rural towns were planned in connection with only eleven, and in most of these cases regional towns -- which play a major role in retaining multiplier effects within the area -- were insufficiently emphasized.

If we examine these eleven cases, the majority pertain to the 1970s, suggesting that the trend is moving in the right direction. Partly this is because of an increased emphasis on area development and regional planning. Welcome as this is, the eleven examples nonetheless relate to only five countries. Furthermore, in most cases there is a tendency to emphasize new towns rather than the enhancement of existing towns, even where suitable existing towns exist. Not only is this a more expensive undertaking, but frequently the old town will continue to out-compete the new one.

C. DIVERSIFYING FARMING SYSTEMS

There are three important reasons for diversifying the farming systems of settler families in terms of multiple cropping and the combination of the crop and livestock components. First, such systems tend to be more resilient and ecologically stable and productive economically. Second, they tend to make better use of family labor providing some farm income and status to various family members in the process. And third, they provide food for nonfarm labor and agricultural produce (including crops, livestock, forest products and fish) for processing.

Under reason one, multiple cropping, including the cultivation of a wide range of essential food stuffs, makes sense for the farm family which can then rely on their own produce where necessary. The evidence is also increasing that multiple cropping and diversification of the farming system tends to increase yields per hectare. According to Innis (1980:7), "Research on three-crop mixtures, which is closer to traditional methods, but more difficult to handle with machines, shows that the closer researchers come to traditional methods the higher the yields are for the same inputs."

As for the second reason, diversification has important economic and social equity advantages as it relates to the farm family as a production and social unit. It also better distributes family labor throughout the annual cycle by providing each family member with a variety of activities which tend to be better distributed throughout the year. As Weitz et al (1978:4) state the case for diversification: "Only through the introduction of properly planned additional enterprises into the crop pattern is it possible to fill the gaps of underemployment in the slack season of the agricultural year."

Thirdly, diversification of settler farming systems is still more directly related to area development in that it provides foodstuffs for nonfarm families and raw materials for agricultural and other industries.

D. NET INCOME OF SETTLER FAMILIES

If new lands settlements are to initiate a process of area development, far more attention need be paid to the net income of settler families than has been the case to date.

The settler family, not the land or the water resources, is the main resource, and the new lands settlement can only catalyze a process of area development if the settler family has the incentive and the opportunity to produce.

Concerning the dynamics of the settlement process, so long as

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settlers remain close to the subsistence level, it is reasonable to expect them to be risk adverse. As net income goes up, however, investment strategies change and consumption goes up, hence increasing demand for goods and services which in turn provide increased nonfarm employment opportunities. This point has been documented time and again. In their Agriculture and Structural Transformation: Economic Strategies in Late-Developing Countries, Johnston and Kilby (1975:301) note that "as per capita output in the economy rises a growing share of household expenditures are devoted to manufactured and processed commodities."

Where planners do take into consideration the multiplier effect of increased agricultural production, the conventional wisdom is that most employment generation will be in agro-industry. But what evidence is available (and only some of this applies to settlement projects) suggests that this is not the case. In their World Bank study of the Muda Irrigation Project in Malaysia, Bell, Hazel, and Slade (1980) reported that for every dollar of direct benefits generated by the project, there were eighty-three cents of indirect benefits. Of that eighty-three cents, fifty cents came from increased farmer demand for consumer goods and services rather than from production linkages (with rice milling accounting for only ten cents of the total). After recounting this case, Carroll adds that agro-industry may not be the best way to generate rural employment. Referring to a summary of research studies (in the form of a undated manuscript entitled "An Approach to Spatial Planning for Rural Development" prepared by U.S. Aid's Working Group on the Rural Poor), Carroll (1980) concluded that "small enterprises for production of local household consumption goods engaged about two-thirds of the nonagricultural labor force" (p. 15).

In the literature on new lands settlements, various authors have stressed the risks associated with net incomes which are either too low or too high. As a general proposition, target incomes both in terms of settler incentives to farm and increasing settler demand for a wide range of producer and consumer goods and services, might be best based on the "average national income" or even the "average urban income," depending on the nature of rural-urban terms of trade. Though raising target incomes in this way would reduce the number of settlers employed on the land, in the long run it would probably facilitate area development, including employment generation. Since settler incomes that are too low are a serious constraint to subsequent development, it is better to err on the higher side than on the lower side.

Almost by definition a successful settlement process will create a new rural elite among both settlers and nonfarm families. As they move from the transition to the stage of economic development, many successful settlers can be expected to pursue dynamic investment strategies as their incomes go up. The challenge for planners is to "set the stage" in such a way that settler initiative is encouraged

without being too exploitative of others. This can be done in a number of ways. Ready availability of credit for annual production needs and strong settler dominated producer and marketing organizations will help spread the benefits of settlement to a larger proportion of the settler population.

E. EMPLOYMENT GENERATION

Empirical knowledge is scant for assessing the potential of different farming systems and settlement designs for generating employment. Yet the topic is a critically important one, especially in countries with rapid population growth and high rates of unemployment and underemployment.

New lands settlement have the potential to increase three general types of employment. These are, first, employment of owner/operators and their families on farm holdings; second, the employment of permanent and seasonal farm labor; and, third, nonfarm employment. With few exceptions the planning and implementation of new lands settlements by government and donor agencies has emphasized the first type of employment while ignoring the other two types.

1. Owner/Operators and Their Families

Owner/operators and their families are the key to subsequent development including increased agricultural production, rising living standards, and employment generation including nonfarm employment.

In terms of employment generation, there is no alternative to emphasizing recruitment of settlers working small holdings versus those working medium and large holdings. Not only does emphasis on small holders increase the number of farm owner/operators, but as Johnston and Kilby note, "where income is more or less evenly distributed over broad segments of the population, the result is large markets for comparatively simple goods" (1974:304). Since the production of these requires little technical and managerial sophistication, such goods can be produced within settlement areas, hence increasing the scope for nonfarm employment.

There is an upper limit, however, to the number of small holders who can profitably be settled in a particular area. Unfortunately planners tend to forget this point — so that increasing the number of settlers beyond a certain level actually reduces employment generation since settler net incomes are insufficient to increase demand for locally manufactured goods and services, and since local production is not sufficiently great or diversified to meet the demand of nonfarm workers for locally produced foodstuffs and raw

materials for local processing, the farm enterprise becoming mainly a subsistence operation which perpetuates rural poverty rather than alleviating it.

2. Seasonal and Permanent Laborers

With only a few exceptions, it is unrealistic to expect successful settlers to continue to employ only family labor. Yet time and again settlements are planned on the assumption that settlement allotments must be cultivated with family labor. This position not only ignores the natural development cycle of the family but it also ignores the dynamic nature of settler investment strategies once Stage Three (economic and social development) begins. As net incomes rise, settlers begin to substitute nonfamily labor for family labor in regard to less desirable and/or less productive agricultural activities. This recruitment of seasonal and permanent labor occurs even on small holdings of several acres.

Furthermore, it is not in the interests of either employment generation or the welfare of farm laborers to pretend that they do not exist or to deemphasize their existence. In large-scale irrigation based settlement projects seasonal workers during the harvesting seasons may outnumber adult settlers. Though hire of laborers is less significant in regard to farming systems based on rains cultivation, even there large numbers of seasonal laborers are used during certain stages of the production cycle.

3. Nonfarm Employment

The general literature on linkages between agriculture and industry, though sparse, suggests two conclusions. First, that the potential multiplier effects of agricultural development would appear to be considerably greater than realized in terms of employment generation in rural areas and, second that national development policies must share much of the blame for the failure of new lands settlements to realize their development potential in terms of employment generation and multiplier effects.

According to the World Bank (1978a), over half of all nonfarm employment in Africa and Asia is still in rural areas — a situation which we tend to forget because of the ongoing influx of rural peoples into urban areas. Furthermore, nonfarm activities in rural areas provide a primary source of employment and earnings to approximately one-third of the rural labor force where rural towns are included (my underscoring), with this proportion rising to 40 percent where town population in rural settings increases to twenty to thirty thousand residents.

Not only do rural nonfarm activities appear to employ more people than previously expected but these activities also provide a significant proportion of the income of rural households. Chuta and Liedholm present data from six countries which show nonfarm earnings accounting for over 20 percent of the income of rural families. Estimates of 22 percent and 23 percent are presented for Korea and Pakistan, respectively, versus 43 percent for Taiwan and 70 percent for Japan. While the Japanese and Taiwanese cases represent special features nonetheless it should be possible to eventually achieve similar results in carefully selected settlement areas in the tropics and subtropics with careful planning and plan implementation.

F. NATIONAL DEVELOPMENT POLICIES

It is very difficult for new lands settlement projects to sustain themselves through time in the face of adverse national development policies and private sector policies. Where rural-urban terms of trade are unfavorable to the rural sector new lands settlements face a major constraint from the start.

The generation of nonfarm employment in manufacturing and other activities is more directly constrained where industrialization policies favor the development of large scale urban based industries through a range of direct and indirect subsidies. Government and private sector credit policies may be especially critical for both the agricultural and industrial components of new lands settlements, Katzman arguing that the increasing proletarianization of the agricultural labor force in the Northern Parana settlement area of Brazil is due in part to adverse government and private sector credit policies.

V. NEW LANDS SETTLEMENT STAGES

A. INTRODUCTION

A major goal of the global evaluation was to develop a framework which could be used for the systematic analysis of new lands settlements and more specifically for their planning, implementation management and evaluation. In attempting to explain the relative success or failure of new lands settlements which have been in existence for at least a number of years, I developed a four-stage framework. Before outlining this, a cautionary warning is warranted about the use of stages. These are merely tools for coming to grips with a complicated and dynamic process. They amount to simplifying assumptions which attempt to break the settlement process into a series of critical time periods during each of which a range of basic issues need be addressed.

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B. SETTLEMENT STAGES

The four stages cover at least a generation and are as follows:

1. Planning, Initial Infrastructural Development, and Settler Recruitment
2. Transition
3. Economic and Social Development
4. Handing Over and Incorporation

In order to be successful, a new lands settlement area must pass through all four stages though the order of the third and fourth may be reversed. These last two stages are "crucial if living standards and productivity are to rise and if continuity and development are to continue" (Scudder, 1981c:13). Though ideally a settlement area should pass rapidly through all four stages so as to realize its development potential at the earliest possible date, in fact a wide range of internal and exogenous factors are apt to interfere, so that a steady movement through the four developmental stages tends to be the exception rather than the rule. Furthermore, many spontaneous and sponsored settlements never reach the third stage of economic and social development but rather evolve directly from Stage Two to Stage Four.

In spite of such variations and various analytical difficulties, it proved to be relatively easy during the global evaluation to place different settlements within a particular stage or between two stages. Furthermore, "the very concept of stages draws attention not only to the fact that new lands settlements have histories but also that these histories are remarkably similar" (Scudder, 1981:13). It follows from this that people and the sociocultural systems in which they are imbedded and interrelated (including settlement agencies) respond to new lands settlement in predictable ways. And these responses have major policy implications.

1. Stage One: Planning, Initial Infrastructural Development, and Settler Recruitment

This stage lends itself to further division into two substages: the first relating to feasibility studies, planning, and design and the second to settler recruitment and the construction of such initial infrastructure as roads and irrigation facilities.

a. Feasibility Studies, Planning, and Design. Ideally, the feasibility studies which are carried out during this substage should consider a wider range of alternatives before a decision is made to proceed or not to proceed with a particular type of settlement. Under planning, a wide range of issues need be considered — including the scope and scale of the intended farming systems and the settlement as a whole in relationship to regional development. Weitz and his colleagues assume, for example, that multiplier effects are correlated with diversification of the farming system, farm family income, and settlement scale and scope. During the planning phase, consideration should also be given to the extent to which the hosts will be included within the settlement project on social equity, economic, and political grounds.

b. Construction of Initial Infrastructure and Settler Recruitment. The wording "initial" infrastructure suggests that infrastructural development should be phased, with planners establishing priorities for implementing in time different types of infrastructure for settler families, administrators, and other nonfarm families.

As for settler recruitment, far too much emphasis in the past has been paid to the recruitment of individual men as opposed to settler families where attention is paid to both spouses. But settler recruitment should be still more broadly linked during the planning process to the consideration of what types of production systems, what types of communities, and what types of societies are desired so that recruitment can seek out both farm and nonfarm families with the necessary aptitude/orientation, experience, and skills.

2. Stage Two: The Transition Stage

The use of the word "transition" is used to emphasize two points. First, that this is a stage of transition for settlers who in many cases are moving from one habitat to another and, second, that this transitional period must come to an end before settler families can be expected to take risks and increase significantly their productivity. While the duration of the transition stage may be less than a year for a minority of families in settlements which subsequently reach Stage Three, for the majority it would appear to last for at least two years and more often for five to ten years.

During the transition stage many settlers are risk-averse, which explains why few technical, organizational, and sociopolitical innovations are adopted at this time. Risk-aversion appears to be a coping response to the stress and uncertainty associated with moving into a new habitat — where settler families need not only come to

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grips with a new physical and biotic environment but also with new neighbors, an increased government presence in the case of government sponsored settlement, and frequently with a new host population. While "learning the ropes," most settlers adopt a conservative stance, their first priority being to meet their subsistence needs. They favor continuity over change; and where change is necessary, they favor incremental change over transformational change. Where possible, they cling to the familiar by moving into new settlements with relatives, former neighbors, and co-ethnics. They also try to transfer area-of-origin house types, farming practices, and other skills even though they may not be suited to the new habitat.

The transition stage comes to an end when enough settler families shift from a conservative stance to a dynamic open-ended one, hence initiating the third stage of economic and social development. This shift is most apt to occur after settler security is increased through the production of sufficient food to meet family needs and the settlers begin to feel "at home" in their new habitat.

At this point it is worth mentioning certain policy issues associated with the transition stage. Granted the security oriented and conservative stance of the settlers at this time, it is unreasonable for governments and donors to expect rapid increases in productivity through agricultural intensification during the first five years.

The logical way to improve project performance during these early years of implementation is to shorten the length of the transition stage. This can be done in a number of ways. One relates to settler recruitment. The advantages of recruiting settlers from different villages within the same locale and ethnic area as opposed to different ethnic areas are overwhelming during the transition stage. There are two reasons for this. The first is that neighbors and co-ethnics are much more likely to form self-help groups for land clearing and house building during the early years of settlement which so often are characterized by serious labor shortages. The second is that the potential stress and uncertainty of having to adapt to new neighbors is lessened when those neighbors come from a similar ethnic background.

Another way for governments to shorten the length of the transition stage is to make a conceptual distinction between settlement and development stages. During the settlement stage, the emphasis should be on helping the settlers feel secure in their new habitat at the earliest possible moment. Such an approach does not mean that development activities should be ignored at that time. Just as land negotiation and tenural arrangements should be completed during Stage One to expedite subsequent development, so too are there a similar range of developmental activities which can be implemented during the transition stage. These include, for example, continual provision of crucial physical and social infrastructure and

construction, equipping, and staffing of schools. Schools are especially important because one of the first investments made by settlers is in childrens' education. If schools are inadequate in number and quality, government sponsored settlers are less apt to bring their families to settlement areas, hence contributing to instability and labor bottlenecks.

Other developmental activities which can be undertaken by sponsoring agencies include fielding of a appropriate unified extension service, the encouragement of appropriate private and public sector marketing services, and setting the stage through extension and training for the emergence of settler-dominated participatory action organizations. All these activities, however, must be carefully formulated and implemented so they actually facilitate settler initiative and independence rather than promote a sense of dependency which can bog a settlement down in the transition stage for years to come.

To sum up, the early years of pioneering a new settlement area are difficult and stressful. They require a period of adaption which is rarely less than two years and usually much longer. Though timely governmental interventions can shorten the length of this difficult period of coping and transition, it cannot be eliminated, hence underlining the unreasonableness of sponsor expectations that settlers will intensify their production from the very start.

3. Stage Three: Economic and Social Development

The contrast between Stage Two and Stage Three is dramatic: the first characterized by a population of risk-adverse settlers and the second by a population of risk-taking settlers. Since the same people are involved, a dramatic change occurs.

While most settlers concentrated previously on a domestic mode of production involving extensive agriculture, during Stage Three we have observed a wide range of investment strategies designed to achieve higher levels of labor productivity through diversification of the family estate. While more data analysis is necessary, it would appear that settlers follow the same sequencing of investment activities in different parts of the tropics and subtropics. Initially they invest in education for their children. Subsequently additional farm land is sharecropped, leased, and/or purchased and the farming system is expanded into cash crops (including labor intensive, higher risk crops), while the crop component is expanded to cover livestock and nonfarm activities.

Nonfarm activities tend to start on the farm homestead, taking the form of small business enterprises such as crafts, baking, and tailoring which are located within the home. Subsequently, investment

expands to nonfarm activities off the homestead but within the settlement area, with these including small general stores and transport for hire in the form of two- and four-wheel tractors, trucks, taxis, and mini and other buses. Still later, investments may be made in urban real estate and businesses.

As incomes go up, many settlers prefer to hire laborers for a increasing proportion of agricultural tasks. Especially in irrigated settlements in Africa, Asia, and the Middle East, the number of seasonal and permanent laborers may exceed the number of settler families.

Farm diversification and increasing net income among settlers also facilitate the development of commercial and service centers which process the produce and serve farm and nonfarm family needs.

4. Stage Four: Handing Over and Incorporation

a. Handing Over. Because of the observed inefficiency of long-established national and special project settlement agencies and because of the frequently negative impact of educational systems on the willingness of settler children to continue farming, I do not consider any settlement to be a success until a degree of handing over control to settlers and other local institutions has occurred and until a second generation of settlers has taken over. Handing over activities to departmental, local government, and settler organizations is a tricky business which can proceed both too rapidly and too slowly. On the whole, however, the problem in the postcolonial era is that settlement agencies retain for too long a period a wide range of activities which could be more efficiently carried out under a policy of devolution to local organizations. Since it is natural for bureaucrats to endeavor to perpetuate themselves in space and time, the problem of inefficient national and special settlement agencies is a major one during the later stages of settlement projects. Indeed, it is so major in some cases as to possibly offset the undeniable advantages of such centralized and hierarchical organizations during the initial stages.

Because of the nature of the educational system and the propensity of settler families to invest in the education of their children, a number of older settlements are having difficulty in passing on farm activities to the children of settlers as the first generation retires.

b. Incorporation. Incorporation refers to the process whereby a new lands settlement become an integrated part (rather than a special enclave) of the region within which it is situated. Physical handing over alone is not sufficient. The incorporating

agencies must have the personnel and capital resources and the will to take over essential settlement services so these services do not subsequently break down.

Part of the problem is political incorporation, since settlement organizations will not be able to compete for regional resources after handing over unless they are integrated within the political economy of the region. So incorporation has a number of aspects which extend beyond the process of handing over. Furthermore, if larger and more diversified new lands settlements are to realize their potential for catalyzing a process of regional development, incorporation must enable the settlement area to play a major role in influencing regional policies and the implementation of those policies.

VI. BASIC ISSUES ASSOCIATED WITH STAGE ONE [Planning, Initial Infrastructure Development, and Recruitment]

A. INTRODUCTION

Each settlement stage is associated with a wide range of basic issues which must be addressed by planners, administrators, and settlers. Though their proportional importance may shift through time, certain issues characterize all stages; others are primarily associated with a single stage.

To avoid repetition, certain major issues (like settler net incomes, employment generation and multiplier effects) which have been already assessed are not dealt with again except in passing. Other important issues which have already received considerable attention elsewhere in the literature are also not emphasized.

B. PLANNING

1. Keeping the Plan as Simple as Possible

Evaluation after evaluation has emphasized the need to carefully prioritize interventions, stressing a relatively small number of "projects" at a given time in order to realize more complicated program goals.

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2. Keeping Financial Costs per Settler Family Within Reasonable Limits

Financial costs are broken down into capital and recurrent expenditures, both of which can be significantly reduced by following courses of action which should actually enhance possibilities for success rather than reduce them. The paragraphs that follow are not meant to be inclusive; rather their purpose is to illustrate a range of policy options which could reduce costs without jeopardizing the chances for project success.

a. Settlement Type. Financial costs go up as the proportion of sponsored settlers increase relative to hosts and spontaneous settlers. According to Judith Tendler, the incorporation of spontaneous settlers within a portion of Brazil's Alto Turi project nearly halved costs per settler family by increasing the number of beneficiaries, on the one hand, and by decreasing the costs of land allocation and settlement per beneficiary, on the other.

b. Location of Settlement. In discussing lessons learned, van Raay and Hilhorst (1981) emphasize that "market proximity is the major determinant of the economic viability of a land settlement scheme, the general rule being that the highest net income per ha can be realized nearest the market centre" (p. iv).

c. Involvement of the Private Sector. Because of the complexity of integrated area development and its financial costs it makes sense to involve nongovernmental organizations in the development process from the very start. As in Malaysia, private lumber and building contractors can play a major role in clearing settlement areas of timber and in constructing major infrastructure. Such private companies might also be willing to shoulder more of the financial costs of settlement if assured of some of the benefits with lumbering companies, for example, contributing to access and feeder road construction in return for lumbering rights.

Joint ventures between government settlement agencies and commercial companies are another way for organizing a type of cooperation between the public and private sectors that would facilitate land settlement. Such ventures could involve forest product and mining companies as well as agribusinesses. Here I am not referring so much to the provision of infrastructure as to the actual settlement of people around the margins of a forest product, mining, or agribusiness enclave — with settlers providing produce to the enclave both as food and, in the case of forest product companies and agribusiness, as products for processing.

There are also other ways in which the services of the private sector can be used both to reduce financial costs to the government

and to provide a range of management and other services. Current government policy in the Mahaweli Basin of Sri Lanka in connection with the Accelerated Mahaweli Programme is of special interest here since the Mahaweli Authority of Sri Lanka is experimenting with a number of mechanisms for involving the private sector. For example, the MASL has arranged for Hatton National Bank Limited to have exclusive lending rights to settlers in part of System H, while the Ceylon Tobacco Company, Limited, has recently begun to manage H-9.

d. Worker/Settlers. Periodically, attempts have been made to recruit worker/settlers who will clear and prepare their own lands for cultivation, and construct the infrastructure serving those lands.

Worker/settlers often arrive without their families simply because living conditions tend to be extremely difficult. Because of these difficulties and because worker/settlers often come alone, every effort should be made to ready the land for cultivation and family occupancy during the first year; otherwise the hardship and suffering of worker/settlers is apt to be reflected in low morale, increased illness, suspicion of government intentions, and high "drop-out" rates.

e. Housing. Roads and government built permanent housing tend to be the two largest single costs associated with settlement based on rainfed agriculture. While the former are necessary, the latter are not. In addition to high financial cost, government provided housing often is both culturally and sociologically inappropriate. It may constrain family activities and the normal developmental cycle of the family because of regulations as to how the housing and the house plots are to be used. Permanent housing also tends to be associated with relatively small house plots on which it is not possible for the settler's heir to build his/her own housing so as to be near aging parents. In effect, government provided housing locks the social organization of the settler family into "concrete," so to speak, while the size of the household plot more often than not is inadequate for the keeping of animals and the planting of household gardens. For all these reasons, it makes sense for settlers to build their own housing wherever possible.

f. Roads. Time and again roads are the major capital expenditure associated with new lands settlement. In Latin America, for example, they accounted for 38 percent of public expenditure in connection with fourteen settlement projects assessed by Nelson. It is best to recognize such high costs from the start before asking in what ways they can be reduced. When cost reductions are then considered, a number of possibilities come to mind. These include location of settlement areas as close as possible to settled areas and major market centers and involvement of the private sector.

g. The Phasing of Infrastructure. The phasing of infrastructure has two major implications in regard to financing of new lands settlement. On the one hand, it can postpone certain major capital expenditures until a later time phase of the settlement process; on the other hand, it may provide a source of income for partially financing subsequent infrastructural investments.

h. Facilitating the Development of Existing Rural Towns. We have already noted the propensity of planners to create new rural towns from scratch, often with unsatisfactory results. Granted the undeveloped state of the art in the planning of new townships, it makes far more sense to stimulate the development of existing rural towns if such exist. Though no comparative data exists, I presume such an approach would also be significantly cheaper financially.

3. Feasibility and Planning Studies for Siting New Lands Settlements

Because new lands settlements are situated in relatively unknown areas there is no substitute for feasibility studies for considering possible development options and for planning studies to explore particular options in more detail. Such studies can be divided into two broad types, the first dealing with the physical and biotic environment and the second with the host and prospective settler populations. Time and again settlements are planned and implemented without adequate information on the physical and biotic environment. Time and again a major reason for their subsequent failure or inability to realize their development potential is due to the failure to carry out appropriate climatic, hydrological, and soil surveys or to utilize available data. Socioeconomic surveys of the hosts should provide data on their numbers, their systems of land tenure and land use, their water rights, and, to an extent, their socioeconomic systems. Surveys of the numbers and lifeways of the host population are needed to establish the total population that will be impacted upon by a possible settlement project and to assess their attitudes toward being incorporated should settlement proceed. Studies of land tenure and water rights are needed to define host concepts of tenure according to customary law. It is fair neither to the hosts nor to the settlers to ignore customary tenure, since future land disputes can jeopardize the entire settlement process. As for the study of host systems of land (and water) use, these can yield invaluable information on the resources of the area and how to utilize them.

Information on prospective settlers has two major uses. First, it can provide data of use in planning and implementing the settlement itself. Second, it can provide information of how the emigration of a significant number of people from a particular locale can be used to facilitate the development of that locale.

C. PLANNING FARMING SYSTEMS

1. Introduction

Agricultural diversification in terms of the integrated planning of farming systems, fisheries, and silviculture is a rare feature of settlement projects. The same applies even to farming systems diversification, both in connection with diversification within a particular farming system and between farming systems. Throughout the tropics and subtropics, new lands settlements have been planned and implemented as agricultural production schemes based on a relatively small number of crops for export and domestic consumption in that order of priority. Yet diversifying agricultural systems and, more specifically, farming systems increases the development potential of new lands settlements. In the discussion that follows, the need for diversification must continually be kept in mind.

2. The Need for Research

There is no substitute for research for agricultural development. Simply because so little is known about new lands settlement areas, this must start at the earliest possible moment. Because most new lands settlement areas will be colonized by small holders, agricultural research should be oriented toward the creation of more productive farming systems. While I am not suggesting that conventional crop research be deemphasized, I am suggesting that every research station should include an area which simulates in size and other conditions the different kinds of settler holdings.

Regardless of focus, all research programs both on the research station and in the field should be both comparative and longitudinal. Researchers should also be on the lookout for "breakthrough" possibilities which could significantly alter or even revolutionize small holder farming in existing settlement areas, and which could open up new agro-ecological zones for settlement.

To sum up, I am suggesting that agricultural research stations in new lands settlement areas not only place more emphasis on appropriate farming systems research, but also that they serve as the institutional base for carrying out a broader range of research relating to the development of agricultural systems. For example, serious consideration should be given to basing monitoring and evaluation activities at agricultural research stations, with the necessary facilities attached. Since effective extension must be research based, it also makes sense to place training facilities for both settlers and extension staff close to research stations.

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D. PHASING INFRASTRUCTURE

A common characteristic of settlement agencies is their attempt to provide instant infrastructure from scratch. Not only is this a very expensive procedure but, also, it seldom works. For the majority of settlers, the first few years tend to be the most difficult. At that time the critical items of infrastructure would appear to include potable water and certain disease control programs, access roads, primary schools, and in the case of irrigation projects the timely completion of the irrigation infrastructure and of land preparation so to ensure the timely delivery of water in the right amounts to the settlers.

Since settler health tends to suffer during the initial years, a clean supply of potable water is a major need which, in fact, is rarely supplied.

All weather access roads service a variety of needs. First, they provide access not just for the settlers but also for a wide range of essential goods and services. If access roads are adequate, private sector entrepreneurs are more apt to move into the area to provide transportation facilities, and to build general stores and other retail outlets. Second, access roads provide exit routes. Their very existence reduces the degree of isolation for the settlers. Like access roads, the early provision of primary schools also encourages settlers to bring their families and to remain in settlement areas during those initial years of hardship. As for the timely provision of water in irrigation based settlements, that is a crucial input which time and again has been delayed with very detrimental impacts on settler morale, departures rates, and relationships with the settlement and other government agencies.

From the settler point of view, the need is much less urgent to provide secondary schools, a hospital, postal and banking services, and other types of infrastructure during the first five years of the transition stage. Such items are far more important from the point of view of administrative staff and other government and private-sector nonfarm personnel. For them, however, it is both cheaper financially and quicker to upgrade existing facilities in established rural towns than to build new towns.

E. SETTLER RECRUITMENT AND POLICY

1. Introduction

Pioneer families tend to be relatively young, often with only one or two small children. Over the years family size can be expected to increase significantly, usually exceeding the national average in terms of the number of children. The planned size of the household plot should take into consideration the needs of this growing family unit, including the probability that in their old age the first generation of settlers will wish their heir to build on the same plot during the period of handing over.

2. Settler Mix

The global experience is that there are more than enough good candidates for sponsored settlement. The desired nature of the mix will vary between countries and from one agro-ecological zone to another. Generally speaking, in countries with large areas of underutilized humid rain forests and with heavily populated old lands with a significant proportion of landless farmers, spontaneous settlers tend to outnumber sponsored settlers by three or four to one. The proportion of spontaneous settlers tends to be significantly less in areas with rainfall deficiencies during the main cultivation season.

If planners are aware of the history of spontaneous settlement within the different agro-ecological zones of a particular nation, obviously they are in a better position to forecast the possible response of spontaneous settlers to the opening up of new areas and, based on such estimates, to work out the settler mix. What the mix should be will also depend on other factors. Since financial costs per spontaneous settler family tend to be lower, this factor alone may be significant -- although a site and service approach to sponsored settlement can also reduce settlement costs. Another factor relates to whether or not sponsored settlers come from within the settlement area or are hosts. In the latter case, the global evaluation suggests that the best strategy is to incorporate them within the settlement area if they are willing.

A major conclusion of the global evaluation is that far more emphasis should be placed on facilitating spontaneous settlement and combining it with sponsored settlement as a mechanism to tap into the initiative of spontaneous settlers, to diversify settlement areas in terms of occupational specialization, and to cut the financial costs of the settlement process.

3. Recruitment

Where new lands settlements are a major development intervention, serious thought should be given to formulating a national set of recruitment criteria. While desirable criteria will vary, some generalizations appear valid. Of these perhaps the most important is to recruit settlers as families rather than as individuals. Another is to use a formal point system whereby both spouses are evaluated according to desirable criteria with recruits being those families with the highest number of points.

Time and again settlers are interviewed by sponsoring agencies or local leaders as if they did not have wives or families, and with little effort made to learn if wives wish to move -- and if they do, as to whether they have appropriate skills. Special planning is needed to correct this situation. As a starting point, it makes sense to recruit only families in which both spouses wish to become settlers. As for establishing a point system, not only does that require more careful thought about the relative merits of different criteria, but it also reduces the possibility of favoritism within the selection process.

Though it is far more difficult to generalize about the desirability of specific criteria, and very difficult to weigh them in relationship to each other, even here the global evaluation has led to some tentative conclusions. Most frequently emphasized are health, education, skills, background, and number of children. Granted the hardship associated with the initial years of settlement, good health is clearly important. On the other hand, the correlation between education and becoming a productive settler is not clear in spite of a good bit of attention to this factor in the research literature. Ideally, sufficient education to be able to read simple instructions and to keep simple accounts makes sense. Other than that, what appears to be more important than the number of years of education is the type of education and the expectations associated with being educated.

If new lands settlement is to initiate a process of integrated area development, obviously people must be recruited with both farm and nonfarm skills. This applies at all community levels, since even small settler communities need barbers, carpenters, masons, blacksmiths, curers, midwives, religious leaders, and other skilled personnel. Background relates more to occupational and class background. Throughout the tropics and subtropics, we found that sponsored settlers were overwhelmingly low income rural residents. In this sense, sponsored settlement is an effective mechanism for assisting low income populations.

The fifth criterion relates to settler family size and more specifically to number of children, a criterion with which most settlement planners are concerned. They are faced with a relatively

difficult choice. While younger couples can be expected to be in better health, and hence more capable of dealing with the early rigors of settlement, older couples with more, and older, children will have a larger labor force of family members during those same critical years. One factor that tends to be ignored are the sociological implications of a settlement population which initially contains very few three-generational families and, in comparison to old lands, a very small proportion of older people. Assuming that a broader mix of people of different ages is desirable, planning consideration could be given to actively recruiting older couples to provide the necessary nonfarm occupational skills. Older women, for example, could be recruited as midwives and older men as carpenters, masons, and blacksmiths. Older couples could also be recruited as health practitioners and religious leaders.

4. Middle-Class Settlers

Periodically governments and settlement agencies have experimented with the recruitment of middle-class settlers, usually as a minority within a settlement dominated by lower-class settlers but occasionally within their own settlement. One or two reasons tend to be used to justify a policy incorporating middle-class settlers. The first is that they will make more successful farmers. The second is that they will provide leadership within the settlement.

There is no evidence that middle-class settlers make better farmers. On the contrary, what evidence is available suggests that yields per hectare generally speaking are lower on middle-class allotments than on peasant holdings within the same settlement.

As for providing leadership, the issue is more complex. Though Farmer (1957) notes that middle-class settlers in Sri Lanka "have on the whole done little or nothing to provide any form of leadership for nearby peasant colonists," small holders at Tahaddi (Egypt) told members of the Pacific Consultants team that graduates were useful in pressuring the settlement authorities to live up to their responsibilities in terms of operating the irrigation system and providing inputs. At Way Abung, an Indonesian transmigration settlement in Sumatra, middle-class settlers had been instrumental in establishing a senior secondary school and other social services which were then available to all settlers. On the negative side, however, is the tendency for middle-class settlers to dominate positions of leadership not just on school boards but also within cooperatives and other production and marketing oriented settler organizations, becoming a new rural elite in the process which impedes the subsequent development of more broadly based settler organizations.

Against this background, there appears to be little justification for combining middle- and lower-class settlers in the

same settlement. Rather settlements of small holders should produce their own leaders, a conclusion which Farmer reached in the 1950s after his analysis of settlement in Sri Lanka.

5. Exclusions

Though the evidence is overwhelming that new lands settlements benefit the poor, nonetheless exclusions occur on sociopolitical grounds. Most frequently such exclusions pertain to host populations, although they may also be more specifically ethnic. Though exclusions may be justified in some cases, these would appear to be the minority. Furthermore, where the hosts are excluded, future conflicts can be expected to threaten the viability of the settlement process. Though the international community of donors has the opportunity to at least question (if not influence) exclusionary policies, rarely have they done so.

6. Settler Homogeneity

Though governments continue to see new lands settlement as a mechanism for integrating and nationalizing a heterogeneous population, the evidence appears overwhelming that settlers prefer to live and work with co-ethnics and that ethnically homogeneous settler populations facilitate cooperation, reduce potentially disruptive conflict, and are a contributory factor to a shorter transition stage.

Cooperation is especially crucial during the early years of the settlement process when settler families often have to clear and prepare their land, build temporary homes, and plant and care for their farms under unfamiliar conditions. Self-help groups for alleviating labor constraints are formed more often among co-ethnics than among settlers from different ethnic groups.

While there is very good evidence to back up the recommendation that co-ethnics from the same locale be settled within the same community, one advantage of large scale settlement is that there is room for a range of ethnic groups within the settlement as a whole. In terms of spatial arrangements, it makes sense for co-ethnics to be clustered around their own rural service centers. Mixing between adult members of different ethnic groups would then occur at the next level in the settlement hierarchy — that of the rural town where their children, for example, would mix in junior and senior secondary schools.

7. Land Acquisition, Land Tenure, and Land Use

a. Land Acquisition. In terms of fairness to both hosts and settlers alike, land acquisition must be carefully undertaken before the first settlers arrive so as to reduce the incidence of subsequent land disputes. Even then, some disputes are almost inevitable. In spite of this, formal land acquisition and adjudication policies tend to be neglected by settlement planners until after conflicts occur.

b. Land Tenure and Land Use. No generalizations are possible as to the relative merits of individual versus communal control and cultivation of land. Rather the key factor is working out a form of tenure which provides sufficient security to the settler family to encourage members not only to maintain their allotment but also to make permanent improvements, and to develop a form of cultivation which the settlers support. Within these limits many possibilities exist — including family cultivation and control of land, family cultivation and settlement agency control of land, family cultivation and communal control of land, and communal cultivation and control of land. There is little doubt, however, that the majority of settlers in the tropics and subtropics prefer family cultivation and control of land. So do the majority of settlement scholars who expressed themselves on this matter.

Settlement agencies, on the other hand, tend to shy away from granting titles to settlers (even where promised), preferring tenancy type arrangements based on annual or longer term leases which theoretically can be terminated at the discretion of the settlement agency. This preference for tenancy arrangements and for long term purchase options can be largely explained in terms of two government concerns. The first is a concern for maintaining certain agricultural production goals -- goals which the settlement agency fears will not be met if settlers have full title to their land. The second is concern that settlers will sell their land to speculators, hence interfering with social equity goals -- or they will subdivide it among heirs, hence interfering with production goals.

Both of these concerns appear exaggerated when compared with the problems associated with lack of settler security over land tenure. Productivity, for example, is more apt to suffer where the settler has a disincentive to produce and to make permanent improvements because of tenural insecurities, while subdivision may occur because of inability to obtain credit -- many institutional donors requiring land title for collateral. Finally, on a disproportionate number of the more successful settlements, settler families own their land.

For such reasons as the above, settlement scholars tend to favor granting land titles to settlers. While they also suggest that safeguards be institutionalized to reduce land sales and subdivision,

I suspect that the best measures are ready availability of credit and the development of nonfarm employment for absorbing the second generation.

8. Target Income and Settlement Pattern

Though target incomes need be carefully thought out in each case, a starting point for consideration is either the average national income per employed person or the average income per person employed in the rural sector.

As for settlement pattern, the general consensus favors a nucleated settlement. Although a dispersed pattern does not preclude integrated area development, as shown by the Northern Parana case, it does make it more difficult to provide a wide range of production oriented services as well as social services.

9. Size of the Household Plot

A strong argument can be made for not reducing the household plot below a size which can support various economic activities for various family members (especially the wife), which can accommodate some family livestock, and which will allow extra rooms to be added as new family members appear.

VII. BASIC ISSUES ASSOCIATED WITH STAGE TWO [The Transition Stage]

A. THE DROPOUT PROBLEM: ILLNESS AND INDEBTEDNESS

During the early years of settlement, it is not unusual for relatively large numbers of both spontaneous and government sponsored settlers to drop out. Though there is no quantitative data as to why settlers leave their new homes, case studies indicate a variety of reasons. Of these, misfortune appears to be a more common explanation than deficiencies on the part of the settler family. Two types of misfortune are mentioned time and again. These are illness and indebtedness, with the first not infrequently leading to the second.

Indebtedness can occur for a variety of reasons, including illness and death, crop failure, such social events as weddings, and fiscal mismanagement. Because of the general absence of other forms of credit, indebtedness is usually to local moneylenders. Though their credit is better than none, it is usually provided at very high interest rates so that debtors may find it virtually impossible to

meet their debt servicing responsibilities, with the result that they either sell out or have their land taken over by their creditors.

B. DEPENDENCY AND SUBSIDIZATION VERSUS PAYING FOR DEVELOPMENT

1. Dependency

While spontaneous settlers frequently suffer because of inadequate government assistance, the amount of assistance and the way in which it is delivered to sponsored settlers may cause them to become dependent on the settlement agency. Dependency is undesirable for a number of reasons. First, it delays the arrival of Stage Three and reduces the development potential of new lands settlement by curtailing settler initiative. Second, where settler organizations do form, there is the danger that their activities will be disproportionately concerned with settlement agency-settler organization relationships. Third, settler dependency increases the financial cost of settlement since the settlement agency must retain a large staff and continue carrying out a range of activities which could otherwise have been handed over to local management. The best way to avoid these disadvantages is to attempt to involve the settlers in settlement decisionmaking and management at the very start.

2. Subsidization, Food Aid, and Paying for Development

While subsidization of settlers should be kept to the absolute minimum at all times, during the initial years of settlement special assistance may be necessary. This is especially the case in regard to worker/settler programs where the settlers need shelter, water, and food while preparing the settlement area. It also applies to cases, of which there are many, where it is unlikely that settlers will be able to meet their food needs during the initial months or years of settlement.

In all such cases planners should carefully assess ways to help the settlers become self-sufficient at the earliest possible moment with the least danger of a settler-settlement agency dependency relationship developing. Special attention should be given to procedures which enable the settlers to plant customary food crops while the official farming system is being developed. More often than not, however, governmental aid will be necessary until the first adequate harvest occurs. A number of options are available here including food aid (both national and World Food Programme), wage labor on the scheme, and subsistence allowances until settlers are self-supporting. Where there is a choice, in my experience government provided food for work produces the best results, provided it is distributed in a timely fashion. The trouble with a food allowance is

that family health may suffer if there is insufficient food for local purchase prior to the first harvest, if food prices are seriously inflated, or if the allowance is spent for other purposes. As for wages, the risk there is that the settlers may come to see themselves as laborers on a government farm rather than as owner/operators preparing their future holdings.

While some form of food aid/wages/allowances usually are necessary for a while on sponsored settlements, other types of subsidization should be avoided wherever possible. As a general proposition, settlers should be taxed to the extent that at the very minimum they pay for recurrent project costs. Whether or not they can be expected to eventually repay the government for capital investment will depend on the nature of that investment. As for recurrent costs, these should be covered by land development and/or water taxes which are carefully explained to the settlers from the start.

C. ORIENTATION

Whether in the form of orientation or extension, settler training is one of the weakest aspects of government sponsored settlement programs. Orientation virtually never occurs; in fact, among our cases I am aware of only one where a carefully thought out orientation program has been executed which is separate from extension. This is the San Julian (Bolivia) settlement orientation program. Heads of newly recruited settler families are brought together to the settlement area where they work communally to prepare the land that subsequently they will cultivate as individual families, and to build family housing. Throughout the four-month period they receive orientation and special training.

D. EXTENSION

Throughout the tropics and subtropics the large majority of settlers are unfamiliar with their new habitat at the time of their arrival. Clearly if they are to avoid costly mistakes and a lengthy period of adaptation, both orientation and extension are crucial. We have already seen that orientation programs are virtually nonexistent in regard to new lands settlements. As for extension, of the thirty-six government sponsored settlements on which we have sufficient information, in nineteen cases (53 percent) extension services were either nonexistent or minimal, and in only four cases (11 percent) were they good to excellent in quality (and in one of those cases they were inadequate in regard to availability). In regard to ten spontaneous settlements on which we have adequate data, on nine (90 percent) extension services were either nonexistent or minimal.

In spite of the fact that most government sponsored settlement areas are planned and implemented as agricultural production schemes, data from the global evaluation show that most of the necessary early inputs into the implementation of viable farming systems are absent most of the time. These include soil surveys and research based extension services.

Though there is no easy solution to the extension problem, some guidelines can be given. First, the advice extended must make sense economically as well as technically in the context of the settlers' farming system or systems. Second, advice must be presented to the settler family in a consistent fashion and in the right way. The logical approach to the second guideline is a unified extension service with one field agent responsible for advising settlers in regard to the entire farming system (with back up advice provided by specialized technical officers at the district, section, or irrigation system level). Presenting extension advice in the right way refers not just to how the extension agent approaches the settlers but also to the sex of the agent. A frequent planning weakness of new lands settlements is that planners are apt to put more stress on the farmer rather than on the farm family. Not only is the agricultural role of women apt to be ignored, but agricultural extension personnel often are exclusively male.

A third guideline relates to terms of service for extension personnel -- whose morale is frequently low because of inadequate salaries, inadequate opportunities for advancement, inadequate housing, and inadequate local transport.

E. COURSES FOR SETTLERS AND TRAINING COMMUNITY EXTENSION AGENTS FROM AMONG SETTLER FAMILIES

The British introduced farmers' training centers in their former colonies, while the French paid special attention to training unpaid village volunteers who were selected by their fellow village farmers. Both types of training are applicable to new lands settlements though the actual mix will depend on the nature of the settlement as well as on the nature of any orientation and extension programs.

F. LOCAL PARTICIPATION AND SETTLER ORGANIZATIONS

It is becoming increasingly clear that project success is associated with active local participation. In their Strategies for Small Farmer Development: An Empirical Study of Rural Development Projects, Development Alternatives (1975) stated as their primary findings that "to maximize the chances for project success, the small

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farmer should be involved in the decisionmaking process and should also be persuaded to make a resource commitment to the project" (Executive Summary:1). Generalizing for irrigation projects, Radosevich states that "at the local level, countries with the most successful irrigated agriculture have adopted some form of Water User Association" (1979), the effectiveness of which appears to increase where federated up to the irrigation systems level or within a river basin. Dealing more specifically with settlement, there is the impressive accomplishments of the San Juan flood refugees in Bolivia, whose local action greatly facilitated their settlement and served as a model for the San Julian orientation program.

Granted the lack of social integration which so often characterizes new lands settlements, settlement agencies should be prepared to facilitate the development of settler participatory organizations; indeed, the importance of these in terms of stimulating development and avoiding dependency is such that their creation should be built into the enabling legislation establishing settlement authorities.

Especially important are a wide variety of training programs for training leaders and staff of community farming associations, water user associations, and cooperatives. Here a word of warning is needed since there is a danger that such training programs may separate the trainee from his or her peers who subsequently view the trainee not as representing their interests but rather the government's. As for the scope of settler organizations which are encouraged by government and other agencies, it is especially important to not overload them with too many functions.

G. SHORT AND MEDIUM TERM CREDIT

The issue of credit is one of the most difficult policy issues associated with new lands settlement areas. Though there may be no correlation, nonetheless on settlement projects where credit is easily available through government channels, there is also apt to be a degree of settler dependency which may retard fiscal responsibility and initiative within the settler family. On the other hand, case after case shows that inadequate institutional credit can cause settlers to lose their land, especially following a wide range of misfortunes. In other words, there are special circumstances where credit is needed if otherwise satisfactory settlers are going to survive as settlers.

1. The Type of Credit

For settlers, the primary need is for short term credit during the first few years, although medium term credit may be equally important where farming systems are based on animal traction. With most farming systems, however, the need for medium term credit increases as the focus of the settler family shifts increasingly from production for consumption to production for the market. This trend should not conceal the probability of an ongoing need for short term credit not just to deal with shortfalls in production and family misfortunes but also to deal with such seasonal activities as purchasing fertilizers and pesticides and recruiting labor for weeding, harvesting, and other activities.

As for small and medium scale entrepreneurs, their need is more apt to be for medium term credit to start up businesses, although small provisioners may need credit for replenishing inventories.

2. Individuals as Sources of Credit

A major function of local elites is to provide credit. Though they provide an important service here in the absence of alternate sources of credit, it is usually provided in a patron-client relationship which often enables the patron to profit at the expense of the client. Another noninstitutional source of funds, and one which should be encouraged since it helps develop fiscal responsibility, is the informal rotating savings association or tontine.

3. Institutional Sources of Credit

There are many possible institutional sources of credit, which can be divided into four general types. These are settlement agency sources, other government agencies (such as agricultural banks and agricultural finance corporations), private banks, and settler organizations.

a. Settlement Agencies. Though one of the more reliable sources, settlement agency credit is not without its problems. First, it tends to be tied to a limited number of cash crops in which the settlement agency has a major interest. Often these are export crops, with the ready availability of such limited credit interfering with farming system diversification. Second, the credit may only be available for a limited range of activities. Third, restrictions on availability, or on funds, often curtail the credit to a relatively small proportion of settlers even in project areas with a major credit

component. Fourth, the ready availability of credit through centralized or even decentralized settlement agencies can increase settler dependency at the expense of settler initiative.

In spite of such limitations, settlement agencies are a crucial source of credit during the start-up period of new lands settlements, when other types of credit institutions are either hesitant to become involved or, in the case of settler institutions, are not yet sufficiently organized.

b. Other Government Agencies and Private Institutions.

Throughout the world a variety of national government agencies make available credit to small farmers. The main limitations associated with such credit are the lengthy bureaucratic procedures which the settlers must follow and the limited funds available. Whether in the form of cash or kind, the credit may be received after the deadline for its use. The possibilities for involving private banks in credit programs for new lands settlements are greater than usually realized, with the result that they are an underutilized source of funds. Private banks tend to hesitate to become involved with small holder agriculture for a variety of reasons, including lack of collateral to secure a loan, the administrative costs of processing many small loans as opposed to a smaller number of larger ones, and low repayment rates. There are imaginative approaches, however, which can be used to solve these problems such as providing loans only to settler organizations rather than to individuals. In Sri Lanka, Hatton National Bank, Ltd. has been given a monopoly on supplying credit to small holders in H-5 on the basis of an earlier experiment during the 1970s whereby they provided credit to farmers on a small scale settlement.

c. Settler Organizations. The global evaluation suggests that one component that not infrequently is associated with more successful settlement areas is settler organizations which provide credit for their members. Possibilities include savings and thrift associations, cooperatives, water user associations, and community farm associations.

4. Interest Rates

These must be attractive enough to secure the participation of private banks and ensure the fiscal viability of other institutions allocating credit. What evidence is available suggests that settlers are willing and able to pay such rates, especially if we bear in mind their willingness to receive credit from individual moneylenders at much higher interest rates.

A word of caution, however, is needed here which once again emphasizes the initially poor information base dealing with new lands settlement areas and the high exposure to risks from crop failure during the first few years. Such risks need be more carefully calculated by planners. Where they are especially high, the need may be more for a food aid program during the first few years than a major credit program, although again some settlers will need credit to cope with such special circumstances as the severe illness of the family head.

5. Repayment

Repayment rates tend to be better where settlers are required to market their crops through the project and where incentives to do so are high enough to forestall the development of too large a black market. Repayment also would appear to be better where loans are given through a settler organization which vouches for loans to members.

In sum, data from the global evaluation supports the Development Alternatives conclusion that factors associated with a good repayment rate include "group rather than individual credit liability; and compulsory marketing through an organization established by the project" (1975:24). Under such circumstances, however, it is important that credit be available for farming systems diversification rather than just for one or two crops.

6. Eviction of Settlers

Even where careful recruitment procedures are followed, inevitably some settler families will prove to be unsatisfactory farmers. This should be anticipated, with minimum standards of adequacy carefully worked out and explained to settler families as they are recruited.

VIII. BASIC ISSUES ASSOCIATED WITH STAGES THREE AND FOUR [Economic and Social Development; Handing Over and Incorporation]

A. MANAGEMENT

1. Introduction

A major conclusion of the global evaluation is that new lands settlements cannot stimulate a process of integrated area development

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without major external assistance — the lack of such assistance being perhaps the major reason why spontaneous settlement has been so unsuccessful as a development intervention. The primary source of such assistance is government agencies.

Having emphasized the need for government assistance, it is also important to emphasize that inadequate government assistance and management capabilities may also become the major constraint facing settlement development. Partly for this reason it is important to stress the need for combining government initiative with local participation, and with private sector and private voluntary organization cooperation. Creating viable new lands settlements is a complicated ongoing task, with case after case indicating that where government agencies attempt to go it alone, they are apt to become a constraint on the very development that they are supposed to foster.

2. Centralized and Autonomous Government Management Agencies Versus Coordinating Agencies

The conventional wisdom is that large-scale settlement projects are best carried out by autonomous specialized or national settlement agencies. While such centralized agencies are parastadals established outside the normal departmental structure through special statute, coordinating agencies tend to be incorporated within a particular government ministry or department.

a. Parastadal Management Agencies. In theory parastadals have the flexibility to plan, implement and manage through time the complicated components associated with settlement and integrated area development whereas government departments do not. At least at the beginning they also are apt to have considerable political support which is translated into funds, personnel and influence. Powerful autonomous agencies are also in a stronger position to lobby for additional resources and to protect settlers against outside interests. To attract staff such agencies also tend to offer higher salaries, hence achieving better staff quality and continuity.

Though the strengths of parastadal settlement agencies have received considerable emphasis, less attention has been paid to their weaknesses. Three types of weaknesses, linked to an extent, appear with relative frequency. The first is an increasing inability to service the settlement area as time goes by, while the second relates to poor relationships with other government departments. The third relates to an inability to hand over managerial responsibilities to settler organizations, rural and municipal councils and other government agencies. This tendency to resist devolution increases the risk that the settlement bureaucracy will become more inefficient as the years go by, especially if political influence wanes with time, so

that financial resources are cut and the better staff seek more rewarding job opportunities elsewhere.

While there are no easy solutions to the weaknesses outlined above, two types of approach exist -- the one political and the other based on budgetary inducements and constraints. Both have the same goal -- to force parastadal settlement agencies to share power not just in the interests of efficiency but also in terms of facilitating integrated area development.

While political pressure from settlers does not appear to be an effective mechanism at this point in time for pressuring parastadal settlement agencies into handing over more managerial responsibilities, budgetary inducements and constraints would appear more promising. Budgetary constraints, for example, could be built into annual reviews in such a way that certain funds would not be allocated if it appeared that insufficient effort was being made to honor timetables relating to handing over and incorporation goals. The international community of donors could apply pressures here by allocating funds to appropriate institution building and training programs for local organizations, by more carefully monitoring their use, and by justifying different approaches on the basis of experience elsewhere.

b. Coordinating Settlement Agencies. The main advantages of using an existing government department to play the lead role in coordinating the settlement process relate to the later stages of the settlement process, and especially to handing over and incorporation. Both actions are less of a problem simply because the various departments being coordinated are usually the ones to which an autonomous settlement agency would hand over responsibility.

The weaknesses of using an existing department or ministry to coordinate the development of a new lands settlement relate to problems of flexibility and of influence. As Dalton (1981) has pointed out for area development projects, the coordination of complex development efforts involving many agencies is an incredibly difficult task demanding "an enormous and steady series of efforts directed at fostering communication and understanding among policymakers, technocrats and technicians," not to mention politicians and leaders of local organizations.

Two types of solutions to the above weaknesses suggest themselves, the first being institutional and the second budgetary. At the national level, the coordinating agency's influence and political clout could be enhanced if the coordinating committee reported directly to a prominent cabinet subcommittee or even directly to the vice-president or president himself. As for the local level, clearly local government agencies should be involved along with the participating government departments while at the district level, the

senior district political official in most cases would be the logical person to chair the coordinating committee.

As for budgetary solutions, here funds could be allocated to the coordinating agency to "encourage" participating agencies to carry out activities that fall within their sphere of influence but which they may have neither the motivation or the staff, equipment and finance to carry out.

B. MARKETING FACILITIES AND SETTLER RUN COOPERATIVES

Marketing facilities include appropriate handling and storage facilities on the settlement and transport from the farm to the market either direct or via government or private sector marketing agents.

Sponsored or spontaneous, marketing inadequacies are associated with the majority of settlements on which we have data. Whether related to availability or cost, frequently these are associated with inadequate feeder and settlement access roads, with the result that the settlers have to pay exorbitant prices to marketing agents to pick up their crops at the farmgate, have to hire laborers and transport to carry produce to the nearest government or cooperative depot, or have to use their own labor and transport.

In discussing possible solutions to marketing inadequacies from the settlers' viewpoint, it is important to emphasize that private traders, like money lenders, provide an invaluable service -- at a high price -- in the absence of other alternatives. Granted the complexity of the settlement process, and the difficulty of government sponsored agencies providing, at a lower price, equally efficient services, settlement planners should seriously consider providing alternative options to settlers as opposed to a strategy which attempts to give government marketing organizations a monopoly from the start by excluding both private traders and settler organizations.

While some government marketing organizations are very efficient, the majority may well be counterproductive in terms of new lands settlements initiating a process of integrated area development. There are three reasons for this. First, concentrating on one, or a small number of crops, such organizations act as a constraint on farming systems diversification. Second, while often evening out price fluctuations, prices offered settlers tend to be low -- government marketing boards being a major mechanism whereby farmers are "taxed" to subsidize the development of the urban-industrial sector (Bates, 1981). Third, they are often highly inefficient, with delayed pickup of crops and delayed payouts.

Effective solutions to marketing problems often must provide not just prices, marketing facilities, and pickup and payout schedules

which meet settler needs, but also help remove other constraints which increase settler dependency on private traders. Credit is a case in point.

Although a number of cases, including the Northern Parana one, indicate that the private sector can provide adequate marketing services without competition from government and settler run organizations, we have argued throughout that settler participation is associated with project success. The emergence of settler run cooperatives is a major way to foster that participation. Indeed, during the global evaluation we came across a number of efficient settler run cooperatives which were able to outcompete private traders by offering members a better price and a range of other services.

C. ECOLOGICAL IMPACTS OF NEW LANDS SETTLEMENT

Of special concern is the replacement of highly diversified humid tropical rainforests with less productive cropping systems. Especially serious in Latin America because of the emphasis placed on conversion of humid forest to grassland for ranching, the elimination of primary rain forest is also occurring at a rapid rate throughout tropical Asia and in West Africa. Of concern is not just the removal of the forest cover but also the techniques being used for land preparation, with mechanical clearance more apt to remove the top soil and cause adverse compaction than hand clearing.

Another problem in the humid tropics as well as in savanna environments is declining soil fertility occurring over both the short and long run. In arid and semi-arid lands, adverse ecological impacts are more apt to relate to problems of salinity and water logging, coupled with declining fertility. A major problem relates to inappropriate farming systems for each agro-ecological zone. In terms of corrective action, a good starting point is the more diversified farming systems of host populations which are characterized by multicropping and interplanting, with both leading to a more intensive form of land use which is not carried out at the expense of soil fertility.

D. RESEARCH

Many of the conclusions in this study are based on a relatively small number of studies. They need testing against the results of further research. There is a special need for farming systems research, for research on the multiplier effects of new lands settlements, and for research dealing with the later stages of the settlement process.

There is a special need for experimental research, for topical research, and for long-term comparative research. Of these three general types, experimental research will deal in large part with appropriate farming systems. It should be based at agricultural research stations, provided the linkages to universities and other research centers exist to ensure that such research is not restricted to agronomic and technical components alone.

As for topical and long-term comparative research, a strong argument can be made that at least part of this research should be carried out by settlement agencies themselves.

IX. AN IDEAL SETTLEMENT PROCESS

Government sponsored settlement has a yet to be realized potential to catalyze a process of integrated area development in carefully selected portions of the tropics and subtropics. For while planners overestimate the magnitude of rates of return during the first five years, they underestimate the long-term potential. This potential relates to both irrigation-based and rainfed farming systems. In stating this I do not wish to give the impression such potential will be easy to realize. The majority of government sponsored settlements cannot be considered a success in terms of either direct or indirect benefits. Furthermore, they are fraught with problems for the settlers who are the major risk takers. For example, of forty sponsored settlements on which we have sufficient data, the majority were characterized by three or more major problems at the time of study, major problems including such factors as settler dependency; on-farm problems due to poor soils, lack of fertilizers and credit etc.; inadequate infrastructure; and institutional problems relating to inadequacies on the part of the settlement and other government agencies.

Such problems are not easily solved especially by government organizations with annual funding and relatively short-term horizons. But they are not intractable. For that reason they should be amenable to solution through more careful planning, implementation, management and evaluation.

The paragraphs that follow outline the distinctive features of an ideal settlement process. This is not presented as a new prototype or "model" to be superimposed on a particular area. Rather it represents a composite, based on features -- drawn from many settlement experiences -- which would appear to increase the possibility for success. These features have never been combined in a single case so that little can be said about how they would fit together and evolve through time. Furthermore, because of the distinctiveness of national ideologies and development policies, of the background and experiences of prospective settlers, and of the

differential nature of agro-ecological zones, some features of the composite would be inappropriate. Notwithstanding these cautions, we know enough now about the settlement process that the presentation of a ideal settlement "model" can help national and international policymakers and planners, administrators, settlers and other settlement participants improve the planning, management, implementation, and evaluation process. At the very least, planners should consider the appropriateness of the "ideal" and not introduce major variations unless these are based on convincing reasons.

The major goal of an ideal settlement process is to catalyze a process of integrated area development with growing linkages between the agricultural and the industrial sectors within the settlement area. Though initially the settlement population numerically is dominated by settler families, as the settlement area evolves the proportion of farm laborers and of nonfarm workers increases until eventually nonfarm workers employed in rural service centers, rural towns, and urban areas predominate. To reduce financial costs and the type of organizational inadequacies which so often dominate sponsored settlements, careful attention should be paid from the start to the proper balance between settlers and other settlement residents, the private sector, and the public sector in the provision of inputs required for settlement success.

To realize such a goal, settler families must number in the thousands. Appropriate farming systems should be sufficiently diversified to provide economic opportunities to all family members to realize a net income high enough to stimulate demand for a wide range of production and consumption goods and services which can be locally provided, and to produce a variety of agricultural commodities for meeting the food needs of farm laborers and nonfarm workers, and for stimulating the emergence of agro-industry. Within the farm system, special emphasis should be placed on the crop and livestock but not the off-farm components.

From the start there should be major government involvement, with very careful attention given to how government inputs can be best provided through time in terms of organizational structure. Where a special or national settlement agency is involved, mechanisms should be built into its enabling legislation or terms of reference which not only encourage the handing over at appropriate times of certain managerial and other functions to the decentralized departments of relevant ministries, rural and municipal councils, and local and participatory agencies, but also include fiscal and other mechanisms to insure that such handing over occurs. Where a decentralized form of administration is established with one agency responsible for coordinating the activities of other agencies, that agency should report to a high enough authority (a council of ministers, perhaps, or the vice president, prime minister, or even president) to ensure compliance from other cooperating agencies in the achievement of clearly stated settlement goals.

Thorough planning should precede implementation, with feasibility studies including climatic, soil and hydrological surveys of potential settlement areas, and socioeconomic surveys of the land tenure, land use, and sociocultural systems of the host population. Once specific settlement areas are selected, more detailed soil, hydrological and host-oriented studies should commence, and appropriate agricultural experiment stations, with a farming system (as opposed to a crop specific) orientation, should be established. The settlement type should attempt to include host, spontaneous, and government sponsored "outsider" settlers not just as mechanisms to provide opportunities for all three populations and to tap into the enterprise and initiative of the spontaneous settlers, but also to cut the financial costs of the settlement process. For settler recruitment, both spouses should be interviewed and settlers with a mix of farm and nonfarm skills should be selected.

All types of settlers should be encouraged to settle in nucleated communities in which household plots are large enough for women to grow vegetables and fruits and raise some livestock and for the second generation heir to build a home next to that of his/her parents. These communities should be articulated to a carefully planned and hierarchically organized network of higher order service centers and townships which should incorporate existing commercial centers wherever possible, and which should be planned with the needs of both settler and nonfarm resident (including government officials) in mind. Orientation courses for at least a minority of settlers (with some drawn from each community) should be undertaken as soon as possible after the arrival of each phase of settlers.

The provision of infrastructure should be phased, with initial emphasis on roads; potable water (and water for irrigation where relevant); preventive medical services (malarial control, for example); appropriate research-based extension advice; and credit. Generally speaking, settlers should be responsible for building their own houses, although in certain agro-ecological zones it may be necessary for government to stockpile local building materials. Throughout, initial government inputs should be targeted to achieve economic and social viability of settler communities at the earliest possible moment so as to facilitate the shift from the second stage of transition to the third stage of socioeconomic development.

Economic assistance should have priority over social infrastructure and housing -- while the provision of such social infrastructure as schools, clinics and other community services should have priority over housing, which is best left to settler initiative. Under economic assistance I have selected out roads, research-backed extension services, and credit for special emphasis during the initial years because of their inadequacy, time and again, in settlement planning and implementation. Properly maintained access roads are crucial not only to move inputs into the settlement area and produce out of it, but also to reduce the sense of isolation from the point of

view of the settlers. Appropriate research-backed extension advice is needed for both production related and ecological reasons, while credit is crucial from the start if the failure or "drop-out" of settler families and the risks of land consolidation in the hands of a few are to be reduced.

There is increasing evidence that production increases and project success in connection with rural development is closely correlated with the participation of local beneficiaries in the development process. For this reason, special emphasis need be placed on how best to involve the settlers in the preparation of the lands that subsequently they will be farming and to facilitate the emergence of settler dominated local organizations during the early years of implementation. In the case of such organizations as water user associations, community organizations, and cooperatives, these should be encouraged to federate up to the project level so as to increase their capacity not only to organize community labor for development purposes and to express settler needs but also to enable the local population to compete more effectively for scarce resources at the district, regional, and even national level.

Though marketing facilities usually are not crucial during the initial years when many settlers are struggling to achieve self-sufficiency, they become crucial as soon as settlers begin to produce for both local, regional, and national markets. As defined here, marketing facilities include those for both storage and transportation. These can be provided by a wide range of organizations, the exact nature of which need be worked out in each instance. The need for market centers right up to the level of the regional town increases as the settlers begin to shift from a Stage Two to a Stage Three orientation. At that time implementation of a policy to develop rural service centers and rural towns should be accelerated through such mechanisms as the provision of credit for business loans. At the same time, efforts should continue to make rural towns attractive places to live not just for the owners of commercial enterprises and their employees but also for settlement agency and other government officials. This can be done through the construction of appropriate housing (in contrast to the policy for settlers) and the phased upgrading and provision of educational and medical facilities, post and banking services, and recreational opportunities — with the upgrading of existing commercial centers favored over the construction of new towns wherever possible.

General prescriptions become more difficult as the settlement area evolves, with the dynamics of the development process becoming more complicated. As more and more factors come into play, with ongoing shifts in their proportional importance, policy prescriptions which are not based on an ongoing process of monitoring and evaluation become more difficult. But a capability for carrying out ongoing monitoring and periodic evaluations should be present from the start since the identification and solution of one set of problems in itself

can lay the basis for new problems. As the settlement area evolves, new constraints and strengths can be expected to continually arise. Rural/urban terms of trade at the national level may shift in such a way that they effect the settlement area either adversely or favorably. New health hazards (such as schistosomiasis in the case of irrigation projects) may sap settler vitality, while the proliferation of rats, wild pigs, and a wide range of insect pests; the appearance of new diseases of crops and livestock; and major environmental changes (owing to increased land degradation and riverine siltation, or to an increased incidence of frost or drought) can bring on major setbacks. Though monitoring and evaluation can not be expected to identify all problems before they reach critical proportions, properly done it can easily pay for itself in terms of what problems are identified and then acted upon in a timely fashion.

One function of monitoring and evaluation is to ascertain the appropriate time for handing over certain managerial responsibilities from the settlement agency or agencies to settlers and local government institutions. This, of course, requires monitoring and evaluating the capabilities of those institutions — with weaknesses identified so that they can be offset by appropriate training and other assistance, and strengths also identified so that they can be built upon. Though aspects of centralized management may have to continue where settlement is part of large-scale river basin development projects or major systems of irrigation, this is not necessarily the case even there. It is still less necessary during Stage Four in the case of settlement based on rainfed systems of agriculture.

The Development Potential of Agricultural
Settlement in New Lands - Institute for
Development Anthropology

The report of Dr. Montgomery, Chairman of the subcommittee that included Drs. Anderson, Falcon, Tanter and Thorbecke, is summarized below:

The subcommittee is in agreement with regard to the merit of the proposal and the excellent qualifications of the principal investigator. There is some disagreement on what the ultimate disposition of the proposal should be.

The question that has been posed is whether it is possible to examine the experience governments have had in resettlement schemes so that performance in future resettling efforts can be improved.

The proposal is to study four types of resettlements: (1) spontaneous migration unaided by government, (2) spontaneous migration aided by government, (3) migration that is initiated by government through the selection of sites and the provision of inducements to migrate and (4) compulsory resettlement. The investigator would examine the behavior of settlers in each of the typologies after relocation. This behavior is believed to follow a predictable pattern where in the first stages the relocated population becomes resistant to innovation and risk and skeptical of government involvement. After economic self sufficiency is achieved the acceptance of change is greater.

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A weak point in the argument is that there is also a need to analyze the nature of the stresses experienced by the settlers after relocation. These should be better understood to help governments respond in a timely manner to the needs of these people.

Timing of activities proposed under the project would be as follows: (1) a three month literature search, (2) two months of field trips to observe some of the re-settlement schemes, (3) one month of additional travel for professional contacts and (4) five months to write the report and prepare a manual from the analysis.

The principal investigator would carry out these activities while on sabbatic. However, this project as designed is bigger than is appropriate for sabbatical.

The subcommittee agreed that the project in its present form is too ambitious for the resources available, and that for the sum requested the issues could not be resolved with much conviction. We are also agreed that the methods and approaches in the proposal are too limited to provide the kinds of insight the project requires.

Three alternative recommendations are suggested in the subcommittee's letters: (1) to reject the proposal without prejudice on the ground that it was not appropriate for AID sponsorship (too theoretical, too academic, not likely to produce findings useful to government or to international donors), (2) to enlarge the scope of the project by adding more personnel and increasing the range and quality of

data to be gathered (a course of action which I would find extremely uncomfortable, since it would essentially require the design of a multi-disciplinary and extremely ambitious project for which the qualifications of the contractor are not immediately obvious), and (3) to reduce the scope of the project and use the resulting research to develop a basis for further field work. I believe the last option, if properly conceived, could be made consistent with the principal arguments advanced by all four of our colleagues.

As to how the project could be redefined and reduced, the members were not fully in accord. I propose the following procedure: (1) Reduce the typologies to be studied from four to two. The first type, "spontaneous migration", is interesting from a theoretical point of view, but it does not become a policy option unless the government decides to offer support, in which case it becomes type 2. The only advantage in studying it as a separate experience would be if some comparisons were to be made between projects receiving government aid and those that survive without it, so that governments could decide whether or not to intervene once spontaneous migration has occurred. This kind of recommendation is much less useful than one that deals with another question: if a government decides to intervene, how should it do so? The fourth type, compulsory relocation, is a policy usually undertaken for reasons not likely to be affected

by a finding that it does not produce socially or economically results. And such a finding is all that one could expect from the proposal under consideration. Since that type has already been studied most thoroughly by the principal investigator, I propose to drop it, too, leaving types 2 (spontaneous migrations that are subsequently facilitated by government agencies) and type 3 (government-sponsored settlements) as the two forms of settlement schemes to be studied under this project, (2) Reduce the field trips to countries in which both Type 2 and Type 3 settlements co-exist, so that a direct comparison can be made. Such a study will permit the principal investigator to explore further his "coping" hypothesis. Both types, according to the principal investigator's expectations, will display the "closed system" responses, along with the involuted culture and minimum technological change syndrome. But I suggest that other features of the settler response will be more important than these characteristics and should be added to the analysis. The investigator should refrain from the temptation to consider only responses that "test" his hypothesis, (3) The range of questions to be studied should include more details about agricultural potentials and decisions. They will require the use of agricultural scientists of various types, preferably from the host government. It is likely that existing programs in

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Indonesia, for example, are already pretty well studied,

(4) Economic data, especially household budgets, should be collected with the aid of an economist experienced in micro analytics and farm surveys so that productivity, income, and consumption data gathered in the field will follow standard formats and procedures. Efforts should be made in advance of the field trips to discover what data already exist so that a strategy for augmenting them can be worked out, (5) The kinds of services rendered by governments to both types of settlements should be carefully studied to discover the extent to which different public investments elicit developmental responses. "Indicators" of developmental responses should be developed by the principal investigator in advance of the field studies, preferably with the assistance of measurement specialists at Cal Tech or the University of California or Stanford, (Eugene Webb, e.g., already worked on such indicators.), (6) Organizational strategies used by governments to encourage responsive behavior on the part of settlers should be studied as independent variables. Such strategies might include the use of semi-public organizations such as public enterprises or cooperatives; the sharing of different kinds of responsibilities with locally elected or otherwise responsive officials; and the kinds of activities central authorities engage in to keep

field operations going. The principal investigator assumes that settler behavior is essentially the same in all four types of situations. That may be, but there may be important differences in their responses to the different situations caused by factors like project implementation and continuity of central support. They, too, should be studied systematically. Although the principal investigator has already indicated that he is no specialist in this kind of analysis, he is to be joined in Indonesia by Colin MacAndrews, who is. There are no exotic or esoteric skills required for this kind of analysis. It might be prudent if the fieldwork began in Indonesia so that the approach developed there can be replicated in the other studies, and (7) Land tenure relationships, hypothesized on page 12 as the principal means by which governments can reduce settler "uncertainty", need to be structured in comparative settings to determine whether productive responses are associated differentially in the short term with various policies. If productivity responses, not "uncertainty", were treated as the dependent variable, it should be possible to derive a better understanding of the relationships, to be confirmed, if they appear strong, by further investigation.

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Dr. Anderson thought the proposal was not a defensible investigation. The topic of the rural poor appears to have been added as an extra side light. The evaluation of services of government is time consuming and requires good data which will not be available. The investigator wants to include agricultural scientists in the investigation, but who are they? How do they fit into the project? There is no evidence that the investigator knows what he wants to study in terms of social structure. Finally, there is the really important question of how important are settlements to development. What policy questions would be resolved?

Dr. Falcon thought the topic was legitimate. Land settlements in S.E. Asia is a central issue. The problem the investigator has is that he promises too much for the professional resources he plans to employ. Agricultural technology is a serious omission in the proposal. The proposed budget is too small by about \$100,000.

Dr. Falcon would narrow the typologies and reduce the number of countries. He would then support the project as sound and because the principal investigator's track record is good.

Dr. Tanter thought the four typologies were needed to decide the issue of what level of government intervention is effective, i.e., to obtain variation in type of intervention. The sample of countries should be selected also to maximize variance in intervention and to minimize confounding variance.

Interaction effects should be a central focus but isn't discussed. The policy implications in the study could be strengthened.

Dr. Thorbecke thought the proposal was too broad in scope. The number of issues to be studied could be reduced and sharpened. Any issue examined should be formulated in a testable hypothesis which should be based on reliable data. Explanations of the statistical tests should be given in the methodology.

Dr. Peterson remarked that more attention should be given to the reason for successes and failures among the settlers. Many settlers leave their original location because of failure. Others have misconceptions about markets and other factors. Also the agricultural potential of the new settlement should not be ignored.

Dr. Carter thought the Agency should not try to respond to a time period fixed by the principal investigators availability. The time period should be increased, the budget enlarged and the project paper revised.

Dr. Heady thought the project could be useful but should be done in a broad perspective. The success of a resettlement project would be determined by many economic variables which would not be included in the project.

Mr. Hobgood of the AID staff described the project as not as rigorous as research in the hard sciences but quite good for an anthropology study. Insights from

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anthropologists are needed on this problem. The project is a serious research effort and would have high policy implications.

Dr. Morton of the AID staff explained that the principal investigator would be taking leave from the University and would not be on sabbatical. Part of the time that he is away from his university position, he will serve as a consultant to PPC. He is a very policy oriented anthropologist. After some discussion, he would like to restrict the scope to the Near East, Africa and Asia and extend the field component to six months. There have been two favorable responses from USAID missions. The principal investigator has tried to address the perceived needs of AID from an anthropological point of view.

Dr. Montgomery suggested that perhaps the RAC had been a little too exuberant in its discussion of the project design. The project is a beginning in this area and needs to be done. He offered the following motion:

Motion: That the proposal be approved subject to the following conditions:

1. The project be focused on relationships between government action and settler responses in "new land" development.
2. The number of countries be reduced and should include Indonesia and Malaysia.

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3. A RAC subcommittee be appointed to review and approve the project design.

Dr. Anderson thought the proposal should be re-submitted to RAC after modification.

Dr. Falcon wanted the proposal to be carried out primarily in Asia.

Mr. Kaschak of the Latin American Bureau said members of that bureau liked the project in principal but wanted to see an elaboration of the design in the economic area. He also asked for clarification as to whether Latin America would be excluded from the research.

Dr. Smuckler suggested that the project required further internal clearance after which the RAC subcommittee could meet to help the principal investigator further develop the project.

Dr. Peterson wanted some assurance about why settlements fail. He was still bothered by the lack of agricultural components in the study.

Vote: 2 opposed; 1 abstention, all others in favor.

The motion carried.

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D R A F T 9/4/81

ACTION MEMORANDUM FOR THE ASSISTANT ADMINISTRATOR FOR AFRICA

FROM : AID/AFR/SWA, Frederick E. Gilbert

SUBJECT: Princeton University Research Proposal "Agricultural Development in Senegal: Prospectives, Risks and Production Strategies"

Problem: A decision is needed by the Assistant Administrator on USAID/Senegal's recommendation that AID approve an unsolicited research proposal from Princeton University / on agricultural development and public policy in Senegal. The research would produce studies of Senegal's agricultural perspectives and risks, economic options, and administrative practices which Senegalese and U.S. decision-makers may use in forming judgements concerning agricultural strategies and investments to implement the Government of Senegal (GOS) Reform Plan of 1980, Guidance and feed-back for the three-year policy-oriented research would be provided by the Ministry of Plan's Interministerial Agriculture Policy Committee. Total cost of the research would be \$450,000, with \$300,000 allocated by the GOS from funds in the P.L. 480 Title III agricultural policies account and \$150,000 provided over three years from the Mission's Planning, Management and Research account, of which \$50,000 would be obligated in FY 1981.

Discussion: Senegal has come to a critical juncture in its economic and political life. As the A.I.D. Country Development Strategy Statement for FY 1983 and papers done for the Joint GOS-US Assessment of U.S. Assistance Program in Senegal all make clear, new directions have to be undertaken in economic policy and administrative structures. A new political leadership is in place and significant reforms are already underway. The U.S. Government is providing the Government of Senegal with balance of payments support, in addition to regular technical assistance, in order to help the country meet its current economic crisis over the next several years.

U.S. non-project assistance for the purpose includes a P.L. 480 Title III three-year \$21 million sales and agriculture development program, a \$25 million Agriculture Sector grant beginning in FY 1982, and possibly an Economic Support Fund grant.

A program/ in an unsolicited proposal from Princeton University should improve the capability of the GOS and USAID to apply the U.S. balance of payments support, and related counterpart funds generated as a result of that support, to effect stabilization and basic reform measures in Senegal's agro-economy. The research findings should be helpful to Senegal in implementing overall budgetary and foreign exchange restraints to which the country committed itself in the 1980 Reform Plan. worked out in consultation with the IMF and IBRD. In addition, the research on "Agricultural Development in Senegal: Perspectives, Risks, and Production Strategies" should specifically help the government refine and implement economic policy in the agricultural sector, as promulgated and accepted by the GOS in the Reform Plan. Under the Plan, for instance, the government is committed to change marketing and producer price policy to promote production of food stuffs and agricultural exports, to overhaul prices of farm supplies and equipment, to re-structure the terms and conditions of agricultural credit, to create and develop village sections within the cooperatives, and to encourage private initiative in marketing. The government in any case will encounter great difficulty in making

these economic changes and even more difficulty if it lacks an understanding of the political, social, and historical dimensions of the challenge. The proposed research will help the government factor these elements into a cohesive agriculture economic reform package.

This is a propitious time for AID to respond affirmatively to Senegal's request for advisers from a university selected by the GOS to assist in shaping programs and investments for the country's Sixth Development Plan. In this connection, the Minister of Plan has requested that the advisers and analysts in the Princeton University research team assist the newly formed Interministerial Agriculture Policy Committee. This Committee is chaired by the Director of Planning and includes representatives Ministry of Rural Development, the GOS agriculture research agency (ISRA) and the Prime Minister's Office.

The research proposal provides that an interdisciplinary team of eight political scientists and economists, in collaboration with Senegalese scholars and officials, conduct policy-oriented research on the country's experience in managing the agricultural economy for economic growth at a time when the govern-

ment itself aims to decentralize operations and disengage from ^{many} direct economic activities. The researchers will assess the institutional balance of forces that leads to policy design for the country's agricultural strategy in rural areas. Various rural growth productionist and community based strategies which the government has attempted since independence in 1960 will be examined as a basis for identifying avenues open to achieve changes required in the 1980 Reform Plan. These changes include/ ^{hastening} decentralization, reorganizing the coops, overhauling large intermediate parastatals such as the Regional Development Agencies, and working primarily with small groupes de producteurs. The researchers will assess the attitudes and goals of institutional factors, past problems, successes, failures and their remedies, and the likelihood that free market forces will be *eased* in the near future. Administrative and other instruments for implementing policy will be appraised, together with political implications of *adjusting* the system.

The research will also examine how the government goes about reconciling legitimate but potentially incompatible goals such as increases in food production and agricultural exports, decreases in agricultural imports, improvements in subsistence agriculture, and provision of urban populations with food. In sum, ^{administrative analysis} the/ component of the research will develop a detailed picture of the institutional actors who will produce answers of their own. It will then ascertain what these actors believe the relevant factors are in setting priorities and estimate their relative weights in the policy process.

The economic analysis component will examine the options available to Senegal in terms of a limited number of issues such as the wisdom of focussing on export crops, domestic production, or a mix of the two; the role of public and private sectors in providing goods to the rural sector; methods for establishing domestic pricing and and overseas marketing of agricultural commodities; questions of private/public finance

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and ownership of productive resources; the role of large-scale private firms; and ways and means to introduce new agriculture technologies. Researchers will examine operations of the agricultural economy as a whole and estimate costs of different types of uncertainty and risks on the part of farm and farm supply operators in different parts of the country.

Together the economists and political scientists will produce a set of policy-relevant papers useful to Senegalese and U.S. development administrators concerning critical problems and options for Senegal over the next decade. The researchers expect a wide reading from development practitioners world wide as has been the case with their other work. The work will be made available to Senegalese and U.S. analysts as it is undertaken. The researchers offer to hold meetings in Dakar and Washington to make their progress known to the policy community.

The Princeton University principals who have proposed the research and will manage the on-site team are uniquely qualified to lead such an effort. Professor Henry Bienen, Director, Research Program in Development Studies, and Professor John Waterbury have carried out four major country studies under A.I.D. sponsorship in the political economy of income distribution in Mexico, Egypt, Nigeria and Turkey. The multi-disciplinary work was carried out as an unsolicited research proposal approved by the A.I.D. Administrator in May 1976 when the Agency concluded that the nature of the research and the receptivity of the researchers by the host country made the normal solicitation process impractical. In the case of the current proposal, Dr. Bienen in 1980 produced research on political and administrative aspects of development reform in Senegal which has been accepted by the Government of Senegal as part of the GOS-U.S. Joint Assessment exercise. Together with three other U.S. academicians, he participated in the Mission's strategy formulation session in December 1980, which formed the basis for the FY 1983 CDSS. Professor Waterbury was in Senegal in July 1981 to explore GOS receptivity to the Princeton research

proposal. The GOS has expressed satisfaction with the qualifications and approach of both professors as team leaders who would have large responsibility for political and institutional analysis. Other Princeton economists will be responsible for specific parts of the economic analysis. It would be im-

possible at this date to obtain proposals from other U.S. sources already knowledgeable about Senegalese economic and political practices and institutions involved in the reform, and acceptable to the GOS. To ^{delay the} start up of the research by inviting others to prepare themselves in a comparable manner and to demonstrate their acceptability to the GOS in this area of political-economic analysis, would not be in the interest of the U.S. Government, given Senegal's need to address the economic crisis at once

USAID/Senegal has reviewed and approved the proposal for technical soundness and methodology and for its potential contribution to public policy. The Mission has determined that Princeton's macro analysis and public policy research should be started by October 1981 so that it will correlate with USDA analysis on micro price policies and production export costs, with Michigan State University research in farming systems, and with operations of A.I.D.'s Grain Storage and Marketing Project. Together this series of powerful analytic tools will be used to effect agricultural policy, both at ^{the} national and farm levels. The Princeton research team will be uniquely able to strengthen the GOS capability and resolve to turn Reform Plan commitments for stabilization and policy changes into in-fact shifts toward government decentralization, deregulation and disinvolvement from business functions. It should also enable the government to discover opportunities for increasing private operations in the field of agricultural marketing and other elements of Senegal's ^{food} industry. The USAID/Senegal Director recommends that the Assistant Administrator approve the research proposal for financing with PM&R funds. In response to SWA's request for a statement from the Project Director to the AA/AFR, the Mission Director, acting in that capacity, has provided the required statement certifying that the proposal was unsolicited.

(Attachment A).

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Since this undertaking will be conducted at the host government's invitation following USAID/Senegal's review and approval of the research scope of work and methodology, and since it serves U.S. interests to have the research begin at the time when the first donor's consultative group meets with the Government of Senegal on October 19-21, 1981, to review Senegal's Sixth Development Plan, A.I.D.'s Research Advisory Committee officer agrees that the Africa Bureau may process and approve this unsolicited research proposal now. It is understood that the RAC will have the opportunity to review and provide comments on the work at a later date. Accordingly, it is planned that RAC review the research undertaking during its regularly scheduled meeting on November 17, 1981. We believe that observations and advice from RAC social scientists concerning the content and approach of the research can be beneficial to both interested parties in Senegal and to Agency development officials who may wish to sponsor similar policy oriented research in other countries. Mission and Africa Bureau administrators of the Princeton University research contract will be able to take account of RAC observations and advice as the work proceeds in FY 1982 and as the research agenda for later years is discussed in Senegal and agreed upon with Princeton researchers.

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Princeton University

Recommendation: That you approve the three year/\$450,000 multidisciplinary, and public policy unsolicited research proposal on agricultural development/in Senegal, to be financed from P.L. 480 Title III GOS-owned counterpart funds (\$300,000), and from the Senegal Mission's PM&R account (\$150,000) beginning with \$50,000 in of PM&R funds FY 1981. The balance/would be made available in FY 1982 and FY 1982, subject to availability of funds, upon submission / by Princeton of a research agenda acceptable to A.I.D.

Approved _____ Date _____
Disapproved _____ Date _____

That you accept the certification of the Mission Director as Project Manager that the proposal was unsolicited, (Attachment A).

Approved _____ Date _____
Disapproved _____ Date _____

Clearances:

AID/AFR/SWA:HGray _____ Date _____
AID/AFR/DP: _____ Date _____
AID/AFR/DR: _____ Date _____
AID/GC/AFR: _____ Date _____
USAID/Senegal (draft) Date 9/2/81 D Shear
AF/W:WHudson (draft) Date 9/2/81
S&T/PO: Rechcigl, M. (draft) Date 9/21/81

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Department of State

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FOR FRANCES JOHNSON, AFR/SWA FROM SHEAR

E. O. 12365: N/A
SUBJECT: PRINCETON UNIVERSITY UNSOLICITED PROPOSAL

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MISSION DIRECTOR CERTIFIES THAT NEITHER HE NOR TO THE
BEST OF HIS KNOWLEDGE AND BELIEF ANY OTHER AID EMPLOYEE
SOLICITED PROPOSAL FROM PRINCETON UNIVERSITY, OR HAD
OTHER PRIOR CONTACT WITH PRINCETON UNIVERSITY REGARDING
THIS PROPOSAL OTHER THAN TO CONVEY TO THE UNIVERSITY
AN UNDERSTANDING OF AID'S MISSION AND NEEDS RELATIVE TO
THE TYPE OF EFFORT CONTEMPLATED IN THE OFFER. BRAY

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PROPOSAL

Agricultural Development in Senegal:
Perspectives, Risks, and Production Strategies

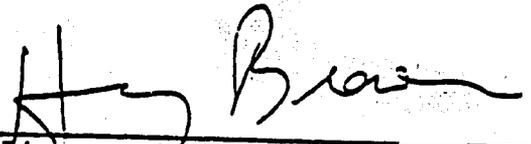
Woodrow Wilson School
Princeton University
Princeton, New Jersey

March, 1981

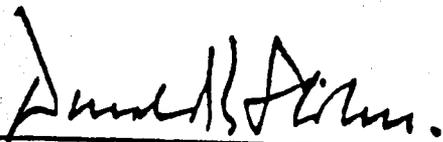
September 1981 - December 1984

U.S. AGENCY FOR INTERNATIONAL DEVELOPMENT

\$512,206



Henry Hienen, Director
Research Program in Development Studies
Woodrow Wilson School
Princeton University



Donald E. Stokes, Dean
Woodrow Wilson School of Public and
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Princeton University



Alan J. Sinigalli, Director
Office of Research and Project Administration

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I. Introduction

Senegal has come to a critical juncture in its economic and political life. As the AID Country Development Strategy Statement for FY 1983 and the various papers done for the Joint Assessment of U.S. Assistance Programs in Senegal all make clear, new directions have to be undertaken in economic policy and administrative structures. A new political leadership is in place and significant reforms are already underway. USAID is also reformulating its activities in Senegal, building on the Joint Assessment evaluations.

Princeton University's Research Program in Development Studies believes that it can make a significant input to the study of agricultural development strategies in Senegal. We have already carried out four major country studies in the political economy of income distribution in Mexico, Egypt, Nigeria and Turkey. The Director of RPDS, Henry Bienen, has been involved in the Joint Assessment exercise. Above all, RPDS has already developed a proposal on the political and economic risks in export orientation, choice of technology, and capital sources, which is before the International Economics Division of the Department of Agriculture. This involves study of a number of problems which are relevant to Senegal's own development strategies. (USAID Senegal and the Senegal desk, USAID Washington, have copies.)

The comparative advantages and costs of producing traditional food stuffs for the domestic market, especially millet, compared to producing food crops such as rice — which are now taking up significant foreign exchange through importation is a terribly important policy issue for Senegal. Similarly, how much revenue can be raised from increasing groundnut

production and thus providing funds for foreign exchange involves an analysis of the costs and benefits of reaching food self-sufficiency through trade. So does the question of diversifying into new exports of either agricultural commodities or manufactures.

These issues have been central to our own concern with analyzing the interrelated aspects of production, storage, transportation and marketing price and trade policies. As our proposal on agricultural development strategies notes:

While the prospects for rural reform strategies cannot easily be generalized to developing countries as a whole and while strategies must be country-specific in order to take account of critical political and cultural, as well as economic variables, we can, at a high level of generality, characterize the options available for countries in terms of a limited number of key (and unresolved) issues:

(1) the extent to which development should focus on the cultivation of export crops rather than on expanding sources of food for domestic consumption;

(2) the extent to which the finance and ownership of productive resources should involve foreign and/or public sources rather than the maintenance of small, largely self-financing farms; and the role of large-scale private farms versus medium or small-scale private farms;

(3) the role of the public sector in providing goods to the rural sector;

(4) the extent to which the public and/or foreign investors should be involved in the domestic pricing and overseas marketing of agricultural commodities;

(5) the rate at which new agricultural technologies should be introduced into the agricultural sector and the form of these technologies.

The context for Senegal, as for most developing countries, is one where trade issues loom very large. Indeed, for Senegal, issues of trade and regional and international development are closely linked because: 1) Senegal remains in the franc zone, and 2) important new development projects involve cooperation with Mali and Mauritania in the OMVS. The issue of joint development of the Senegal River Basin involves coordinating price and tariff policies with neighboring countries. Since capital investments are going to be undertaken which will have effects on at least three countries, the costs and benefits of OMVS projects must be weighed in the regional context.

We feel we have a very strong comparative advantage in building on work done at Princeton and elsewhere on models of risk and opportunity through which to consider problems of trade, investment, agricultural strategies. Moreover, our own comparative advantage at Princeton has been to work on issues that lend themselves to joint efforts by economists and political scientists. Analysis of the development and implementation of agricultural strategies requires sensitivity to political and administrative concerns as well as to economic development and trade issues.

These issues could not be more relevant to Senegal. Senegal's Plan de Redressment has the two major objectives of: 1) bringing about increased financial rigor in management of the economy as a basis for increased growth; 2) decentralizing decision-making within the economy which entails the progressive disengagement of the government from many of the kinds of activities in which it has been involved in the rural sector.¹

¹See Donald Brown, "Senegal's Plan de Redressment," Dakar, October 15, 1980.

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Senegal is now engaged in a far reaching and consequential attempt to alter its administrative structures operating in the rural areas. These have been some of the most elaborate and developed, although surely not efficient and productive, set of administrative structures in Africa. Yet there is a widespread recognition within Senegal, among Senegalese policy makers, donors, and producers, that fundamental alterations in responsibility for farm inputs, credit and seed, and marketing will have to take place. Any discussion of food self-sufficiency and greater productivity in the rural sector must take account of the questions of decontrol and commercialization in Senegal's rural sector. Thus to the analyses of risk, private initiatives, both domestic and foreign, and trade issues must be added an analysis of the potential for reform of the administrative structures for rural development and agricultural growth in Senegal. Again we believe we are well placed to carry out such studies through our interdisciplinary team at Princeton and in collaboration with Senegalese scholars and officials.

II. Institutional Aspects of the Development and Implementation of Agricultural Strategy.

When nations, LDCs or others, announce development strategies, set priorities, and allocate investments, it is only natural that dominant policy makers try to propagate an image of unanimity and immutability. But these policy packages are the result more of bargaining among institutional and less formal interests than of consensus, and as such tend to be fragile. As the economic situation they claim to address changes, for good or ill, the policies are changed or scrapped.

It is important in the case of Senegal to have a clear picture of the institutional balance of forces that leads to policy design for the rural areas. To come up with that picture would require some or all of

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the following steps listed in Sections A through E.

A. Draw lessons from the four major phases of rural growth strategies to date:

1. 1959-62 -- the socialist-oriented strategies of rural transformation, aimed as much at equity as at increased production. Why was this judged a failure? What conclusions were drawn from it? What institutional actors felt that it was not mistaken?

2. 1962-72 -- the productionist phase with an emphasis on efficiency and the regrouping of the agricultural sector into large administrative and production units. What, other than the Sahelian drought, called this approach into question?

3. 1972-79 -- an effort was made through the Administrative Reform of 1971 to return some administrative and political power to regional and sub-regional units. In terms of production and standards of living, however, the situation continued to deteriorate. Was it a question of local leadership at the level of the commune rurale not understanding the powers at their disposal, or of over-zealous sous-prefets unable to work with local groups in a cooperative spirit? (Geller's work will be useful here)

4. 1979 -- the Plan de Redressment contains policies that would hasten decentralisation, reorganizing and revitalizing the coops, overhauling large intermediate institutions such as the RDAs and ONCAD, and working primarily with small groupes de producteurs. Is this a return in part to the philosophy of the 1959-62 period? Is it the first step in the retreat of the public authorities from the agricultural sector?

B. An assessment of the goals of institutional actors

At the broadest level outside observers have difficulty in seizing the true direction of the government since 1978/80. Don Brown posits that the Plan de Redressment calls for "the progressive disengagement of the government from the economy, particularly in the rural sector." Galler presents a very different view: "Most GOS cadres are anti-business, especially commerce. Their attitudes are not likely to change. Hence, there is little likelihood of "unleashing" free market forces in the near future. Government regulation is thus going to remain a feature of Senegal's economic institutional structures for quite some time."

It is probable that current plans will bring about a regrouping of governmental efforts rather than a retreat. The crux of the matter lies in elaborating a strategy for regrouping, and the strategy for that regrouping will be the result of intense bargaining among institutional actors, including the ruling party and the Brotherhoods.

It will be essential, therefore, to map these interests and detail their images of past problems, successes and failures, and their remedies for the future. An attempt must be made to assess their relative weights in the bargaining process.

C. Target Groups and Levels

Institutional actors will have preferences for aiming at specific groups as the best operating units in effecting policy goals, and will select the instruments of intervention according to the nature of the group or level.

There appears to be some preference, perhaps only rhetorical, for small clusters of cultivators, below the commune level. These are referred to vaguely as groupes de producteurs, or, in the case of the OMVS PID, hydraulic

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units. How in fact are these groups likely to be composed? There will of course be marked differences by region and by the nature of rural production: Cassamance rice cultivators, peanut farmers, cattle raisers, participants in newly-irrigated perimeters in the Fleuve, etc. Target groups and policy instruments will and should vary according to these regional and production characteristics.

Beyond such variations, however, is the question of whether or not the groupes de producteurs will be anything more than a mask for the local notables that Carvin and others feel most benefitted from the 1971 administrative reform. This question must be understood both for its equity implications and because other proposed policies may in fact enhance the power of local notables.

Thus it will be important to understand if the government wants to work with existing interests and local power structures (for example the OMVS PID talks of encouraging clan/village/cooperative participation) or if it will seek to erode or break them up. If the latter, the question then is, how?

D. Instruments.

There is not yet a consensus among Senegalese planners on the best instruments, administrative or other, for implementing policy. There will probably continue to be a great deal of tinkering with the system. Other components of this proposal will deal with the economic implications of these instruments; this component intends only to address some of their political implications.

1. Pricing and marketing. There will continue to be strong public participation in both processes although market forces and the private sector are slated to play a growing role. What will be the impact of that course? For example, how will the two-track pricing system whereby the government sets prices for export crops and allows the market to determine the price of local food crops impact on various segments of the society? Will there be a flight from export crops? Will the government have to "indenture" peanut growers to their crop? What will rapidly rising food prices mean for the urban poor? If the pricing of inputs, including eventually fertilizers, is to be left to the market, who will be able to afford the inputs? What interests will supply them? As more marketing functions devolve upon the private sector, who will assume them? These questions should be linked to those in Section C. We shall seek not so much to answer them as to ascertain how Senegalese policy-makers assess them.

2. Credit. How will the terms, volume, and private/public mix of agricultural credit be determined? What will be the leading criteria for public credit and who will be able to meet them?

3. Institutional levels. Where will financial resources and extension efforts be directed? -- to groupes de producteurs? cooperatives? CERPs?, communes rurales? RDAs? Will the sous-prefet continue to be the linchpin between policy from Dakar and local implementation?

4. Performance criteria. There is an unmistakable emphasis, flowing from the overall economic crisis, upon efficiency and orthodox cost-benefit criteria in assessing investments and project performance. The government may enter into performance contracts with parastatal organisms such as the RDAs, and it is claimed that investments of any magnitude (\$500,000+) will be subject to strict cost-benefit analysis. Can these concerns be made

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compatible with concerns for equity and the greater involvement of small underfinanced producers in the development effort?

E. Performance Goals.

The most difficult task facing the Senegalese Government will be in reconciling several fully legitimate but potentially incompatible goals:

1. increased agricultural production -- what cultivators or agents (state farms? foreign agribusiness?) are most suited to bring this about now? What will be the cost to the less-suited?

2. increased exports of agricultural products -- what price incentives must be introduced to promote such exports and what are the trade-offs in terms of local food production?

3. reduction of imports of agricultural products -- is this to take place largely through increased rice production and the vulgarisation of millet consumption?

4. making subsistence agriculture more viable -- can this be done short of subsidizing a sector characterized by low productivity?

5. servicing the needs of urban populations for cheap food -- what is the degree of political risk involved in not servicing these needs?

To reiterate the basic strategy involved in this component of the project -- it is not to produce answers to the questions posed, but rather to develop a detailed picture of the institutional actors that will produce answers of their own and to ascertain what they believe the relevant factors are in setting priorities. Finally, an estimate of their relative weights in the policy process will be made.

We now turn to the economic analysis component of our study where questions noted above will be analyzed with regard to production choices in the context of trade and risks.

III. Uncertainty and an Economic Strategy for Senegal

1. The Problem

The dominant sector in the Senegalese economy, agriculture, is exposed to large uncertainties from several sources. First, extreme variability in climatological conditions from year to year make the output of crops highly variable (see, for instance, Niane, 1980). Second, world price fluctuations create uncertainty about the terms at which the export crop, peanuts, can be traded for foodgrains and other imports. Recognition of the uncertain nature of the Senegalese economic environment raises questions about implications for economic policy.

2. Scope of the Analysis

An understanding of the implications of different types of uncertainty for Senegalese economic policies requires both theoretical and empirical research.

The theory of decision-making under uncertainty is not so well developed as to provide immediate answers to many questions raised by the Senegalese situation. Crucially important to making any theoretical research enlightening for decision makers will be the strategy choice of assumptions appropriate to Senegalese conditions. The development of this theory can be informed by the work of other analysts who have previously described the economy. A selection of the works cited in Kostinko and Dione (1980)

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will be particularly valuable in this regard. An outline of proposed areas for theoretical investigation is contained in the next section of this proposal.

Informed policy making, however, requires more than a highlighting of relevant issues at the theoretical level. Knowledge of the actual magnitudes of different phenomena and of the quantitative impacts of different policies is also needed. The empirical work necessary to provide this information is limited by the data available on the past operation of the Senegalese economy. At this stage it is too early to know how far this type of empirical work can be pursued. A proper inventory of Senegalese data resources is extremely time consuming and can only be undertaken in Senegal after discussions with Senegalese researchers and government officials. In Section 4 we outline a number of possible empirical projects, their data requirements, and comment on their probable feasibility. We are confident that some significant empirical research is possible.

3. Theoretical Research

Uncertainty is an especially important problem in Senegal due to the absence of well developed capital markets in which individual farmers can diversify risk. With no opportunities to buy insurance, producers are likely to choose their techniques and affect the allocation of resources in such a way as to lessen their exposure to risk. Thus, the classical arguments for free trade may fail to hold in the absence of well-functioning markets to share risk (see Helpman and Razin, 1979).

Several policies have been proposed to allow Senegal to deal with the problems associated with instability. First, it has been suggested that Senegal follow a policy of import substitution aimed at achieving self-sufficiency in food. We propose to investigate the issue of optimal trade policy in an environment without risk-sharing markets. In a

country like Senegal commercial policy can be used to provide insurance to producers who otherwise are exposed a great deal of risk. The cost of such a policy are the usual ones; interference with production incentives causes a misallocation of resources. There are different short-run and long-run potentialities for commercial policy as insurance. In the short-run, commercial policy affects only the allocation of mobile resources, such as labor (see Eaton, 1979). In the long-run the allocation of land to the various crops can be affected as well. The optimal degree of import substitution may also depend on the relative contributions of terms-of-trade variability and weather variability to overall uncertainty, as the former exposes consumers as well as producers to risk, whereas the latter does not.

Integration into international capital markets offers a vehicle for the provision of the required diversification of risk. One way in which Senegal could achieve such integration is by encouraging foreign direct investment in Senegalese agro-business. It may be possible to analyze the gains to host countries from direct investment when domestic risk-sharing markets are absent. In such cases the return to foreign capital will represent both the physical productivity of the factor invested and the amount of risk that is borne by foreigners. This may yield larger than normal gains to the host country, since foreigners with their well-developed capital markets are sure to have a comparative advantage in risk-bearing.

A related opportunity for smoothing year-to-year variability in the economic situation of Senegal is to provide by financial borrowing from the Eurodollar market. Eaton and Gersovitz (1980, 1981a, and 1981b) have undertaken considerable research at both the theoretical and empirical level into certain aspects of this type of problem. Further theoretical

research seems justified by the particular question of resource allocation between crops and sectors suggested by the Senegalese case.

Another option available to Senegal is to develop a buffer stock program. This program could be an integrated one implying cooperation with some of its neighbor countries. One question that may then be posed is: which set of countries would form the optimal group for such a buffer stock. This question bears some similarity to earlier ones posed in the international economics literature concerning optimal currency areas and optimal customs union size. In this case an important consideration seems to be the correlation of the weather patterns across countries, as well as the correlation of world prices for the major export crops of the various countries. Regional buffer stocks may provide a useful institution for a group of LDC's to supply insurance for each other.

The riskiness of economic activity in Senegal may have important implications for different types of investment projects. For example, irrigation works affect not only the average level of output, but also its variability. Similarly, transportation improvements have an important role to play in a situation where different regions of a country may alternately be in surplus and deficit with other regions. The profitability of processing agricultural output for export may also be affected by conditions of riskiness.

An unorthodox proposal to deal with risk is to set up an international insurance scheme. It may be difficult to insure output because monitoring procedures would be required to ensure that they do not systematically reduce inputs and then claim a shortfall due to bad weather. However, it should be possible to insure weather directly. For instance, an international insurance company like Lloyd's could promise to pay certain amounts to Senegal depending on the level of rainfall as measured over

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a certain period at a certain place. In return, Senegal would pay an insurance premium. We propose to investigate the feasibility of this program.

Several of the preceding proposals may depend on the nature of Senegal's relationship with its neighbors. For instance, tariff policies which might otherwise be attractive in dealing with uncertainty may become unusable if crops can be smuggled in and out of Senegal from neighbouring countries. The economists involved in the project will leave to the political scientists the analysis of the political determinants of this cooperation. However, the economic analysis can provide valuable insights into the types of cooperation required and the associated gains, and we intend to explore these issues.

The large projects proposed for OMVS development make all the more essential consideration of cooperative arrangements with Senegal's neighbors.

A final important set of issues to consider in the context of all of the above proposals is the interaction of risk and the design of these proposals with the organization of agriculture. There may already be traditional institutional arrangements in Senegal which mitigate the effects of risk. For instance, Moslem brotherhoods are organizations which involve the coordination of individual producers. Rural families may have members in urban areas who can participate in the pooling of income generated outside agriculture. Analysis is needed to see how these traditional organizations have altered the nature of risk in Senegal and how best to design national programs to complement these institutions.

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4. Empirical Research

In the context of this research proposal, two types of data sets are valuable, each providing a different perspective on the behavior of the economy and allowing projections of the response to policies.

Aggregate data can be used to build a small model of the operation of the economy as a whole. This model would be similar in conception and size to, but quite different in formulation from, the first macro-econometric models of the U.S. Production in the groundnut, rice, millet/sorghum and non-agricultural sectors would be related to the use of inputs (land, labor, capital, fertilizers, water and meteorological conditions) in these sectors. The demand for the output of these sectors and imports depends on prices, incomes and other variables such as population and its composition. The effect of various price controls and possible black market operations would require special attention given the past history of Senegalese agricultural policy. These relationships would be estimated using annual time series data at least as far back as independence. Many of these time series are easily available in the U.S., others should be obtainable in Senegal.

Using this aggregate model, estimates can be made of the costs of different types of uncertainty. It will also be possible to evaluate a number of policy experiments suggested by the theoretical research described in Section 3.

While the aggregate model is important in indicating gross magnitudes, it can say little about the finer detail of economic behavior. To what extent do different areas of the country suffer the effects of uncertainty with different intensity? In particular, how does the experience of irrigated areas contrast with that of unirrigated areas? How does the

experience of areas with poor transportation contrast with that of more accessible areas? What are the magnitude of the implied returns to investments in irrigation and transportation? How have traditional organizations been able to cope with risk?

These are some of the questions which can only be answered using detailed micro surveys providing information on individual farms and farm families. This type of data may be available for some regions of the country in special studies such as evaluations done by ENEA in its beneficiary survey and from USAID's small irrigated systems. A national agricultural census may soon be completed which could be of great help. We are dependent on others for these data and can only know about availability after discussions in Senegal with appropriate people and in discussions with colleagues at Purdue, Michigan State and other U.S. institutions that have received USAID support for work on Senegal.

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IV. Personnel

The Research Program in Development Studies has a team of political scientists and economists who have carried out their own extensive work on problems of economic and political development and trade in many developing countries. Professor Henry Bienen, Director of RPDS, has worked for many years in Kenya, Tanzania, Uganda, and Nigeria. He participated in the Joint Assessment for USAID, Dakar. Professor John Waterbury has worked in Morocco and Egypt and has excellent French and Arabic. Professor Waterbury hopes to spend the year in Senegal from September 1981 - June 1982. Professor Mark Gersovitz has worked in Zaire, Nigeria and Mexico. He was born in Montreal and is French speaking. Professor Joseph Stiglitz worked in Kenya. He is a prominent economic theorist and student of economic development.

Professor John Lewis, former USAID Director in India, former Dean of the Woodrow Wilson School and presently head of the Development Assistance Committee of OECD will return to Princeton in August, 1981. He will be an invaluable participant in our work. Professor Gene Grossman and Professor John Eaton are economists who have done important work on trade and risk.

There will also be at the Woodrow Wilson School a significant number of regular staff and visitors with strong interest in development and agricultural strategies. A great resource is Sir W. A. Lewis, Nobel winner in economics, who is a constant participant in our seminars and intellectual work.

Professors Bienen and Waterbury will have large responsibility for political and institutional analysis and the economists will be responsible for economic analysis. But we have found a great deal of interdisciplinary

effort has gone on in our past empirical work on income distribution in Turkey, Egypt, Mexico and Nigeria. The large income distribution projects funded by USAID, show that RPDS can produce high quality policy-relevant research under difficult conditions. The studies were collaborative efforts with large groups of scholars from Turkey, Nigeria, Mexico and Egypt. We have every confidence that we have a group that can work productively in Senegal, in harmony with Senegalese institutions and scholars.

V. Output

Our intention is to produce a set of papers, and possibly a book, that will make major contributions to the scholarly literature on development strategies and on Senegal. Above all, we intend to produce policy relevant work for thinking about the critical problems and options for Senegal over the next decade. This work should be policy relevant for Senegalese policy makers as well as USAID and other US Government officials. We would expect a wide reading from academics worldwide as has been the case with our other work.

We stand ready, as we did during our income distribution projects, to make work available to Senegalese and U.S. analysts as we go along. We will hold meetings in Dakar and Washington, if desirable, to make our progress known to the policy community.

We will also be happy to make work known to international agencies such as the IBRD, IMF, various development banks and donor institutions. Other academics can, of course, have access to our work where appropriate.

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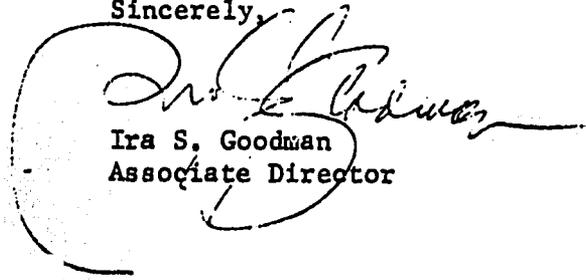
August 18, 1981

James Erickson, Ph.D.
Agency for International Development
(DF-HEA), RPE
Office of Health
Washington, D. C. 20523

Dear Dr. Erickson:

Enclosed please find one original copy with institutional approval of Dr. Ruth Nussenzweig's program project application entitled "Sporozoite Induced Immunity in Simian Malaria - Use of Hybridoma for the Characterization of Protective Sporozoite Antigen(s)"

Sincerely,



Ira S. Goodman
Associate Director

enc.
ISG/cek
cc: Ms. Cari Gaskins

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LEGAL NAME AND ADDRESS:

New York University Medical Center
550 First Avenue
New York, New York 10016

TITLE OF PROPOSAL:

"Characterization of the Protective Antigens of Sporozoites of Rodent, Simian and Human Malaria. Studies on their Immunogenicity."

PROJECT DIRECTOR:

Ruth S. Nussenzweig, M.D., Ph.D., Professor
Head, Division of Parasitology
Department of Microbiology

CC-DIRECTOR:

Victor Nussenzweig, M.D., Ph.D., Professor
Department of Pathology

PRINCIPAL INVESTIGATORS:

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Department of Microbiology

Savanat Tharavanij, M.D., Professor,
Head, Department of Microbiology and Immunology

PERIOD OF PROJECT:

January 1, 1982 - December 31, 1984



Ruth S. Nussenzweig, M.D., Ph.D.
Project Director



T.A. Fitzgerald, Director, Office of
Grants Administration & Institutional Studies

8/12/81

(Date)

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CHARACTERIZATION OF THE PROTECTIVE ANTIGENS OF SPOROZOITES
OF RODENT, SIMIAN AND HUMAN MALARIA
STUDIES ON THEIR IMMUNOGENICITY

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* (A) is title page.

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CHARACTERIZATION OF THE PROTECTIVE ANTIGENS OF SPOROZOITES
OF RODENT, SIMIAN AND HUMAN MALARIA

STUDIES ON THEIR IMMUNOGENICITY

Project 1. IMMUNIZATION WITH PURIFIED ANTIGENS OF P. berghei
SPOROZOITES

Principal Investigator: Ruth S. Nussenzweig*
Co-Investigators: Emmanuel Ojor Amaize*
Fidel Zavala
Luiz Shozo Ozaki*

Project 2. CHARACTERIZATION AND ISOLATION OF THE PROTECTIVE
ANTIGENS OF P. knowlesi SPOROZOITES

Principal Investigator: Alan H. Cochrane*
Co-Investigators: Victor Nussenzweig†
Robert Gwadz*

Project 3. NATURE OF THE PROTECTIVE ANTIGENS OF SPOROZOITES
OF DIFFERENT PLASMODIAL SPECIES

Principal Investigator: Arturo Ferreira†
Co-Investigators: Victor Nussenzweig†
Alan H. Cochrane*
Ferruccio Santoro†

Project 4. CHARACTERIZATION OF THE PROTECTIVE ANTIGENS OF
P. vivax SPOROZOITES

Principal Investigator: Elizabeth H. Nardin*
Co-Investigators: Ruth S. Nussenzweig*
Jurg Gysin*

Project 5. SERO-EPIDEMIOLOGICAL INVESTIGATION OF SPOROZOITE-
INDUCED IMMUNITY IN Thailand

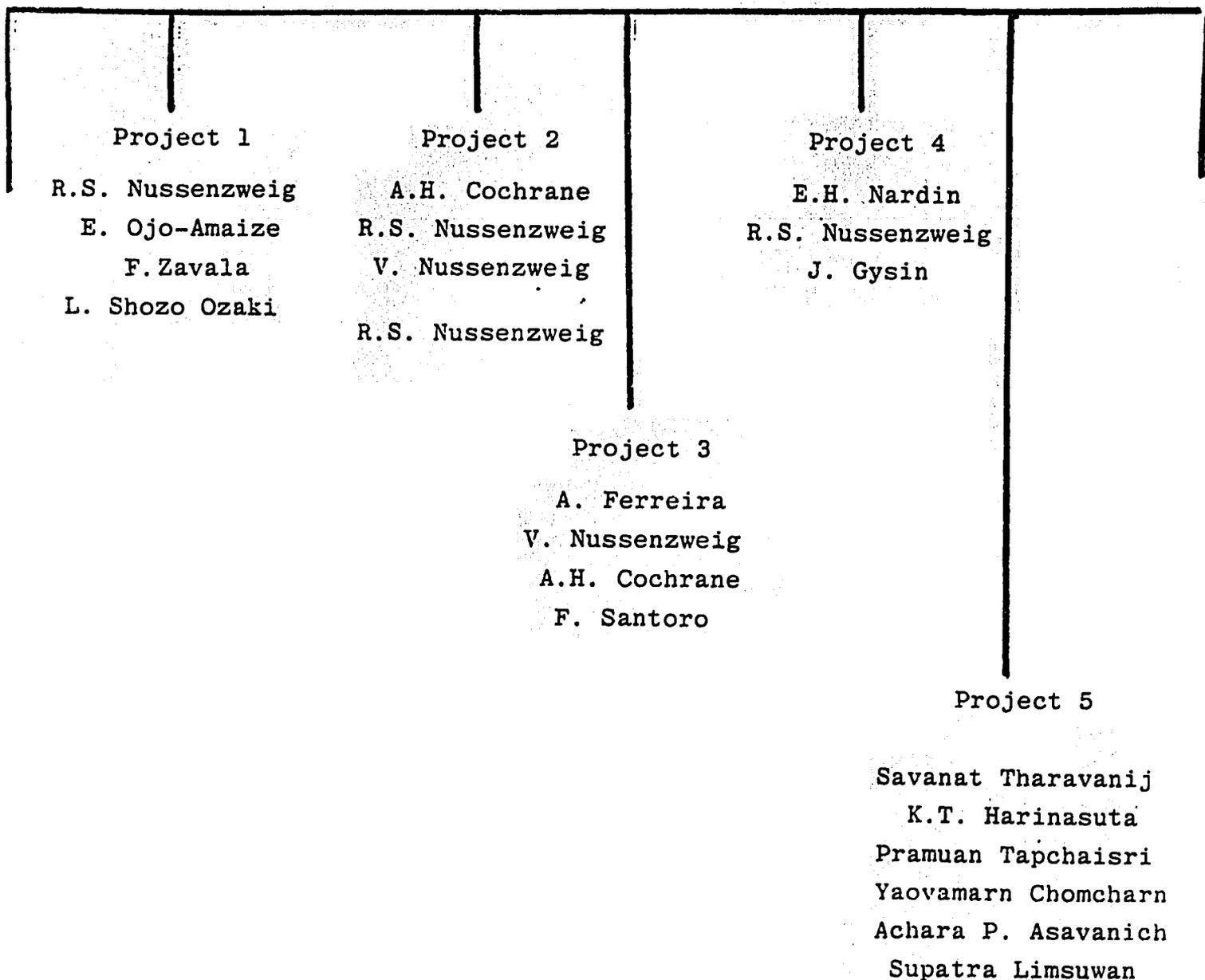
Principal Investigator: Savanat Tharavanijs
Co-Investigators: K. T. Harinasuta§
Pramuan Tapchaisris
Yaovamarn Chomcharns
Achara P. Asavanich§
Supatra Limsuwan§

* Division of Parasitology, Department of Microbiology
† Department of Pathology
§ Mahidol University (several departments)
° Consultant, NIH, NIAID, Laboratory of Parasitic Diseases

Table I

CORE ADMINISTRATION

Program Director
Program Co-Director
Administrative Assistant



- a. Projects conducted at the Division of Parasitology, Department of Microbiology
- b. Project primarily conducted at the Department of Pathology
- c. Project primarily conducted at the Mahidol University (Bangkok)

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B)* RELEVANCE OF THE PROPOSED WORK TO AID'S MISSION

1) Statement of the problem and pertinence of work to AID's Mission

The ultimate goal of our research is to develop the experimental basis for a malaria vaccine of man, based on the use of purified protective sporozoite antigens. This involves the identification, characterization and isolation of the protective antigens of sporozoites of various rodent and primate malarias, including those which infect man. These purified sporozoite antigens will then be used in vaccination attempts conducted in experimental animals. The protective antigens of sporozoites of different plasmodial species appear to share a series of common properties, and might well turn out to belong to a family of related proteins. The occurrence of such analogies would, if confirmed, simplify the task of mass producing these antigens by genetic cloning, or by other means.

Support of research directed towards the development of a malaria vaccine has, since 1966, been part of AID's strategy and overall effort towards global control of malaria. This commitment was formally stated by Drs. E. Smith and J. Banta, in the course of a malaria workshop co-sponsored by AID and the National Academy of Sciences (Washington, July, 1974). During a subsequent International Meeting on Malaria and Babesiosis (Mexico City, May, 1979), Drs. E. Smith and J. Erickson reviewed the areas to be investigated in relation to vaccine development and the corresponding priorities based on the then prevailing state of the art (1).

Recently, considerable research advances have been made in other areas, such as the development of hybridoma methodology and genetic cloning, both of which are expected to have a considerable impact on vaccine development and in particular improve the prospective of mass producing a malaria vaccine. The use of hybridoma methodology has led to a series of novel research approaches directed toward the identification of the "protective" malaria antigens. The first results obtained in this area were reviewed at an AID-sponsored Malaria Workshop (Bethesda, January, 1981).

Two pert charts prepared by AID consultants were presented and updated in the course of this meeting. The charts reflect the present state of the art, and the sequence of foreseeable steps which should lead to sporozoite and merozoite vaccine development. These charts will, undoubtedly, facilitate the assessment of research progress

and future research needs. Our own research activities in the area of sporozoite-induced immunity coincide to a considerable degree with the respective pert chart.

The proposed Program Project, and its individual components, are a logical follow-up of our earlier research in these areas, and of our more recent findings on the protective surface antigen of sporozoites of rodent malaria (2-4). We consequently trust that our Program Project is consistent with AID research priorities, and is entirely relevant to AID's mission in this field.

2) Potential for effective utilization of research results in developing countries worldwide

Our research orientation continues to be directed toward establishing experimental models, as analogous as possible to human malaria, in order that these studies may provide information directly applicable to malaria vaccine development. Our recent work, using radiolabeled sporozoites and monoclonal antibodies, is aimed at characterizing the protective antigens of these parasites and detecting their similarities. This approach is now being extended to sporozoites of human malaria, namely, P. falciparum and P. vivax.

Characterization of the protective sporozoite antigen(s) is a prerequisite for selecting, among the sporozoite components, those which will induce a protective immune response, and which are most likely to provide good candidates for use in a malaria vaccine. These protective antigens will therefore be the targets of genetic cloning in attempts at mass producing these parasite components for vaccination purposes.

Collaborative research, such as our joint project with Drs. K. T. Harinasuta, Savanat Tharavanij and Pramuan Tapchaisri, and others in Thailand (see Project #5), is designed to provide basic information on the level of ant sporozoite immunity of populations living under well-defined epidemiological conditions in areas of high malaria endemicity. These data will be essential for adequately monitoring the immune response to a sporozoite vaccine. It will also be useful for determining the age group which is at greatest risk, and for which vaccination should first be initiated. Furthermore, this study will provide immune sera and sporozoites, essential for the identification of the protective antigens and their corresponding antibodies.

Our AID-supported research activities have provided ample opportunity for the training of investigators from developing countries, as well as from the United States.

This training has, in several instances, contributed to originating or reinforcing existing research teams, which are presently active in sporozoite-related investigations.

In September, 1981, Dr. J. Gysin (The Pasteur Institute, French Guyana) and Dr. E. Ojo-Amaize (Nigeria) are both scheduled to join our research group. In 1982, Dr. T.H. Cheng, a senior investigator from the People's Republic of China, is expected to also join the Division. We are also expecting Dr. L. Shozo Ozaki to join us shortly.

Our collaboration with several investigators and laboratories in the United States, has also, we believe, contributed to AID's network activities. In the course of the upcoming contract period we fully expect to continue our extensive collaboration with Dr. R. Gwadz (Laboratory of Parasitic Diseases, NIH). We also expect to continue our collaborations with Dr. M. Aikawa (Case Western Reserve University) and with Drs. W. Collins and K. Campbell (Center for Disease Control, Atlanta). None of these collaborations are incumbent upon AID funds.

Just recently we have initiated some collaborative research with Dr. M. Hollingdale (Biomedical Research Institute, Rockville, Md.). In addition, a joint research effort is in the process of being initiated in the area of genetic cloning with Dr. N. Godson (Department of Biochemistry, New York University Medical Center), and J. Ellis, a graduate student in the Division of Parasitology. Finally, within our own Division, the combined efforts and shared ideas with Dr. J. Vanderberg, especially in view of his expertise in culturing the sexual stages of P. falciparum, will undoubtedly facilitate our efforts at mass producing sporozoites of this malarial species.

C) SCIENTIFIC ASPECT OF PROPOSED WORK

Cl. Overall Objectives and Rationale of Research Approach

Our present overall research objective, as stated earlier, is to characterize the functional sporozoite antigens of rodent and primate malaria, i.e., those antigens which upon purification and administration to experimental animals will induce protection against sporozoite challenge. Such antigens should function as good candidates for use in a malaria vaccine.

The fact that extensive protection has been obtained by sporozoite immunization in a series of vertebrate hosts, including man, without the need for adjuvants, supports our choice of this developmental stage, namely, sporozoites, as the target for vaccine development.

Our research project was originally limited to sporozoite-induced immunity in rodent malaria. The knowledge obtained as a result of the initial years of this research, namely, the verification that immunization with X-irradiated sporozoites results in a considerable, frequently total protection of the rodents against sporozoite challenge, and that this protection was stage specific and effective against various species of rodent malaria sporozoites, led us to reformulate and broaden our objectives.

During the preceding years of AID-sponsored research, these studies were extended to include two simian malaria systems. The purpose was to verify the general validity of the findings obtained using rodent malaria, and also to establish an experimental primate malaria model related as closely as possible to P. falciparum malaria in man.

The serological methods developed in the course of these studies have, in recent years, been used to investigate the occurrence of ant sporozoite immunity in individuals living in an area of high P. falciparum endemicity. This has led to the detection of a high frequency of specific ant sporozoite antibodies in this adult population (5) and the finding that these antibodies were congenitally transferred from mother to new-born (6).

Using methodology developed during the current contract period, namely isotope labeling of sporozoites and development of monoclonal antibodies to sporozoite surface antigens, it has become feasible to characterize the protective antigens of these parasites. These antigens were identified first on the surface of P. berghei sporozoites (2, 3); more recently a protective antigen has also been identified on P. knowlesi sporozoites.

Characterization of the protective sporozoite antigen(s) and clarification of the effector mechanisms of immunity are undoubtedly among the most important goals to be reached during the development of a malarial vaccine. We consider that these research objectives can now successfully be undertaken and we are fully committed to this aim.

In view of considerable similarities of the basic characteristics of sporozoite-induced immunity in rodent, simian and human malaria (7, 8), and of our initial findings regarding the molecular basis of these immune mechanisms, it is very likely that further studies will uncover analogies among the protective antigens of sporozoites of different malarial species. Under these conditions, the findings in the experimental malaria models will basically be applicable to sporozoites of human malaria, and thus facilitate their study, which otherwise would be severely limited in view of the present difficulties in obtaining adequate numbers of these parasites.

This concept provides the basis of the approach to be followed in the presently proposed Program Project, and the rationale for the choice objectives to be pursued in the individual projects. The various research objectives and individual research projects, listed herein, are closely interrelated, although they utilize diverse methodology and different malaria parasite-host systems. Our past experience has shown that this multifaceted, integrated approach is rather effective, as advances in one area generate the tools and data necessary to explore another aspect of our overall objective. The participation in our project of investigators of diverse training and expertise, working in very close collaboration, certainly is the single most important factor which contributes to the efficacy of the overall approach.

C2). Individual Projects of Proposed Program

The following is a list of the five related Research Projects, which are an integral part of our presently proposed Program Project, and a brief description of their respective goals:

PROJECT 1. IMMUNIZATION WITH PURIFIED ANTIGENS OF P. berghei SPOROZOITES

The overall aim of this project is central to our entire research approach, namely to demonstrate that protection against sporozoite challenge can be obtained by immunization with purified protective sporozoite antigen(s). These experiments will be performed in the P. berghei-rodent system, since most of the available information on protective sporozoite antigens has been generated using P. berghei sporozoites. In addition, the rodent host, to be used for immunization and subsequent sporozoite challenge, lends itself best for the purpose of defining optimal conditions of immunization, i.e., to compare the efficacy of a variety of antigen preparations, as well as of different routes and schedules of immunization. The radioimmunoassay, which is being developed to detect and measure minimal amounts of the protective sporozoite antigens, will also be modified to measure the levels of antibody produced as the result of immunization, and directed against this protective antigen.

PROJECT 2. CHARACTERIZATION AND ISOLATION OF THE PROTECTIVE ANTIGENS OF P. knowlesi SPOROZOITES

The overall goal of this project is to identify the protective antigen(s) and the respective epitopes of P. knowlesi sporozoites, to determine their functional role

through the use of monoclonal antibodies, and isolate these antigen(s) for a variety of related purposes including the structural characterization of the protective antigen. We also plan to further investigate the cross-reactions with surface antigens of other primate malarias, which are detected by these monoclonal antibodies, and the possibility of protection in these heterologous systems. Since sporozoites of human malaria are so difficult to obtain, limiting severely the experimental approaches which can be undertaken, the characterization of the protective antigens of simian malaria is particularly important as a source of information for vaccine development.

PROJECT 3. NATURE OF THE PROTECTIVE ANTIGENS OF SPOROZOITES OF DIFFERENT PLASMODIAL SPECIES

The overall goal of this project is to define the structure of the protective antigens of sporozoites of P. berghei, P. knowlesi and P. cynomolgi and to compare their physico-chemical properties and their peptide maps. It also aims at the identification of the structural features of the epitope of Pb44 which reacts with the monoclonal antibody 3D11.

PROJECT 4. CHARACTERIZATION OF THE PROTECTIVE ANTIGEN(S) OF P. vivax SPOROZOITES

The overall aim of this project is to identify the protective antigen(s) of P. vivax sporozoites, so that they might, in the future, be used as a malaria vaccine. The identification of these antigens will be pursued by the use of monoclonal antibodies, selected for their reactivity with sporozoite surface antigens. Their functional activity will be ascertained by using the sporozoite neutralization assay, i.e., by determining if the incubation of these monoclonal antibodies, or their Fab fragments, with viable P. vivax sporozoites abolishes their infectivity for squirrel monkeys or splenectomized chimpanzees. Sporozoites for these experiments will be obtained from different sources, corresponding to diverse geographic isolates, in order to determine whether these different isolates share the same antigen(s).

PROJECT 5. SERO-EPIDEMIOLOGICAL INVESTIGATION OF SPOROZOITE-INDUCED IMMUNITY IN THAILAND

The overall goal of this project is twofold: a) to establish the patterns of the antibody response to sporozoites of P. falciparum and P. vivax of individuals living in areas of high malaria endemicity of Thailand; and b) to obtain sporozoites of these two Plasmodial species as well as selected antisera from individuals with high ant sporozoite

antibody titers, for the purpose of identifying, in collaboration with the New York University Medical Center group, the protective antigen(s) of P. falciparum and P. vivax.

Data to be obtained on the incidence of antibodies to P. falciparum and P. vivax sporozoites in the sera of individuals living in an endemic area will provide baseline information on the level of the ant sporozoite immunity of this population. Such baseline data will be invaluable for monitoring the effects of a sporozoite vaccine as soon as such a vaccine becomes available for pilot studies.

C3). Rationale for Incorporation of the Individual Research Projects into a Program Project

a. Scientific Merit of Program Project

The various projects of this application are indeed closely related. The individual projects benefit not only from the information and methodological developments obtained in the other experimental systems, but are inter-related and are a common source for essential experimental materials.

For example, Project #4, "Characterization of the Protective Antigens of P. vivax sporozoites" will benefit from the information and materials obtained from Projects #1-3. The usefulness of these rodent and simian malaria projects as a source of information for the investigation of the protective sporozoite antigens of P. vivax is considerable. This is true because of (a) the striking analogies in the properties of the protective antigen(s) of sporozoites of different malarial species, and (b) the very limited availability of sporozoites of human malarias.

Furthermore, Project #4, will benefit from the data and the research materials (immune sera and sporozoites) obtained in the course of the proposed Project #5, "Sero-epidemiological Study in Thailand." The reverse is also applicable as Project #5 will undoubtedly benefit from the availability of hybridomas and the information obtained in Project #4, as well as Dr. E. Nardin's earlier sero-epidemiological work in the Gambia (5, 6).

Reciprocity also exists between Project #3, "Nature of the Protective Antigens of Sporozoites of Different Plasmodial Species" and Projects #1 and 2. These two projects will provide the necessary background information on the antigens of P. berghei and P. knowlesi, as well as the corresponding experimental materials.

b. Justification for Existence of Core Facility

All of the projects to be conducted at New York University will rely on the use of a common Core Facility located within the Division of Parasitology (for detailed information, see pg.

One essential component of the Core Facility is the three insectaries used for the breeding and maintenance of both infected and non-infected mosquitoes. These are operated by a staff of experienced and well-trained personnel, responsible for breeding of the Anopheles, as well as the maintenance of the sporogonic cycle of the various malarial species, and the dissection and purification of all the salivary gland sporozoites used in the various projects.

The Core Facility also includes a major part of the Division's Animal Quarters, which houses most of the animals, primates and rodents to be used in the experimental work proposed in Projects #1-4. Furthermore, the major pieces of equipment listed, and housed within the Division of Parasitology, and in Dr. V. Nussenzweig's laboratory, Department of Pathology, will be used by all the investigators involved in the projects to be performed at the University.

As all our various projects involve the production of additional hybridomas and/or maintenance and recloning of existing hybrid cell lines, the overall Program Project and all of its investigators would benefit greatly from a well-equipped hybridoma laboratory to be set up at the Division of Parasitology as proposed in the present application.

C4) Background Data

This review has a two-fold purpose: (a) to summarize present knowledge on immunization with sporozoites in rodent, simian and human malaria, and (b) to present recent findings obtained during the current contract period, related to the proposed research (Progress Report). Both of these provide the basis of much of the presently proposed projects.

a. Immunization with sporozoites

The demonstration of the immunogenicity of the sporozoite stage and of the occurrence of sporozoite-induced protection was first provided by the classical experiments of Mulligan et al. in avian malaria (9). Since then, a considerable amount of information on sporozoite-induced immunity has been obtained through experimental work using the rodent, and more recently, the simian malaria system. These findings, as well as the data obtained in preliminary vaccination attempts in humans performed by immunization through the bite of infected, irradiated mosquitoes (10), have been the

subject of various recent reviews (8, 11).

For the purposes of the present proposal, we will therefore merely summarize the essential findings in the various systems:

1. The sporozoite stage of malaria parasites is strongly immunogenic. The intravenous injection of small numbers of attenuated, X-irradiated sporozoites induces a high degree of protective immunity and formation of anti-sporoantibodies in rodents (12, 13).

A relatively large number of X-irradiated sporozoites, administered in repeated doses, is required to produce complete protection of rhesus monkeys against challenge with P. cynomolgi or P. knowlesi sporozoites. Only a certain percentage of rhesus are "good responders" to sporozoite antigen and become protected as a result of the immunization (14).

In man, protective immunization through the bite of P. falciparum or P. vivax-infected, irradiated mosquitoes also required multiple exposure to a large number of infected mosquitoes (10).

2. Sporozoite-induced immunity in rodent, simian and human malaria is highly stage-specific. Its effects are mainly, if not exclusively, directed against the sporozoite stage. The erythrocytic stages of the parasite are not affected by this immune mechanism.

3. Considerable cross-protection and serologic cross-reactivity occur among sporozoites of certain rodent malaria strains and species (15). This is not the case for either simian or human malaria, where protection thus far has consistently been found to be strictly species-specific, but very effective against different strains or isolates of the same species (10, 16).

4. Sporozoites undergo antigenic changes during their process of maturation which seem to be correlated with the development of sporozoite infectivity. Sporozoite surface antigens are barely detectable on rodent oocyst sporozoites, are present in considerable amounts in salivary gland sporozoites and can be detected only in the early phase, but not in the late phase of development of exoerythrocytic stages (17). They are absent from erythrocytic stages.

5. The spleen does not play an essential role in sporozoite-induced protective immunity in mice. The immunization of splenectomized mice renders them resistant to sporozoite challenge in the absence of antibodies detectable by the CSP reaction (18).

6. Sporozoite-induced immunity is, at least in part, serum-mediated. The in vitro incubation of sporozoites of rodent malaria with immune serum neutralizes their infectivity (SNA). The passive transfer of immune serum from hyper-immunized mice increases the rate of sporozoite clearance observed in normal rodents. Serum antibodies also display neutralizing activity against sporozoites of simian malaria, i.e., abolish parasite infectivity upon in vitro incubation.

7. The non-specific stimulation of the reticuloendothelial system, which occurs during sporozoite immunization, is very shortlived and does not play an important role in protection against sporozoite challenge in rodents (19).

b. Progress Report. Recent findings obtained and/or published during the current contract period.

(i) Immunization with viable, attenuated or non-attenuated sporozoites

Studies on vaccination of rhesus monkeys with irradiated sporozoites of P. knowlesi and characterization of surface antigens of these parasites (by R.W. Gwadz, A.H. Cochrane, V. Nussenzweig and R.S. Nussenzweig) Bull. WHO 57 (Suppl. 1): 165-173, 1979.

Studies were conducted to develop an effective method of inducing protection against sporozoite-induced malaria in a primate system and to obtain information regarding the surface membrane antigens of sporozoites. Immunization of rhesus monkeys was performed with gamma-irradiated sporozoites of P. knowlesi. Levels of ant sporozoite antibodies were monitored by immunofluorescence, sporozoite neutralization and the circumsporozoite precipitation reaction, and appeared to correlate well with protection. Only the intravenous route was effective in inducing both protection and ant sporozoite antibodies. Immunization with sporozoites mixed with Freund's complete adjuvant failed to induce protection and resulted in a minimal antibody response.

Mechanisms of resistance to sporozoites probably involve the interaction of the host's immune system with the parasite's surface antigen(s). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of surface-labeled, partially purified sporozoites of rodent malaria (P. berghei) followed by autoradiography revealed the presence of a small number of labeled proteins in the extract. Immunoprecipitation with specific antisera to P. berghei detected primarily one of these membrane components, with an apparent molecular weight of 41,000. The molecular weight of this main surface antigen in sporozoites of P. berghei was different from that in sporozoites of P. knowlesi.

Sporozoites of mammalian malaria: attachment to, interiorization and fate within macrophages (by H.D. Danforth, M. Aikawa, A.H. Cochrane and R.S. Nussenzweig) J. Protozool. 27: 193-202, 1980.

Sporozoites of P. berghei and P. knowlesi, incubated in normal serum, readily interact with peritoneal macrophages of mice or rhesus monkeys, respectively. Interiorization of the sporozoite requires that both serum and macrophages be obtained from an animal susceptible to infection by the malaria parasite. Serum requirements for sporozoite attachment to the macrophage are less specific. Phagocytosis is not essential for the parasites to become intracellular. Our findings indicate that active penetration of the sporozoites into the macrophages does occur.

Antibodies present in the serum of sporozoite-immunized mice are important in determining the fate of both the intracellular sporozoites and the macrophages containing the parasite. Sporozoites coated with antibodies degenerate within vacuoles of the macrophages, which have no morphologic alteration. In contrast, sporozoites incubated in normal serum do not degenerate within macrophages, but the parasitized macrophages become morphologically altered and are destroyed. Preliminary experiments indicate that sporozoites appear to interact with rat Kupffer cells in the same way as with the peritoneal mouse macrophages. It is postulated that Kupffer cells play a dual role in sporozoite-host interaction. In normal animals these cells might serve to localize the sporozoites in the immediate vicinity of the hepatocytes. In the immunized animals, macrophages would remove and destroy the antibody-coated parasites, thus contributing to sporozoite-induced resistance.

Plasmodium berghei: Suppression of antibody response to sporozoite stage of acute blood stage infection (by A.U. Orjih & R.S. Nussenzweig) Clin. Exp. Immunol. 38: 1-8, 1979.

Mice infected with the erythrocytic stages of P. berghei show impaired host response to immunization with irradiated sporozoites of the same malarial parasite. The stage-specific ant sporozoite response was measured by indirect immunofluorescence, upon adsorption of the sera with parasitized red blood cells. P. berghei-infected mice, immunized with irradiated sporozoites on the 4th day of blood-induced malaria infection, developed a normal ant sporozoite antibody response. However, this antibody response was more short-lived, compared with the antibody response in normal mice immunized with a similar dose of irradiated sporozoites. The immune response was severely depressed when the animals were immunized on day 7 or later after malaria infection. None of the sporozoite-immunized animals, including those which responded to the first immunization,

developed a secondary antibody response on reinoculation with irradiated sporozoites. A fully established anti-sporozoite immune response, obtained after multiple immunization with irradiated sporozoites and which resulted in stage-specific protection of the animals, was not affected by a superimposed blood stage malaria infection. The titers of the anti-sporozoite antibodies in these animals were unaltered, in spite of their high parasitemias. Reduction of the malaria parasitemia by chloroquine treatment abolished the immunosuppressive effects of the disease. These observations are discussed in relation to anti-sporozoite immunity and immunosuppression in man in malaria-endemic areas.

Immunization against rodent malaria with cryopreserved irradiated sporozoites of Plasmodium berghei (by A.U. Orjih & R.S. Nussenzweig) J. Trop. Med. Hyg.-29: 343-347, 1980.

The preparation and storage of Plasmodium berghei sporozoites for immunization purposes is described. The sporozoites were harvested from the salivary glands of infected mosquitoes and maintained in cold tissue culture medium M199 with or without mouse serum. They were irradiated and frozen either at -75°C or in liquid nitrogen. After various periods sporozoites were thawed and injected into A/J mice. At the end of the immunization period the animals were challenged with infective sporozoites of P. berghei and monitored for parasitemia. It was found that the storage did not appreciably alter the ability of the irradiated sporozoites to induce protective immunity in the recipient animals. The highest protection (80 to 100%) was induced with sporozoites maintained in 10% serum and stored at -75°C.

Active immunization and passive transfer of resistance against sporozoite-induced malaria in newborn mice (by A.U. Orjih, A.H. Cochrane, and R.S. Nussenzweig). Nature 291: 331-332, 1981.

Newborn mice, ranging in age from 2 to 24 days, and adults were immunized intramuscularly (i.m) with γ -irradiated sporozoites, administered in 2 to 4 dosages. After the immunization, the animals were challenged with infective sporozoites. It was found that 88% of the young animals and 33% of the adults were protected. These experiments document that an effective sporozoite immunization is feasible in infants. Furthermore, our results indicate that, whereas i.v. administration of sporozoites is crucial for the successful immunization of adult animals, a more acceptable route, namely, i.m., is equally effective in

Litters born to and nursed by sporozoite-immunized mice acquired a high level of antisporozoite antibody, which was found to be of the IgG class. A considerable number of these newborn mice (42/69) were found to be protected against sporozoite challenge. These findings demonstrate, for the first time, that sporozoite-induced protection can be transferred naturally, from mother to offspring.

Comparative studies on the immunogenicity of infective and attenuated sporozoites of Plasmodium berghei (by A.U. Orjih & R.S. Nussenzweig) Trans. Roy. Soc. Trop. Med. & Hyg. (In press).

Experiments to characterize the immune response induced by the inoculation of infective sporozoites were undertaken in an experimental rodent malaria system. Since earlier work had shown that the blood phase of the infection suppresses the humoral antisporozoite response, the development of parasitemia was completely prevented by treating the animals with either chloroquine or primaquine shortly after sporozoite inoculation. The results obtained by immunization with infective *P. berghei* sporozoites were compared with those obtained using -irradiated sporozoites, by measuring the antibody levels of the immunized mice and determining their degree of protection against challenge.

The level of protection in animals immunized with infective sporozoites and submitted to chloroquine prophylaxis was found to be comparable to that induced with irradiated sporozoites. Under both conditions, a single intravenous inoculation of 10^4 sporozoites protected approximately 50% of the immunized mice from challenge with infective sporozoites. When two immunizing doses were given, over 90% of the animals from both groups were protected. Immunization of primaquine-treated animals with infective sporozoites resulted in a somewhat lower level of protection. The antibody response to inoculation of infective sporozoites was either comparable to or somewhat greater than that induced by irradiated sporozoites.

In vitro incubation of sporozoites with a high concentration of chloroquine abolished their infectivity. These chloroquine-attenuated sporozoites, however, were immunogenic and induced a protective immune response.

Antibodies to sporozoites: their frequent occurrence in individuals living in an area of hyperendemic malaria (by E.H. Nardin, R.S. Nussenzweig I.A. McGregor and J.H. Bryan) Science 206: 597-599, 1980.

Serum samples from 158 West Africans were tested for antibodies against sporozoites, the vector stage of the

malaria parasite. Antibodies specific for P. falciparum sporozoites were detected by means of the CSP assay and indirect immunofluorescence. More than 90 percent of the serum samples from adults gave positive immunofluorescent reactions against falciparum sporozoites, whereas most of the samples from children gave low or negative reactions.

Congenital transfer of antibodies against malaria sporozoites detected in Gambian children (by E.H. Nardin, R.S. Nussenzweig, J.H. Bryan and I.A. McGregor) Am. J. Trop. Med. Hyg. (In press).

A survey of the antibody response to sporozoites of P. falciparum was carried out using plasma samples obtained from twenty mothers and their newborn infants living in The Gambia, West Africa. Serological assays detected ant sporozoite antibodies in 90% (18/20) of the mothers and similar titers were detected in 17/18 of their infants. The ant sporozoite antibodies were gradually lost from the circulation of the babies until by approximately six months of age positive reactions against P. falciparum sporozoites were no longer detected. A similar pattern of acquisition and persistence of maternally-derived ant sporozoite antibodies was noted in a neonatal rhesus born to a female monkey immunized with sporozoites of P. knowlesi.

(ii) Studies involving the use of monoclonal antibodies

Our most recent area of research, supported since September, 1980 by an AID supplement, has involved the characterization, through the use of monoclonal antibodies, of sporozoite surface antigens, of rodent and more recently also of primate malaria. Some of these results have been published and are therefore only briefly summarized, others are in press, or in preparation.

Hybridoma produces protective antibodies directed against the sporozoite stage of malaria parasite (by N. Yoshida, R.S. Nussenzweig, P. Potocnjak, V. Nussenzweig and M. Aikawa) Science 207: 71-73, 1980.

Hybrid cells secreting antibodies against sporozoites of P. berghei were obtained by fusion of plasmacytoma cells with immune murine spleen cells. The monoclonal antibodies bound to a protein with an apparent molecular weight of 44,000 (Pb44), which envelopes the surface membrane of sporozoites. Incubation of sporozoites in vitro with antibodies to Pb44 abolished their infectivity.

Monovalent fragments (Fab) of monoclonal antibodies to a sporozoite surface antigen (Pb44) protect mice against malarial infection (by P. Potocnjak, N. Yoshida, R.S. Nussenzweig and V. Nussenzweig) J. Exp. Med. 151: 1504-1513, 1980.

Monoclonal antibodies (IgG1,) directed against a surface component of P. berghei sporozoites (Pb44) confer complete protection to mice against a lethal inoculum of parasites. The degree of protection is a function of the number of parasites used in the challenge and of the antibody concentration in serum. Passive transfer of 10 mg of antibody per mouse abolished or profoundly diminished the infectivity of 10^3 sporozoites, but much higher amounts of antibody were required for complete protection against challenge with 10^4 parasites.

Fab fragments of the monoclonal antibodies were as effective as the intact antibodies in mediating protection as determined by the neutralizing assay. This observation suggests that the antibodies interfere with a parasite function necessary for its infectivity, such as, for example, the ability to penetrate into the target cell or to multiply in the hepatocytes.

When sporozoites are incubated with the intact monoclonal antibodies at 37°C, a long filament appears at its posterior end (circumsporozoite precipitation (CSP) reaction). Fab fragments are ineffective at high concentrations. However, if after treatment with Fab, the sporozoites are incubated with rabbit antibodies to mouse κ -chains, a strong CSP reaction is observed. We conclude that the CSP reaction can result from the cross-linking of Pb44 and that it has the characteristics of a capping reaction followed by the shedding of the immune complexes.

The protective antigen of malarial sporozoites (Plasmodium berghei) is a differentiation antigen. (by M. Aikawa, N. Yoshida, R.S. Nussenzweig and V. Nussenzweig) J. Immunol. 126: 2494-2495, 1981.

Pb 44, the protective antigen of rodent malaria sporozoites (P. berghei) covers the entire surface of mature salivary gland sporozoites. This antigen is undetectable in approximately 50% of immature, i.e., oocyst sporozoites. On the surface of the remaining oocyst sporozoites, Pb44 is found in patches. Pb44 is present in early exoerythrocytic liver stages of P. berghei, but becomes undetectable at 30 hrs of intrahepatocytic development. It seems likely that Pb44 is a differentiation antigen associated with an unique function of the sporozoites, perhaps, penetration in the target host cells..

Biosynthesis of Pb44, the protective antigen of sporozoites of Plasmodium berghei (N. Yoshida, V. Nussenzweig & R.S. Nussenzweig) J. Exp. Med. (In press).

In a previous paper we identified a protective antigen (Pb44) of the surface membrane of sporozoites of P. berghei by means of a monoclonal antibody. Immunoprecipitation of extracts of mature salivary gland sporozoites metabolically labeled with ³⁵S L-methionine using the same monoclonal antibody revealed three specific polypeptides: Pb44,

Metabolically labeled Pb44 is probably identical to the protective antigen previously identified by surface labeling. Both proteins have the same molecular weights and isoelectric points under denaturing conditions and they share an epitope. Moreover, Pb44 also seems to be located on the cell membrane.

The results of pulse-chase experiments strongly suggest that Pb52 is the precursor of Pb44. The relationship between Pb54 and the protective antigen is unknown. The three polypeptides seem to be strictly associated with only one of the developmental stages of the parasite. They were not detected in blood forms and were found in minute amounts in sporozoites from the midgut of mosquitoes. In contrast, in mature salivary gland sporozoites they constitute main products of protein synthesis.

Monoclonal antibodies against a surface antigen of P. knowlesi sporozoites (by A.H. Cochrane, R.W. Gwadz, V. Nussenzweig & R.S. Nussenzweig) Fed. Proc. 40: 1011, 1981

We have recently obtained nine hybridomas by fusing spleen cells of mice immunized with P. knowlesi sporozoites with a mouse plasmacytoma cell line (NS-1). All of these monoclonal antibodies react with viable sporozoites of this simian malaria, as detected by indirect immunofluorescence. However, only 5 of these monoclonal antibodies produce a CSP reaction. The various hybridomas produce at least two different patterns of immunoprecipitation upon interaction with a metabolically labeled sporozoite extract. The infectivity of P. knowlesi sporozoites for rhesus monkeys was abolished upon incubation of the parasites with two of the hybridomas. A third, CSP negative, hybridoma failed completely to alter sporozoite infectivity. Five of the hybridomas also reacted with viable P. cynomolgi sporozoites. This cross-reaction had not been observed by using the sera of mice or rhesus immunized with P. knowlesi sporozoites.

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PROJECT 1. IMMUNIZATION WITH PURIFIED ANTIGENS OF
P. berghei SPOROZOITES

Overall goals and specific aims

The overall aim of this project is to induce protection in rodents against sporozoite challenge by immunization with Pb44, a purified surface antigen of P. berghei sporozoites. The internal sporozoite proteins Pb54 and Pb52, which are antigenically related to Pb44 (see Progress Report, pg. 17) will also be used in these vaccination procedures.

To achieve this goal we will pursue the following specific aims:

- a) develop methods to measure relative concentrations of Pb44, Pb52 and Pb54 based on the reactivity of these antigens with the monoclonal antibody 3D11.
- b) assay purified preparations of Pb44, Pb52 and Pb54 for their potential to induce a protective immune response, using different antigen preparations and a variety of schedules for immunization.
- c) monitor the response of immunized animals by measuring their antibody titers and cell-mediated immunity to Pb44 in order to determine whether they correlate with protection.

Rationale and significance

The present emphasis on the identification of "protective" sporozoite antigens, so that they might be used as a vaccine, is based on the assumption that immunization with purified antigen(s) will lead to protection against sporozoite challenge. The demonstration that passive transfer of monospecific antibodies directed against a defined surface antigen confers complete protection against sporozoite challenge (1), lends support to this hypothesis.

The proposed experiments will hopefully provide direct experimental evidence of the immunogenicity of purified sporozoite antigens, and establish optimal conditions for immunization to achieve protection. This approach will permit comparison of the efficacy of different antigen preparations.

Furthermore, we will attempt to measure the immune response to Pb44 in animals injected with X-irradiated

sporozoites. We wish to determine whether a good correlation can be established between protection and the levels of antibody to this antigen. This is to be expected if Pb44 is indeed the major immunogenic moiety of mature sporozoites.

Background and work in progress

To date, most of the successful attempts to achieve protection against sporozoite challenge have been based on the use of intact, attenuated sporozoites (2,3). Inoculation of non-attenuated viable sporozoites also induces protection, in addition to antibody formation. This was demonstrated experimentally by repeatedly infecting rodents with P. berghei sporozoites, and suppressing the blood phase of the infection by drug treatment (4-7). Of relevance to these experimental observations is the finding of anti-sporozoite antibodies in the sera of individuals in a malaria-endemic area (8). The titer of these antibodies was significantly higher in adults as compared to children, who are known to be more susceptible to malaria.

Very little experimental evidence is available, however, on other methods of immunization. Previous attempts to vaccinate mice by injecting mechanically disrupted sporozoites produced only minimal levels of protection (9). These experiments, though, were done under conditions which we presently know lead to rapid enzymatic degradation of Pb44, the protective antigen. Therefore, these negative results do not preclude the possibility of successfully immunizing mice with purified antigen(s).

The surface antigen of sporozoites of P. berghei (Pb44) and its precursor(s) have only recently been identified. In the course of these experiments, it was found that Pb44 shares a common antigenic site or epitope with the internal sporozoite proteins Pb52 and Pb54 (10). It would be reasonable, therefore, to assay all three sporozoite components as immunizing antigens.

Methods of purifying Pb44, Pb52 and Pb54, based on their characteristically low isoelectric point and their binding to monoclonal antibodies, have recently been developed. Furthermore, we have found that these antigens can be eluted from SDS-PAGE slab gels with a high degree of purification and very good yields. Under these conditions, the purified antigens retain their capacity to combine with the monoclonal antibody 3D11 (10). In view of these results, we will use these partially purified sporozoite antigens for immunization purposes. Hopefully, this will diminish the risk of fragmentation of the antigens by contaminating proteolytic enzymes.

Methods and procedures

a) Development of methods to measure Pb44, Pb52 and Pb54

We are presently standardizing a radioimmunoassay to measure Pb44 and the other immunologically-related polypeptides Pb52 and Pb54. The assay is based on the inhibition of the reaction between the monoclonal antibody 3D11 (which recognizes Pb44) and another monoclonal antibody, anti-3D11. We have found that sporozoite antigen very effectively inhibits the idiotype/anti-idiotype reaction. Indeed, the assay is sensitive enough to detect the antigen contained in about 100 sporozoites, and in sporozoites from individual mosquitoes. It is also very specific. No inhibition is observed with extracts from salivary glands of non-infected mosquitoes, nor with sporozoites of P. knowlesi or P. cynomolgi. Further work is necessary to develop a solid-phase radioimmunoassay which could be used routinely to perform large numbers of tests. It should be pointed out that this assay may also allow the detection of small amounts of protective antigens synthesized from the corresponding mRNA by in vitro translation systems and therefore facilitate cloning of the Pb44 gene.

b) Protection by immunization with purified sporozoite antigens

The demonstration that immunization with purified sporozoite antigens can indeed confer protection against sporozoite challenge is an essential and critical step within the context of developing a vaccine based on the use of isolated sporozoite antigens.

Two antigen preparations will be used for immunization: Pb44 and the mixture of Pb52 and Pb 54. Both will be obtained by immunoprecipitating the antigens from extracts of ³⁵S-methionine-labeled P. berghei with the monoclonal 3D11. The immunoprecipitates will be run on SDS-PAGE and the corresponding band will be eluted from the gel by the method described in (10). Initially, we will not attempt to separate Pb52 from Pb54 because they run very close to each other in the gels.

In view of the lack of any prior information, immunization will have to be approached empirically, using a variety of modes of antigen presentation (intravenous, subcutaneous, intradermal) and a series of adjuvants. Since Bordetella pertussis enhances the immune response to a variety of antigens, in addition to being non-toxic to man, we will attempt to combine the administration of this adjuvant with purified sporozoite antigens.

c) Correlation between protective immunity and humoral responses to sporozoite antigens

In order to verify whether Pb44 is indeed the major protective antigen of mature sporozoites, we will attempt to correlate protective immunity with levels of antibody to this antigen in animals injected with X-irradiated sporozoites. In addition, we will measure serum levels of antibodies to Pb44 in animals vaccinated with the purified antigen.

For this purpose we plan to develop a radioimmunoassay to measure antibodies to Pb44. The antigen will be purified from sporozoites as described in a previous section (pg). Microtiter plates will be pretreated with 1% glutaraldehyde solution and then incubated with purified antigen to obtain a satisfactory solid-phase coupled coat. The plates will be treated with 2% bovine serum albumin to eliminate nonspecific reactions to the solid support, after the plates have been treated with the fixative. The serum samples will be diluted in 0.5% bovine albumin containing 0.05% of Tween-20. The dilutions of serum samples will be added to the wells, and after appropriate incubation and extensive washings, excess of radiolabeled affinity-purified antibody to mouse Ig will be added. The wells will be washed and radioactivity counted in a gamma counter.

This is an assay which measures binding of antigen to antibody and is, in principle, independent of the class of antibody to serum. Therefore, we should expect to obtain a good correlation between protection and antibody titers if indeed our working hypotheses are correct; that is, that (i) protection is mediated mainly or exclusively by antibodies to Pb44 and (ii) that antibody functions by binding to Pb44 and not by initiating complement fixation, phagocytosis, etc. (1).

Alternatively, it is conceivable that immunization of mice with purified sporozoite antigens may also induce cell-mediated immune responses. In this case we may find that the animals immunized with the purified antigens develop resistance to sporozoite challenge in the absence of detectable levels of antibodies to Pb44. In this instance, we will attempt to develop methods for evaluating cell-mediated immune responses to Pb44, such as measuring foot-pad swelling in the immunized mice, and/or adapting in vitro correlates of CMI to this system.

Facilities Available

This research will be carried out in 400 square feet of laboratory space. All basic equipment to perform this work is already available or being requested.

PROJECT #1

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1961 - 1962: Research Fellow, Escola Paulista de Medicina, Department of Microbiology and Immunology.

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Publications:

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Publications:

Ojo, E. and Wigzell, H. 1978. Natural killer cells may be the only cells in normal mouse lymphoid cell populations endowed with cytolytic ability for antibody-coated tumor target cells. Scand. J. Immunol. 7: 297-306.

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- Doctoral disserations (monograph):
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Chilean Society of Immunology, 1976. Paper delivered: Dissociation of immunological memory and antibody secretion by treatment of the antigens with glutaraldehyde.

IX International Congress of Alergology, Buenos Aires, Argentina, November 1976. Paper delivered: Loss of immunogenicity of glutaraldehyde treated cells.

Publications:

Zavala, F. 1978. Rol de los macrofagos en la inducción de la respuesta immune. Rev. Medica de Chile 106: 58-61.

Ramos, A., Zavala, F. and Hoecker, G. 1979. Immune response to glutaraldehyde treated cells. I. Dissociation of immunological memory and antibody production. Immunology 36: 775.

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Publications:

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Ozaki, L.S., Maeda, S., Shimada, K. and Takagi, Y. 1980. A novel ColE1::Tn3 plasmid vector that allows direct selection of hybrid clones in E. coli. Gene 8: 301-314.

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Abstracts:

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PROJECT 2. CHARACTERIZATION AND ISOLATION OF THE PROTECTIVE ANTIGEN(S) OF P. knowlesi SPOROZOITES

Overall goals and specific aims

The overall goals of this project are to identify the protective antigen(s) of P. knowlesi sporozoites, to determine their functional role through the use of monoclonal antibodies and to isolate these antigen(s) for a variety of purposes.

Our research in this area will focus on the following specific aims:

- a) to obtain monoclonal antibodies directed against antigenic surface determinants of sporozoites of P. knowlesi.
- b) to determine the protective role of the monospecific antibodies and to define the relationship between their sporozoite neutralizing activity (SNA) and their capacity to induce the circumsporozoite precipitation (CSP) reaction.
- c) to surface and metabolically label sporozoites of P. knowlesi and to identify, by immunoprecipitation and gel electrophoresis, the surface membrane antigens and their precursors.
- d) to determine, using a solid phase inhibition radioimmunoassay, whether the various monoclonal antibodies recognize different epitopes of the protective antigen.
- e) to screen the monoclonal antibodies for cross reactivity with sporozoites of other malarial species, in particular with sporozoites of human malaria.

Rationale and significance

These studies with a simian malaria model will permit us to verify the more general validity of the findings obtained in relation to Pb44, the protective sporozoite surface antigen of P. berghei (see Progress Report pgs. 15-17 and Project #1 of this proposal). Comparison of the protective antigens of these rodent and simian malaria sporozoites might reveal common features, in spite of the dissimilarity of their antigenic determinants. Identification and isolation of the protective antigens of P. knowlesi sporozoites are also prerequisites for the further biochemical characterization of these antigens by peptide mapping (Project #3 of this proposal), characterization of the

antigenic determinants, amino acid sequencing, etc. all of which represent future necessary steps toward vaccine development.

The P. knowlesi ant sporozoite hybridoma system will also allow greater flexibility in experimental approach than hybridomas against sporozoites of the human malarial parasites. Sporozoites of P. falciparum, for instance, are still not readily available in large numbers in spite of the fact that recent advances in culturing the blood stages of these parasites have now yielded gametocytes that are infective to mosquitoes (1). Sporozoites of P. knowlesi, on the other hand, are easily obtainable in the laboratory in large numbers. In addition, P. knowlesi infections in rhesus monkeys provide a reasonably good experimental model for falciparum infections in humans.

Furthermore, mosquitoes infected with P. knowlesi carry large numbers of sporozoites in their salivary glands allowing for maximal parasite yield, with minimal amounts of microbial and mosquito tissue contaminants.

Finally, comparison of results obtained in the P. berghei system with preliminary data obtained with both P. knowlesi and anti-P. vivax hybridomas (Project #4 of this proposal), indicates a number of similarities among the three systems. Thus, future findings and data obtained with the P. knowlesi hybridomas will facilitate the characterization of the protective antigens of sporozoites of human malaria.

Background and work in progress

We have earlier shown that the multiple intravenous inoculation of sporozoites of P. knowlesi into rhesus monkeys induces a sterile immunity in these animals (2). A strong correlation was observed between ant sporozoite antibody levels in the immunized animals and their degree of protection. Using both ultrastructural observations (3, 4) and indirect immunofluorescence (5) we have shown that ant sporozoite antibodies are directed primarily, if not exclusively, against the surface membrane components of sporozoites of both rodent and primate malarial parasites.

Our findings on the protective surface antigen of P. berghei sporozoites using monoclonal antibodies, which have been the subject of various publications (6-8), have provided the background for the approach we are currently using with P. knowlesi sporozoites.

We initiated this research approximately one year ago and have obtained, by fusion of spleen cells of P. knowlesi sporozoite-immunized mice with a plasmacytoma cell line, a

series of 9 hybrid cell lines secreting antibodies to P. knowlesi sporozoites. The hybridomas were screened and selected by immunofluorescence, i.e., by the production of antibodies to sporozoite surface antigen(s). Most of these hybridomas have now been successfully cloned. Five of the hybridoma antibodies produce a positive CSP reaction when incubated with viable sporozoites. Several monoclonals have been tested in the sporozoite neutralization assay and some have been found to abolish sporozoite infectivity. These initial results were reported at the Hybridoma Workshop, during this year's AID meeting, and at the mini-symposium on "Characterization of Parasite Surface Antigens" at this year's FASEB meeting (9).

We have also obtained preliminary evidence indicating that some of the P. knowlesi hybridomas cross react with both glutaraldehyde-fixed and viable P. cynomolgi sporozoites, indicating the presence of shared epitopes between sporozoites of these two Plasmodial species.

Methods and procedures

a) To obtain monoclonal antibodies against antigenic surface determinants of sporozoites of P. knowlesi

We are using the method of Kohler and Milstein (10), as modified by Pontecorvo (11) to obtain monoclonal antibodies to P. knowlesi sporozoites.

In this procedure, Balb/c mice are immunized by multiple intravenous injections of viable sporozoites of P. knowlesi. The immune status of the mice is monitored by antisporezoite antibody levels as determined by immunofluorescence and the CSP reaction. Four days after the last immunizing dose, i.e., when high levels of these antibodies are reached, an animal is sacrificed. Immune spleen cells are recovered and fused with NS-1 plasmacytoma cells using polyethylene glycol-1000 as the fusing agent.

The assay used for the determination of antisporezoite antibody producing hybrid cell lines is the indirect immunofluorescent test. The antigen for the assay consists of glutaraldehyde-fixed sporozoites of P. knowlesi.

Most of the 9 cell lines secreting antisporezoite antibodies have now been cloned by the method of limiting dilution and have been injected intraperitoneally into Balb/c/DBA2 mice to obtain ascites. This assures the maintenance of the cloned cell lines and also results in very high monospecific antibody levels in the ascites and serum of the tumor-bearing mice. To accelerate and increase the production of ascites, mice are injected intraperitoneally with pristone several hours prior to the intraperitoneal injection of the cloned cells. The clones have also been frozen in liquid nitrogen as assurance

against possible loss of their capacity to produce antibody. Some of the monoclonal antibodies have been purified from the ascitic fluid by ammonium sulfate precipitation followed by anion exchange chromatography and filtration through a Sephadex G-200 column.

We are planning to perform additional fusions to obtain more monoclonal antibodies to further characterize sporozoite surface antigenicity of P. knowlesi and to determine the extent of shared surface epitopes between sporozoites of P. knowlesi and other primate malarias, in particular human malarias.

b) To determine the protective role of the monospecific antibodies and to define the relationship between their sporozoite neutralizing activity and their capacity to induce the CSP reaction

This specific aim is based on the findings that sera of sporozoite-immunized and protected mice or rhesus specifically abolish the parasite's infectivity. This is antibody mediated, as has been clearly documented by the fact that the incubation of sporozoites of P. berghei with small amounts of the purified monoclonal anti-Pb44 antibody, or its Fab fragments, results in the loss of infectivity of the parasites (7).

In both sporozoite-immunized rodents and rhesus monkeys, protection against challenge corresponds closely to the presence of sporozoite neutralizing activity in the sera of these animals. The monoclonal anti-P. knowlesi antibodies will be assayed for SNA and the relationship between this activity and CSP reactivity will be determined. In this procedure, infective sporozoites of P. knowlesi will be incubated with the various antibodies, or their Fab fragments, for one hour at room temperature and will then be injected intravenously into rhesus monkeys for determination of loss of infectivity. Four of the nine monoclonal antibodies have already been tested in this assay. Preliminary results indicate that a CSP positive hybridoma and two CSP negative hybridomas possess sporozoite neutralizing activity. A third CSP negative monoclonal failed to abolish sporozoite infectivity.

We plan to also determine the protective role of the monoclonal antibodies by the passive transfer of the purified antibodies to rhesus monkeys prior to sporozoite challenge. In the P. berghei-rodent system, passive transfer of rather small amounts of purified monoclonal antibody completely protects mice against subsequent sporozoite challenge (7).

c) To surface and metabolically label sporozoites of P. knowlesi and to identify, by immunoprecipitation and gel electrophoresis, the surface membrane antigens and their precursors

As mentioned above, three monoclonals have shown pro-

protective properties and they react with surface antigen(s) of sporozoites. Our aim is to identify the antigen(s) by immunoprecipitation of surface or metabolically labeled sporozoites.

Initial attempts to label the protective membrane antigen(s) of P. knowlesi sporozoites, using lactoperoxidase mediated iodination (12), have not yet yielded conclusive results. Modification of this technique is being considered, as well as the use of other iodination procedures, such as that described by Markwell and Fox (15).

With regard to metabolic labeling, ^{35}S -methionine, with high specific activity, will be added to P. knowlesi sporozoites suspended in RPMI-1640, modified by the omission of L-methionine and the addition of bovine serum albumin. Kinetics of the ^{35}S -methionine incorporation into TCA-precipitable protein will be determined as described by Burgess and Metcalf (14).

The sporozoite extracts, obtained by the addition of 0.5% NP40, will then be immunoprecipitated by the monoclonal antibodies, as well as by various control sera, including anti-P. knowlesi antisera obtained from an immunized and protected rhesus monkey and from immunized mice. A suspension of formaldehyde-treated Staphylococcus A, Cowan I strain (Staph A), will be used to bind immune complexes (15). SDS-PAGE will be performed in slabs as described by Laemmli (16).

The initial results indicate that the three protective monoclonals all immunoprecipitate the same three polypeptides from the extracts of metabolically labeled parasites. The CSP negative, non-protective hybridoma does not immunoprecipitate any ^{35}S -labeled moiety.

These findings have to be extended, using other monoclonals, to determine whether all protective antibodies recognize the same three polypeptides. In addition, we plan to determine which of the three proteins is present on the surface of the parasite, and whether there is a precursor-product relationship between them.

Two approaches can be used to determine if one or more of the polypeptides is found on the membrane of P. knowlesi sporozoites: (1) viable metabolically labeled sporozoites are incubated with the monoclonals, washed by centrifugation and extracted with NP40. In this situation the antibody should bind mainly to external antigen(s)--which should be the only one detected by immunoprecipitation. In contrast, when extracts of metabolically labeled parasites are incubated with the monoclonals, both external and internal antigens will be recognized. A comparison of the results

of immunoprecipitation obtained by SDS-PAGE of both kinds of extracts should indicate which polypeptide is on the membrane; (2) treatment of metabolically labeled sporozoites with low concentrations of proteolytic enzymes to remove or cleave surface proteins. After the treatment, the parasites will be extracted and immunoprecipitated. The gel patterns will then be compared with those obtained using control sporozoites not exposed to the enzyme.

In order to determine whether the two larger polypeptides are precursors of the smaller one, we will use the pulse-chase technique, as was used to study the protein synthesis of P. berghei sporozoites (17).

Finally, using the same methodology, that is, metabolic labeling and immunoprecipitation, we will try to determine whether these protective antigens are also present in sporozoites obtained from mosquito midgut oocysts. This may be important in order to obtain large amounts of m-RNA for the purpose of genetic cloning.

d) To determine, using a solid-phase inhibition radioimmunoassay, whether the various monoclonal antibodies recognize different epitopes of the protective antigen.

In this study, each monoclonal antibody will be assayed for its ability to inhibit the binding of a second monoclonal antibody to the sporozoite extract. The methodology to be employed is currently in use in Dr. V. Nussenzweig's laboratory, and is being applied to the study of P. berghei sporozoites.

In this procedure, microtiter wells are first coated with sporozoite extract obtained by repeated freeze-thawing of the parasites. Each well is washed with PBS containing BSA (1 mg/ml) to prevent non-specific binding of the monoclonals to be assayed.

Purified monoclonal antibodies will be radiolabeled with ^{125}I , using solid-phase lactoperoxidase-glucose oxidase to mediate the reaction (18, 19) and the amount of each labeled antibody required to saturate antigen binding sites will be determined. Inhibition of binding of labeled antibody by an excess of cold antibody of a second hybridoma will indicate competition between the two antibodies for the same or a spatially related determinant. No inhibition will indicate that the two antibodies are directed against separate epitopes.

These experiments may provide clues regarding the relative importance of the various domains of the antigen molecule in providing protection. For example, if one particular epitope is involved in entry of the sporozoite into the

target cell, certain hybridomas may be more effective than others in mediating protection.

e) To screen the monoclonal antibodies for cross-reactivity with sporozoites of other malarial species, in particular with sporozoites of human malaria.

This aim will be investigated by a number of approaches, including the CSP reaction and immunofluorescence, using both viable and glutaraldehyde-fixed sporozoites. Should shared antigenic determinants be detected, the anti-knowlesi monoclonals will be used for immunoprecipitation of labeled extracts of sporozoites of the various Plasmodial species, to allow comparison of the antigens being recognized. Cross reacting monoclonals will also be assayed for their ability to detect functional surface membrane antigens of the various sporozoites and to abolish parasite infectivity.

Facilities Available

The work proposed in this project is mainly performed in a 400 square foot laboratory, shared by two, and frequently more investigators. The laboratory is equipped for hybridoma work, containing our single laminar flow hood, and a CO₂ incubator. It also contains the equipment for gel-electrophoresis, a gamma counter, two refrigerators, and is undoubtedly very crowded. A Beckman brand scintillation counter is available for use in an adjacent corridor. A fluorescent microscope, cold room, insectaries, dissecting and phase contrast microscopes, etc. are all part of our common shared facilities.

This project, as well as our other proposed research in Projects #1 and #4, would benefit greatly from the availability of a separate hybridoma laboratory complete with adequate freezing facilities.

PROJECT #2

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PROJECT 3. NATURE OF THE PROTECTIVE ANTIGENS OF SPOROZOITES
OF DIFFERENT PLASMODIAL SPECIES

Overall goals and specific aims

The long-term goal of this project is to compare the structure of the protective antigens found on the surface of sporozoites of different Plasmodial species. Our current working hypothesis, based on observations to be described in the section entitled "Rationale and Significance," is that a single protein is the major constituent of the surface membrane of sporozoites and that this protein is involved in the penetration of the parasites into the target cells. If this were to be true, this molecule must have maintained through evolution certain common structural features essential for its function, in addition to species-specific characteristics. In other words, this family of molecules, which cover the entire surface of mature sporozoites of different species, may have common and variable domains.

A related goal is to determine the nature of the antigenic determinants (epitopes) recognized by monoclonal protective antibodies against these surface antigens. Our hope is that these antigens may contain immuno-dominant regions and that if a smaller immunogenic moiety is identified, the development of a vaccine against malaria will be facilitated. In the past, when monoclonal antibodies were not available, this might have been an impossible task. However, using hybridoma products we can now assay simultaneously for protection against infection and for the presence of an epitope on the immunogen.

For example, in the case of P. berghei we have identified a hybridoma (3D11) with protective properties and have found that it reacts with a surface antigen of sporozoites, Pb44. In principle, the portion of the molecule of Pb44 containing the epitope recognized by 3D11 could be used as a vaccine, provided, of course, that it retains its immunogenicity.

Another structural feature of Pb44, which may be of direct relevance to vaccine development, is the question of whether the molecule is glycosylated. If Pb44 is a glycoprotein, the sugar residues could be part of the protective epitope. This would be a complicating feature for the production of a vaccine by recombinant DNA technology.

Our specific aims, therefore, are twofold:

- a) to purify the protective antigen from three species

of Plasmodium (P. berghei, P. knowlesi and P. cynomolgi), to compare some of their physico-chemical properties and peptide maps.

b) to determine whether Pb44, the protective antigen of P. berghei, is glycosylated, and to determine the nature of the epitope of Pb44 which is recognized by the monoclonal antibody 3D11.

Rationale and Significance

What is the rationale for the hypothesis that a single polypeptide is the major constituent of the surface membrane of sporozoites and that the surface antigens from sporozoites of different species are structurally related?

To date, hybridomas secreting monoclonal antibodies against surface antigens of several species of malaria parasites have been obtained in our laboratory; that is, hybridomas against P. berghei, P. yoeli nigeriensis, P. knowlesi, P. vivax and P. falciparum. In every case, the assay which was used to obtain the hybridomas aimed at detecting, by immunofluorescence, antibodies reacting with any surface antigen of the parasite. A remarkable finding was that in most instances the monoclonal antibodies bound to antigens with similar characteristics (1,2,3), as summarized below:

a) The surface antigen uniformly covers the cell membrane of sporozoites as determined by immunofluorescence and by ultrastructural studies.

b) With few exceptions all hybridomas give CSP reactions after incubation with viable parasites. This indicates that the corresponding antigen is cross-linked by the monoclonals, and more importantly, that the cross-linking leads in every case to capping and shedding of the antigen. It should be pointed out that in other systems the surface events which lead to capping and shedding require energy and appear to involve the participation of intracellular organelles.

c) In three instances (P. berghei, P. knowlesi and P. y. nigeriensis), hybridomas which induce CSP reaction have protective properties. The hybridomas against the other species of malaria sporozoites have not been tested in neutralization assays.

d) By the two-dimensional O'Farrell technique the antigens defined by some of the hybridomas (P. berghei, P. knowlesi, P. y. nigeriensis) have low isoelectric points and molecular weights between 40,000 and 60,000. Actually, the positions of the spots in 2D gels, originating from the

immunoprecipitation of extracts of different parasites, are remarkably similar.

e) Finally, in two cases a structural similarity does indeed exist between these molecules. A hybridoma against P. y. nigeriensis surface antigen reacts with Pb44 from P. berghei. Also, a hybridoma against P. knowlesi surface antigen recognizes a membrane antigen of P. cynomolgi (4). Very encouraging is the observation that the cross-reacting hybridoma against P. y. nigeriensis protects mice also against challenge with P. berghei sporozoites (1).

Taken together these observations suggest that we may be dealing with a family of closely related surface molecules, which have an important function in the life history of mature sporozoites. However, direct evidence supporting this view is still missing. Some of the experiments which are part of this proposal may clarify this issue in a definitive fashion.

If our hypothesis is correct this could be of relevance to several aspects in the future development of a malaria vaccine. As already mentioned elsewhere, our hopes for obtaining large amounts of the putative protective antigen from sporozoites rely on the usage of recombinant DNA technology. If the experiments proposed in the present plan show that the protective antigens of all sporozoites are structurally related, the cloning of the relevant genes would be simplified. It would be likely that a fragment of one of the genes could be used as a probe to clone all the others. Equally important, many practical aspects of development of a vaccine, such as dosages, toxicity, route of immunization, etc. could be studied in animal models, using the product of the corresponding cloned gene, with greater assurance that the information obtained might be applicable to humans.

The rationale for aiming at the identification of the structural features of the epitope of Pb44 which reacts with 3D11 is obvious. If we can identify a small fragment of Pb44 which retains the immunogenicity of the whole molecule, this peptide could perhaps be synthesized. We are aware that the antigenic determinants reacting with 3D11 may be associated with a three dimensional structure originating from the folding of the polypeptide chain. In this case, a peptide resulting from the digestion of Pb44 may not be recognized by 3D11. For this reason, we plan to fragment Pb44 with several enzymes aiming at limited proteolysis. One attractive feature of this project is that Dr. Pedro Potocnjak has recently developed a quantitative assay for measuring the binding of antigen fragments to 3D11, which should render our task much easier (see following section).

Work in progress

Two recent developments in our laboratories have direct relevance to this project, since they should greatly facilitate the experiments proposed. First, we found that we can purify, perhaps to homogeneity and with excellent recoveries, the protective antigens from two malarial species, P. berghei and P. knowlesi. The technique used involves:

- (a) radiolabeling the antigens either metabolically or by surface iodination of the parasites
- (b) immunoprecipitation of the antigen with the corresponding hybridoma
- (c) subjecting the immune complexes to SDS-PAGE and radioautography
- (d) using a modification of the procedure described by Beemon and Hunter (5), to identify and elute the relevant antigen(s) from the dried slab gel.

We found that using this technique we can recover between 50 to 70% of the specific counts loaded on the gels and that more than 60% of these counts can be immunoprecipitated specifically by the hybridoma; that is, the eluted fragments retain the relevant epitope (6).

The second development is the recent isolation by Dr. P. Potocnjak of a hybridoma directed against the combining site of 3D11; that is, a hybridoma against an idiotype of 3D11. The technique for the production of this hybridoma will be described, in detail, elsewhere (7). In short, it involved first the production, in rabbits, of a polyclonal anti-idiotype of 3D11 by classical methods; then, using this polyclonal antibody to assay for the production of a mouse anti-idiotype monoclonal antibody. It should be pointed out that this is a unique reagent, since both 3D11 and the new hybridoma, which we call 1D12, were produced in Balb/c mice. The evidence that 1D12 (IgG2a, κ) reacts with or close to the combining site of 3D11, is given in Fig. 1, which demonstrates that the binding of 3D11 to 1D12 is specifically inhibited by extracts of P. berghei.

The sensitivity of this reaction seems high, since extracts from $2-5 \times 10^2$ sporozoites inhibit the interaction between 3D11 and 1D12. This radioimmunoassay appears particularly well suited to the identification of an epitope of Pb44, which reacts with 3D11. We expect that fragments of Pb44 containing the relevant epitope will also effectively inhibit the reaction between the two monoclonals.

Methods and Procedures

- a) Purification of protective antigens from P. berghei,

P. cynomolgi and P. knowlesi, and comparison of physico-chemical and peptide map characteristics

Partially purified sporozoites of different species will be metabolically labeled with different amino acids, as recently described (6). In addition to ^{35}S L-methionine, the use of ^3H -phenylalanine may also be convenient. This amino acid can be obtained with very high specific activity (more than 100 Ci/mM) and in many instances it is more common than tyrosine in proteins.

When P. berghei is metabolically labeled by incubation with ^{35}S -methionine for two hours at room temperature, three labeled polypeptides react with 3D11, the monoclonal antibody against Pb44. One of these polypeptides is the membrane-associated Pb44. The second polypeptide has a molecular weight of 52,000 and by pulse-chase experiments seems to be a precursor of Pb44. The third polypeptide, Pb54, shares some properties with Pb52, but its function is unknown (6). Preliminary experiments show that very similar results are obtained when P. knowlesi and P. cynomolgi are metabolically labeled (4).

The peptide mapping of the three polypeptides originating from the different species of parasites should provide answers to two important questions:

- a) the structural relationships between Pb54, Pb52 and Pb44. This may confirm the precursor-product relationship between Pb52 and Pb44, and clarify the nature of Pb54.
- b) the possible homology between the corresponding polypeptides in sporozoites of different species. The general methodology to be followed is already in use in our laboratory and is summarized below.

The radiolabeled parasites will be solubilized with non-ionic detergents in the presence of protease inhibitors. This material will be immunoprecipitated using monoclonal antibodies and Staphylococcus aureus, Cowan I. Immune complexes will be eluted from the bacteria with denaturing sample buffer and subjected to SDS-PAGE, under reducing conditions. Different concentration gradients of acrylamide will be used to get the best resolution of the specific bands. The electrophoretic run, processing of the gel, elution and tryptic digestion of the relevant protein bands will be done essentially as described by Beemon and Hunter (5). As already mentioned, we have eluted the purified polypeptides from SDS-PAGE slab gels, and the recoveries have been between 50-70% of the total specific counts loaded. After tryptic digestion of the denatured proteins, the method of choice for peptide analysis will be high performance

liquid chromatography (HPLC). Among the advantages of this method are the extreme reproducibility of the chromatograms obtained, the short time required for separation (between 10 to 90 minutes per injection), the excellent resolution capacity and the possibility of recovering fractions (peptides) with a high degree of purity.

If our hypothesis is correct we should find similarities in the HPLC profiles of antigens originating from different species of parasite. Although the HPLC-generated peptide maps are extremely reproducible, we will verify the identity of peaks by analyzing tryptic digests of mixtures of ^{35}S -methionine and ^3H -phenylalanine-labeled polypeptides from different species of Plasmodium.

b) To determine whether Pb44, the protective antigen of P. berghei, is glycosylated and the nature of the epitope of Pb44 recognized by 3D11.

To date we have no evidence that Pb44 is a glycoprotein. The intact parasite does not bind to lectin columns (8), nor is it stained by various fluorescein-labeled lectins (E. Nardin, unpublished observations). Also of some relevance is the observation that neuraminidase treatment of Pb44, Pb52 or Pb54 does not affect its isoelectric point. However, it could be argued that the sugar moiety of Pb44 is not accessible on the cell membrane, as occurs, for example, in the case of the glycoprotein which covers the surface membrane of African trypanosomes. Since the presence of carbohydrate in Pb44 can have practical importance in the development of a vaccine, some experiments will be performed in an attempt to clarify this issue.

Different experimental approaches will be used:

1. With regard to the question of whether Pb44 is glycosylated, two types of experiments will be performed. First, we will label membrane-associated Pb44 with ^{125}I and after immunoprecipitation we will isolate the purified polypeptide from slab gels as explained in the previous section. According to our preliminary results, we can recover more than 4×10^5 specific counts of purified material from a single preparation of about 4×10^6 sporozoites. The isolated labeled Pb44 will then be passed through columns bearing various lectins such as Con A, lentil lectin, wheat germ agglutinin, etc. If binding of Pb44 occurs, we will try to elute this antigen with specific sugars.

Second, we will study the incorporation of labeled galactose, fucose and glucosamine into Pb44 and related polypeptides (Pb54 and Pb52). Partially purified parasites

from salivary glands will be incubated with the labeled sugars, extracted with NP40, immunoprecipitated with the monoclonal 3D11; then the immune complexes will be subjected to SDS-PAGE and radioautography. A positive result will constitute strong evidence for glycosilation of the polypeptides. This material could be eluted, digested and chromatographed in the HPLC system for identification of carbohydrate-carrying peptides.

2. The other experimental approach aims at verifying whether carbohydrates are part of the Pb44 epitope recognized by the monoclonal antibody 3D11. To evaluate the dependence of serological reactivity on the polypeptide structure we will digest denatured Pb44 into smaller peptides with high concentrations of trypsin. The extent of digestion can be followed by SDS-PAGE in 20% gels and radioautography. These experiments will be performed with labeled Pb44, purified from slab gels. After denaturation and enzyme treatment, the digests will be immunoprecipitated with 3D11. If the antigenicity of Pb44 is resistant to the enzymatic treatment, this would imply that the epitope may be a carbohydrate or less likely, that it is part of a polypeptide chain resistant to proteolysis. Additional information will be gained by treating either purified Pb44 or the parasites with periodate, which oxidizes 1,2 dihydroxyethyl linkages of glycosyl residues. If this treatment abolishes or inhibits the antigenicity it will indicate that the epitope is a carbohydrate. Further experiments can be done treating the parasites or purified Pb44 with exoglycosylhydrolases, such as β -glucuronidase and β -galactosidase, which remove a terminal glycosyl residue from the non-reducing end of carbohydrate chains. If after these treatments the antigenicity is still preserved, the participation of carbohydrates in the reactivity of Pb44 with 3D11 should be considered unlikely. If this is found to be the case, our next aim will be to identify and isolate fragments of the Pb44 polypeptide chain which retain the epitope. For this purpose we will digest isolated radiolabeled Pb44 with proteolytic enzymes under conditions of limited reactivity, to produce large peptides. For example, we will subject Pb44 to trypsin for short periods of time at 25°C hoping that Pb44 may have a few unusually sensitive lysil or argynil peptide bonds. Since there is no standard set of conditions for limited proteolysis, we will also have to use various concentrations of other enzymes such as papain, subtilisin, Staphylococcal protease, pepsin, etc. Alternatively, large fragments of proteins can be obtained by selective chemical cleavage. For example, methionyl peptide bonds can be broken under relatively mild conditions using cyanogen bromide.

The reactivity of the mixture of resulting Pb44 peptides with 3D11 can be monitored in two ways: by immuno-

precipitation, or preferably by a radioimmunoassay in which we will estimate the inhibitory activity of the digest on the 3D11 idiotype/anti-idiotype reaction. In our experience, this reaction, which involves the inhibition of interaction between two monoclonal antibodies, is quite sensitive, specific and reproducible. If the relevant epitope of Pb44 resists limited proteolysis, we will try to isolate the reactive polypeptide by using reverse phase and/or size exclusion HPLC.

Facilities Available

This research will be carried out in 1,500 square feet of laboratory space in the Department of Pathology, New York University Medical Center, and in the Division of Parasitology, Department of Microbiology. All basic equipment to perform this work is already available in either laboratory, including an HPLC apparatus.

INHIBITION OF IDIOTYPE — ANTI-IDIOTYPE INTERACTION
 BY EXTRACTS OF P. BERGHEI

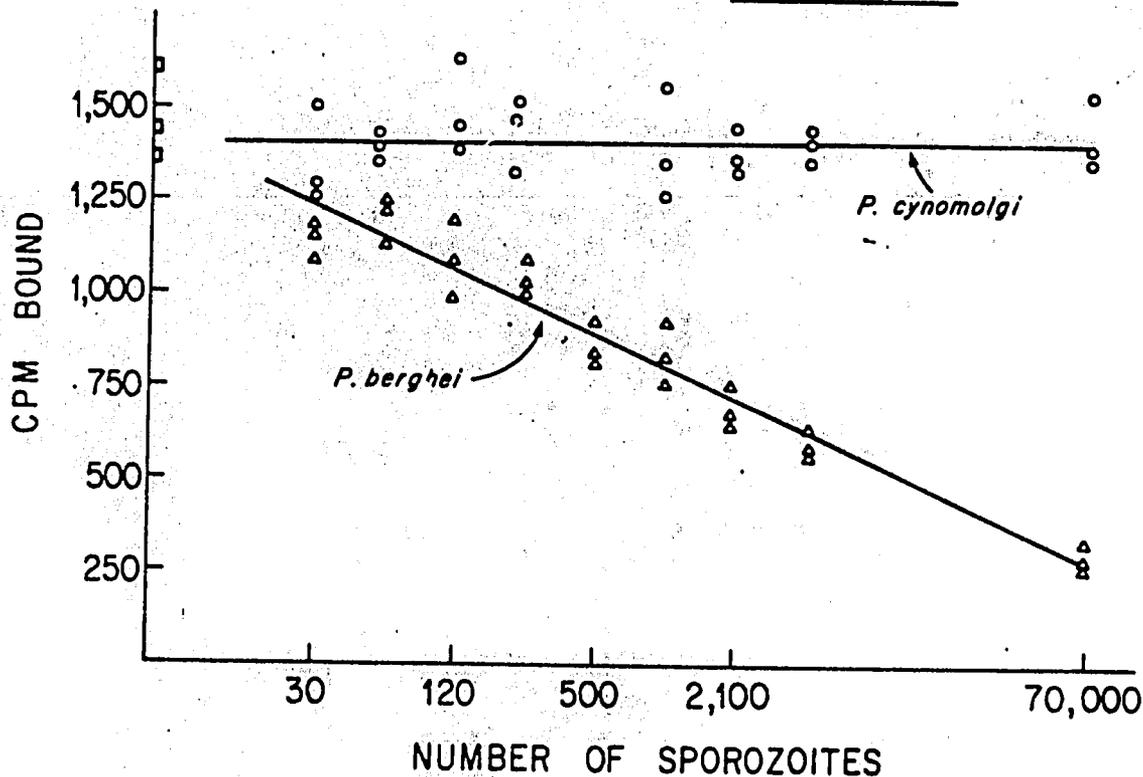


Figure 1. Extracts from purified sporozoites of P. berghei and P. cynomolgi were obtained by disrupting the parasites in a French pressure cell. 25 μ l of the dilutions of extract were added to wells of flexible plastic plates, which had been previously coated with the monoclonal antibody 3D11. After 2 hrs of incubation at room temperature, 1.5×10^4 cpm of 125 I-anti-idiotypic (specific activity, 2×10^7 cpm/ μ g) were added to the wells and the incubation proceeded overnight at 4°C. The plates were washed 3x with PBS containing 1% BSA and counted. The symbol \square represents controls in which only PBS-BSA was added to the 3D11-coated well.

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PROJECT #3

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PROJECT 4. CHARACTERIZATION OF THE PROTECTIVE ANTIGEN(S)
OF P. vivax SPOROZOITES

Overall goals and specific aims:

The overall aim of this project is to identify the protective antigen(s) of P. vivax sporozoites, for their future use in a malaria vaccine.

The specific aims of this project are:

a) to develop a series of monoclonal antibodies against the surface antigens of P. vivax sporozoites and to test the ability of these hybridomas to react with sporozoites of different geographic isolates of P. vivax and to cross-react with sporozoites of different species of simian and rodent malaria.

b) to test the functional activity of the hybridomas by determining if, after in vitro incubation of the P. vivax sporozoites with the monoclonal antibodies, sporozoite infectivity for a susceptible primate host is abolished.

c) to examine Saimiri sciureus (squirrel monkey) as an experimental host for the study of P. vivax malaria in order to:

1. utilize these animals to test the functional activity of the monoclonal antibodies (SNA).

2. ascertain the value of Saimiri as gametocyte donors to provide P. vivax sporozoites for the investigation of parasite antigens.

3. test the immunogenicity of purified antigen(s) of P. vivax sporozoites and define the conditions of immunization which will result in protection of squirrel monkeys against sporozoite challenge.

d) to use the monoclonal antibodies to characterize the surface antigens of P. vivax sporozoites and to compare these findings with the pattern of antigens detected by using polyvalent serum from sporozoite-immunized primates and serum of naturally infected individuals living in a P. vivax endemic area.

Rationale and significance

Studies on the immunity induced in man by the sporozoite stage of P. vivax and P. falciparum have shown that total protection can be obtained by exposure to the repeated bite of infected irradiated mosquitoes (1-4). Man, as well as rodents, immunized with sporozoites of P. vivax produce antibodies that react with the surface membrane of these parasites, as demonstrated by the (circumsporozoite precipi-

tation reaction). CSP reaction (4-6) and by indirect immunofluorescence (IFA) (7).

In rodent malaria, it has recently been demonstrated that passive transfer of monoclonal antibodies to a sporozoite surface antigen (Pb44) confers protection against sporozoite challenge (8). Based on these findings, and the recent identification of protective antigens of P. knowlesi sporozoites (9), we feel that using basically the same approach should lead to the identification of the protective antigen(s) of P. vivax sporozoites which is an essential step for mass producing antigens for use in a vaccine.

Standardization of P. vivax sporozoite-induced infections in a reliable non-human primate host will be necessary in order to assay sporozoite infectivity and determine the functional activity of the monoclonal antibodies. Earlier data on the susceptibility of splenectomized chimpanzees and squirrel monkeys to sporozoite-induced P. vivax infection indicate that these animals might provide promising experimental models (10-12). Vaccination of these animals with irradiated P. vivax sporozoites will also provide information on the antisporozoite response in an experimental non-human primate.

The prospect of vaccination with a purified protective antigen of P. vivax is based on the identification and characterization of this antigen which will then have to be mass-produced, possibly through the cloning of the gene coding for the protective antigen. For this purpose, viable P. vivax sporozoites must be obtained for immunizing mice as a source of immune spleen cells for hybridoma production, as well as for metabolic and surface labeling of the sporozoites with radioisotopes. The Saimiri/P. vivax model will hopefully provide a source of infective gametocytes for mosquito feeding and sporozoite production, in addition to those sporozoites which will be obtained in the course of the collaborative studies with Dr. Savanat and others (see Project #5).

Background and work in progress

The identification of a protective antigen of sporozoites of rodent malaras, P. berghei (8) and P. y. nigeriensis (13), and of a simian malaria, P. knowlesi (14), provides the basis for the work on the human malaria parasite P. vivax. Using the hybridoma technique of Kohler and Milstein (15), we have obtained a monoclonal antibody against P. vivax sporozoites (16). Viable sporozoites, obtained from Anopheles mosquitoes fed on a P. vivax-infected Aotus monkey, were used to immunize

Balb/c mice. Fusion of spleen cells of these immunized mice with NS-1 myeloma cells resulted in the formation of hybrid cells producing antibodies to P. vivax sporozoites. These hybrid cells were subsequently cloned and injected into mice. Serum from the hybridoma-bearing mice had high titers of CSP and IFA reactions with P. vivax sporozoites. These sera also reacted with sporozoites of P. vivax of different geographic isolates.

For the development of a malaria vaccine, it is important that protection would function against a broad range of parasite strains or isolates. Cross protection against different strains of P. vivax has been demonstrated, in vivo, in a sporozoite immunized volunteer (3). This cross-resistance was reflected in the humoral response of the sporozoite immunized host which gave positive CSP (3) and IFA (7) reactions with both homologous and heterologous isolates of P. vivax sporozoites.

The presence of serological cross-reactions between sporozoites of different P. vivax strains, detectable by both the serum of a sporozoite immunized individual and the anti-P. vivax monoclonal antibody, suggests the presence of shared antigens among sporozoites of different isolates of P. vivax. Preliminary findings using an extract of labeled P. vivax sporozoites indicate that both the polyvalent serum and the monoclonal antibodies immunoprecipitate the same set of P. vivax sporozoite antigens.

In contrast to the cross-reactivity between strains of P. vivax, protection and serological activity in the sporozoite immunized host is species specific (3, 4). Consistent with this species specificity, the polyvalent serum from the P. vivax sporozoite immunized host and the anti-P. vivax monoclonal antibody failed to react with P. falciparum sporozoites.

In some cases, however, monoclonal antibodies have detected cross-reactions between different species of malaria sporozoites which were not detectable using polyvalent serum from sporozoite immunized hosts. In the rodent system, one out of three monoclonal antibodies directed against sporozoites of P. y. nigeriensis cross reacted with P. berghei sporozoites (13). This monoclonal antibody was found to give a positive CSP reaction and to neutralize the infectivity of P. berghei, as well as the homologous P. y. nigeriensis sporozoites. In contrast, no cross-reactions were detected when the polyvalent serum from the sporozoite immunized mice was tested, nor was any cross-resistance to challenge with P. berghei sporozoites noted in the P. y. nigeriensis immunized mice.

A similar species cross-reactivity has also been detected using monoclonal antibodies directed against P. knowlesi

sporozoites which were found to cross-react with sporozoites of another simian malaria, P. cynomolgi (14). The polyvalent serum from the P. knowlesi sporozoite immunized rodents or rhesus did not show this cross-reactivity. The cross-reacting hybridomas did not react with sporozoites of P. berghei or P. coatneyi, another simian malaria.

Therefore, based on the findings in the rodent and simian system, we feel that by testing numerous hybridomas of P. vivax, it may be possible to detect monoclonal antibodies which cross-react with sporozoites of other primate malarias. The cross-reacting hybridoma could then be used to characterize the common antigenic determinants shared by sporozoites of different species of malaria.

In order to test whether the cross-reacting or homologous monoclonal anti-P. vivax antibodies are recognizing a protective surface antigen, it will be necessary to determine if the monoclonal antibodies abolish sporozoite infectivity. Neutralization of sporozoite infectivity in vitro (SNA) by monoclonal antibodies has been demonstrated in both the rodent and the simian systems (8, 13, 14). In the case of P. vivax, an experimental primate host model must be used to assess monoclonal antibody SNA activity. Chimpanzees have been shown to be susceptible to P. vivax infections initiated by the i.v. injection of salivary glands of heavily infected Anopheles, containing an unspecified number of sporozoites (10, 17). Splenectomy of the apes was required in order to allow the erythrocytic forms of vivax malaria to become detectable in blood smears. The splenectomized apes, injected i.v. with sporozoites, had a prepatent period similar to humans exposed to the bite of vivax-infected mosquitoes (10).

However, although the splenectomized chimpanzee appears to be a good model for functional assays of the activity of the monoclonal antibodies, the cost, handling and maintenance of the apes present a problem for more extensive studies of the host/parasite relationship. In view of the difficulties in using relatively large numbers of chimpanzees in experimental studies, it is important to attempt to establish a more practical simian model. Squirrel monkeys (Saimiri sciureus) have proven to be good candidates as experimental hosts for blood-induced infection with the human malarias (12, 18, 19). These monkeys have also been shown to be susceptible to sporozoites of P. vivax introduced by i.v. injection or the bite of infected Anopheles (11, 12). All stages of the parasite are detectable in the monkey and gametocytes infective to Anopheles mosquitoes have been obtained (11). Early studies, however, did not quantitate the number of viable P. vivax sporozoites required to initiate a sporozoite-induced infection. Therefore, it will be necessary

to define the susceptibility of the squirrel monkeys prior to their use in testing the sporozoite neutralizing activity of the monoclonal antibodies.

Squirrel monkeys will also provide a readily available host for the study of the antigenicity of P. vivax antigens isolated by using the protective monoclonal antibodies. The parameters of the Saimiri response to intact sporozoites will be assayed initially by immunizing the monkeys with irradiated P. vivax sporozoites. Squirrel monkeys have been found to develop ant sporozoite antibodies to a simian malaria, P. brasilianum, under natural conditions and can be immunized with irradiated sporozoites of this species (20). In addition, Saimiri monkeys can be readily karyotyped (21) and the anti-sporozoite response to P. vivax can therefore be studied in a defined host. It has been shown in the rhesus-P. knowlesi system that some monkeys are "good responders" to sporozoite antigens and make high levels of ant sporozoite antibodies, which are associated with immune resistance to sporozoite challenge (22). The ability to karyotype the squirrel monkeys may allow future studies on the in vivo role of humoral versus cell-mediated immunity in sporozoite immunized animals.

Methods and procedures

a) To obtain hybridomas and test for cross-reactivity:

The methods for obtaining monoclonal antibodies against antigenic surface determinants of P. vivax sporozoites are essentially the same as those described for rodent and simian malaria (see Project #2). Balb/c mice will be hyperimmunized with viable sporozoites of P. vivax and their spleen cells fused with myeloma cells. Screening for positive hybridomas will be carried out using the IFA assay, with glutaraldehyde-fixed P. vivax sporozoites as antigen. After cloning and expansion in mice, the pooled sera and ascites of the hybridoma-bearing mice will be purified by ammonium sulfate precipitation and passage on a Sephadex G-200 column. Fab fragments of the monoclonal antibodies will be obtained by papain digestion of the purified monoclonal antibody using the method of Nisonoff (23).

Strain cross-reactivity and species specificity of the anti-P. vivax monoclonal antibodies will be assayed using the CSP and IFA reactions. Viable sporozoites of P. falciparum and P. vivax of Thailand origin will be obtained through collaboration with Drs. Harinasuta and Savanat and their colleagues, as described in Project #5. Sporozoites will be brought back to New York University, or purified monoclonal antibodies will be sent to Thailand, for CSP and IFA assays

and testing using radiolabeled sporozoite extracts. Sporozoites of P. vivax of South American and other origins hopefully also be obtained from infected squirrel monkeys.

b,c) To test the functional activity of the hybridomas:

Functional assays of sporozoite neutralizing activity (SNA) will be carried out using Fab fragments of the monoclonal anti-P. vivax antibodies. These fragments have been shown to be more effective than intact IgG molecules in mediating sporozoite neutralization (14). As control, P. vivax sporozoites will be incubated in Fab fragments of non-cross-reacting monoclonal antibodies. Following incubation with the Fab fragments for 45 minutes at room temperature, the P. vivax sporozoites will be injected intravenously into splenectomized chimpanzees and/or squirrel monkeys. Blood smears will be examined daily to determine if the sporozoite infectivity was effectively neutralized by the anti-P. vivax Fab fragments.

Splenectomized chimpanzees can be obtained through our ongoing collaboration with Dr. Moor-Jankowski of LEMSIP (Laboratory of Experimental Medicine and Surgery in Primates) at New York University. Squirrel monkeys of known karyotype and of specific geographic origin will be provided through collaboration with the Pasteur Institute (Guyana). For the infection of squirrel monkeys, we will use a monkey-adapted strain of P. vivax obtained by Dr. J. Gysin. Infections of mosquitoes by direct feeding on vivax-infected monkeys will be attempted using A. stephensi from our mosquito colony and other Anopheles species, if necessary. Immunization of squirrel monkeys will be done through multiple exposure to the bite of irradiated P. vivax infected mosquitoes and/or by the intravenous injection of irradiated P. vivax sporozoites using the techniques described for the rhesus-P. knowlesi model (22).

d) To characterize the surface antigens of P. vivax sporozoites:

The characterization of P. vivax sporozoite antigens will be carried out using the techniques described for P. knowlesi (Project #2). The viable P. vivax sporozoites will be surface labeled with ^{125}I or biosynthetically labeled with ^{35}S -methionine (24). Immunoprecipitation of the radiolabeled extract will be carried out using the monoclonal antibodies, polyvalent serum from sporozoite-immunized primate hosts and serum of individuals living in endemic areas of malaria. After immunoprecipitation, the antigen/antibody complexes will be bound to Staph A (25), washed, eluted and processed by SDS-PAGE (26). The dried gels will be exposed to films and the pattern of the radioautographs compared.

Facilities Available

This research will be carried out in approximately 300 square feet of laboratory space. All basic equipment to perform this work is available or being requested.

PROJECT #4

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PROJECT III DETERMINATION OF THE ANTIBODY
RESPONSE TO SPOROZOITES OF P.FALCIPARUM
AND P.VIVAX OF POPULATIONS LIVING IN
ENDEMIC AREAS OF THAILAND

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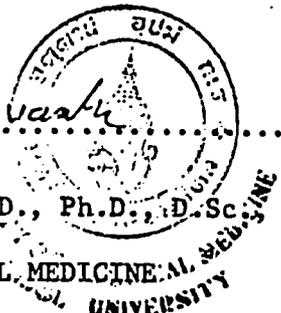
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17 April 1981

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PROJECT 5. SERO-EPIDEMIOLOGICAL INVESTIGATION OF SPOROZOITE-INDUCED IMMUNITY IN THAILAND

Overall Goals and specific aims

The overall goal of this project is twofold: a) to establish the patterns of the antibody response to sporozoites of P. falciparum and P. vivax of individuals who live in areas of high malaria endemicity in Thailand; and b) to obtain sporozoites of these two Plasmodial species as well as selected antisera from individuals with high ant sporozoite antibody titers, for the purpose of identifying, in collaboration with the New York University Medical Center group, the protective antigen(s) of P. falciparum and P. vivax.

Data on antibodies to P. falciparum sporozoites, to be obtained in various regions of Thailand, will be compared with earlier results obtained in the Gambia (1, 2). This will be of considerable interest, in view of the profound differences in the patterns of malaria transmission, as well as in the use of chemotherapy in these two countries.

No prior information is available on the immune response to P. vivax sporozoites, except on a small number of immunized volunteers (3, 4). The planned study will therefore provide the first sero-epidemiological data on the ant sporozoite antibody levels of individuals exposed repeatedly under natural conditions to the bites of P. vivax-infected mosquitoes.

The proposed research will focus on the following specific aims:

a) To determine the incidence and titers of ant sporozoite antibodies in individuals of different age groups, repeatedly exposed to malarial infection

All sera will be tested for their interaction with sporozoites of P. falciparum and P. vivax using both the indirect immunofluorescent antibody test and the circumsporozoite precipitation reaction. In the future, these results might be compared with those to be obtained by using a radioimmunoassay to detect ant sporozoite antibodies. Such an assay, based on the use of monoclonal antibodies and identification of the respective immunodominant sporozoite surface antigen, is presently being developed for the P. berghei system. (See Projects #1 and #3). Additional monoclonal antibodies and further information on the antigens of sporozoites of human malaria to be obtained in the course of Project #4 and Drs. R.S. Nussenzweig and E.H. Nardin's ongoing NIH-sponsored study on P. falciparum sporozoites, should, in the future, make it feasible to adapt this radioimmunoassay to the human malaria system.

b) To investigate the persistence of ant sporozoite antibodies

The sera of individuals who left an endemic area for long periods of time will be analyzed with regard to anti-sporozoite antibody levels. These findings will be compared with the corresponding data obtained from individuals of the same age group, who have remained in the endemic area.

c) To ascertain the occurrence of common antigens shared by sporozoites of different geographic isolates of the same malarial species

Sera which react with sporozoites of various Thai isolates of P. falciparum and/or P. vivax will also be tested with African and South American isolates of these parasites, using both the CSP and IFA reactions. If available in sufficient amounts, these parasites will be radio-labeled and immunoprecipitated with monoclonal antibodies for comparison of their antigenic make-up.

d) To compare the patterns of immunoprecipitation produced by the interactions of P. falciparum and P. vivax sporozoite extracts with individual immune sera

These patterns will define the immunodominant sporozoite antigens, i.e., those antigens which in most individuals induce antibody formation.

This work will be performed in close collaboration with Drs. E. Nardin and R.S. Nussenzweig at New York University. Such an approach, in addition to allowing for corroboration of experimental findings, will enable us at the same time to work with sera of individuals with high ant sporozoite antibodies selected in the course of the sero-epidemiological study.

e) To characterize the protective antigen(s) of sporozoites of P. vivax and P. falciparum

This objective will be approached by testing monoclonal antibodies, directed against defined sporozoite antigens, for their sporozoite neutralizing activity. This research objective will be undertaken in collaboration with the investigators working on Project #4 in New York, namely, Drs. E. Nardin, R.S. Nussenzweig and J. Gysin.

Rationale and significance

Data to be obtained on the incidence of antibodies to P. falciparum and P. vivax sporozoites in the sera of

individuals living in an endemic area will provide baseline information on the level of the antsporozoite immunity of this population. Such baseline data will be invaluable for monitoring the effects of a sporozoite vaccine as soon as such a vaccine becomes available for pilot studies. Furthermore, these sero-epidemiological data will provide a means for monitoring the effects on malaria transmission of control measures such as drug treatment and/or insecticide application.

Determination of baseline data on antsporozoite antibody levels in individuals of different age groups is also a prerequisite for investigating a number of related questions, namely, the seasonal variation of antibody titers, and the persistence of antsporozoite antibodies.

The sero-epidemiological study using blood samples obtained from individuals living in an endemic area will also provide a source of positive sera for determining the species specificity of the IFA and CSP reactions, and the sharing of sporozoite antigen(s) among different geographic isolates of the same Plasmodial species. It is necessary to further explore and corroborate earlier data on the sharing of surface antigens of sporozoites of P. falciparum of different geographic isolates, particularly in light of the recent findings of extensive strain heterogeneity detected in blood stages of P. falciparum (5). Variation in blood-stage parasite proteins, detectable by two dimensional gel electrophoresis, has been observed not only among isolates from different geographic areas, i.e., The Gambia and Thailand, but also amongst isolates from the same Thai area.

The availability of positive sera will also permit us to establish the repertoire of sporozoite antigens recognized by antibodies present in individual sera and detected by immunoprecipitation of a labeled sporozoite extract. Those antisera, having high levels of antsporozoite antibodies based on their CSP and IFA reactivity, will be selected for immunoprecipitation. The corresponding patterns, detected by autoradiography, will be compared with those obtained using the sera of volunteers immunized and protected against challenge with either P. falciparum or P. vivax sporozoites. They will also be compared with the patterns of immunoprecipitation resulting from the interaction of labeled sporozoite extract with the respective monoclonal antibodies recently obtained by Dr. E. H. Nardin at New York University. These findings should clarify whether sporozoites of human malaria have a single or a small number of immunodominant antigens, as seems to be the case for P. berghei and P. knowlesi sporozoites.

At a later stage, when the protective antigens of P. vivax and/or P. falciparum sporozoites will have been identified, it will become feasible using immunoprecipitation and radiolabeling techniques to determine if individuals living in malaria endemic areas produce antibodies directed against these functional antigens. In the future, it will be particularly important to determine whether these "protective" antibodies are present prior to and/or upon vaccination, and to measure their serum levels.

Furthermore, the age distribution of ant sporozoite antibodies in the sera of individuals from an endemic area will be an important factor in determining the optimal age at which vaccination should be initiated.

Background

Sporozoites, in spite of being short-lived, are highly immunogenic, so that the repeated exposure to the bite of infected mosquitoes induces a detectable antibody response and a sterile immunity (3,4,6,7).

The occurrence of ant sporozoite antibodies in the sera of individuals living in an area of high malaria endemicity has recently been demonstrated in serological studies, performed in The Gambia, West Africa (1). Sera of villagers living in an area in which P. falciparum is endemic, were found to have significant levels of antibodies against the sporozoite stage of this parasite. The level of ant sporozoite activity was shown to increase gradually with age. Positive CSP reactions and high IFA titers (1:512) against P. falciparum sporozoites were observed in a large percentage of adults.

In contrast, little or no CSP activity and low (1:8) IFA titers were noted in the majority of children in the 10 to 15 year old age group. In several of the adult Gambians studied, high titers of anti-P. falciparum sporozoite antibodies were detected in the absence of both parasitemia and a significant antibody response to P. falciparum-infected red blood cells.

The species specificity of the serological reactivity was demonstrated by the failure of glutaraldehyde-fixed or viable P. falciparum sporozoites to react with antisera raised against sporozoites of P. vivax and P. knowlesi. The falciparum sporozoites also failed to react with normal sera obtained from individuals living in a non-endemic area.

Immunosuppression has for years been recognized as one of the serious side effects of malaria. It has been found to occur as a selective suppression of the antibody response

to some, but not all antigens, and has been reported to lower the immune response to measles vaccine and tetanus toxoid (8). In rodents we have found that this malaria-induced immunosuppression also markedly affects the host response to plasmodial sporozoite antigens. Mice inoculated with blood forms of P. berghei fail to develop antisporezoite antibodies and protection upon subsequent immunization with irradiated sporozoites (9, 10). If the blood phase of the infection is curtailed by drug treatment, antisporezoite antibodies appear or increase in titer, and the immunized animals develop resistance to sporozoite challenge.

These findings raise the question whether the acute phase of malaria infection in man also interferes with his immune response to certain plasmodial antigens. If so, the clinical course of the disease would modulate, and in the absence of treatment, delay the development of antisporezoite antibodies and protective immunity. We suspect that this might in fact be occurring in The Gambia, where until recently, only the severe cases of malaria were treated upon hospitalization. If this interpretation of the role of immunosuppression is correct, early intervention with chemotherapy in the course of a malaria infection would result in a more rapid occurrence of antisporezoite antibodies, i.e., their presence in younger age groups. This question may be clarified by determining antisporezoite antibody levels in the sera of individuals living in areas of Thailand where chemotherapy is routinely administered.

Work in progress

The determination of antisporezoite antibody levels in the sera of individuals living in an endemic area, namely, Khao Kan-Riang, Kanchanaburi in Thailand, has in fact been initiated late last year. A total of 54 serum samples have been screened for CSP reactivity, and the positive sera have been titrated. The overall percentage of CSP reactivity seems to be relatively low.

These and additional sera from individuals of different age groups will subsequently be tested by immunofluorescence, using glutaraldehyde-fixed sporozoites of both P. falciparum and P. vivax. A research microscope, equipped for both phase contrast and fluorescence, will be used for this purpose. This microscope, provided by a WHO Director's Initiative Fund, has very recently been received at the Department of Microbiology and Immunology, Mahidol University.

A few months ago, a batch of P. vivax sporozoites was obtained by feeding mosquitoes through a membrane on infected blood drawn from a hospitalized patient.

These have been metabolically labeled with ^{35}S -methionine, and have been taken to New York University for antigenic analysis, using monoclonal antibodies produced against P. vivax sporozoites of South American origin.

A number of preparations of P. falciparum sporozoites have also been labeled with ^{35}S -methionine and sent to the Division of Parasitology of New York University. One of these labeled P. falciparum sporozoite preparations has been extracted and subsequently been immunoprecipitated with a monoclonal antibody produced against P. falciparum sporozoites. Another monoclonal antibody, reacting with P. vivax sporozoites, was used as control. This was followed by SDS-gel electrophoresis of the immunoprecipitates and autoradiography. Under these conditions, the anti-P. falciparum hybridoma detected one major labeled antigen. The anti-P. vivax hybridoma failed to react with the labeled P. falciparum extract.

Viable P. falciparum sporozoites, of Thai origin, are being used in an ongoing neutralization experiment at the Laboratory for Experimental Medicine and Surgery in Primates (LEMSIP) at New York University. In this study, sporozoites incubated with monoclonal antibodies to falciparum sporozoites were subsequently inoculated into splenectomized chimpanzees for determination of loss of infectivity.

Methods and procedures

a) Preparation of sporozoites

Anopheles balabacensis mosquitoes will be reared in the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, and infected with blood of malaria patients with either P. falciparum or P. vivax, according to the technique of Chomcharn (11). Infected salivary glands containing mature sporozoites will be dissected in either M199 or methionine-free RPMI-1640 medium, pooled, triturated, and then centrifuged at 800 rpm for 5 min. at 4°C to remove major mosquito tissue contaminants. The supernatant will then be centrifuged at 8000 rpm for 20 min. at 4°C. Sporozoites, contained in the sediment, will be used in the CSP and IFA tests and for metabolic labeling purposes.

b) Preparation of hyperimmune sera in laboratory animals

Mice will be immunized against sporozoites of either P. falciparum or P. vivax by intravenous injections of approximately 10^5 partially purified parasites given at weekly intervals. The ant sporozoite response of the mice will be monitored by the CSP reaction and the IFAT. One week following the last booster, when ant sporozoite antibody titers are high (CSP 1:20; IFA 1:4,000) the animals will be bled for sera to be used as positive controls for the CSP and IFA tests.

c) Antisporozoite antibodies occurring in individuals repeatedly exposed to malarial infection

Sera to be used in this study will be recovered both from patients hospitalized with malaria and from individuals living in malaria-endemic areas.

With regard to hospitalized patients, we plan to use one hundred individuals of different age groups, with either falciparum or vivax malaria, who have been admitted to the Bangkok Hospital for Tropical Diseases. Upon verification of infection by blood film examination, patients will be immediately treated with appropriate antimalarials, and kept in the hospital for a period of observation. Control subjects will include individuals who have not been in a malaria endemic area. They will be recruited from the Faculty members of Mahidol University and/or blood donors of the Thai Red Cross.

For determination of antisporozoite antibody levels of the hospitalized patients, we will use sera collected on days 0, 30 and 60 of patient observation as part of a separately funded sero-epidemiological and ongoing study on antibodies to blood-stage parasites. All sera will be stored at -20° C aliquots to be used for a variety of purposes. Whenever there is a recrudescence or reinfection, blood samples will be taken and the follow up period extended accordingly. Clinical and epidemiological data to be obtained by questioning the patients, their relatives or government agencies will include: 1) determination of the endemic area(s) frequented by the patient (2) duration of stay (3) chronology of the first, subsequent and last malarial attacks (4) chemotherapy, i.e., drugs used, schedule and duration of treatment and (5) the malarimetric index and intensity of transmission in each endemic area.

The occurrence of antisporozoite antibodies in the sera of individuals living in malaria endemic areas will also be determined. The areas envisaged for study include (1) Khao KanRiang, Kanchanaburi, West Thailand (2) Nong Rhee, Kanchanaburi, West Thailand and (3) a village in Chanthaburi, East Thailand. At least 100 finger-pricked samples will be taken approximately every four months from individuals in each study area in Nateson capillary tubes (270 μ l), and the plasma obtained will be stored at -70° C until use. Samples collected in this manner will allow us to determine the seasonal variation in levels of antisporozoite antibodies.

The presence of antibodies to sporozoites of P. falciparum and P. vivax will be detected by CSP (12) and by indirect immunofluorescence (13). For both of these tests, undiluted sera will initially be used. When positive reactions occur,

titers will be determined. Serological tests to detect antibodies to blood stage parasites of P. vivax and P. falciparum will include the IFAT, using the Druilhe modification of the Ambroise-Thomas (1974) technique (14) currently being standardized in our laboratory, and the indirect hemagglutination antibody test of Farshey and Kagan (15). This latter procedure is routinely used in our laboratory.

d) Persistence of antisporozoite antibodies

Antisporozoite antibody levels occurring in the sera of individuals living in malaria endemic areas will be compared with those found in the sera of individuals of the same age group who, at earlier time points, had left endemic areas. For this study, we plan to use one or more of the endemic areas previously proposed. The collection of plasma samples and determination of antisporozoite antibodies will be as described above.

e) The sharing of common antigens by geographic isolates of sporozoites

Sera, having positive CSP and IFA reactions against Thai isolates of P. falciparum and P. vivax sporozoites, will be selected from serum samples obtained both from people living in endemic areas and from hospitalized patients. Subsequently they will be tested, using the IFAT, against sporozoites of Gambian and South American isolates of P. falciparum and P. vivax. If feasible, the study will be extended to sporozoites isolated from North Thailand through collaboration with Dr. Barbara Jacobs, a Peace Corps volunteer in The Malaria Division, Ministry of Public Health, and to sporozoites of African origin to be provided by Dr. P. Druilhe, Paris.

f) Immunoprecipitation Patterns of P. falciparum and P. vivax sporozoite extracts obtained by interaction with individual immune sera

Serum samples obtained from a number of patients with high levels of antisporozoite antibodies against vivax or falciparum sporozoites will allow us to determine the repertoire of sporozoite antigens recognized by these antibodies. Extracts of sporozoites of either P. falciparum or P. vivax, metabolically labeled with ³⁵S-methionine, will be immunoprecipitated with the various sera as well as with sera of volunteers immunized and protected against challenge with either falciparum or vivax sporozoites. The immunoprecipitated antigens will be subjected to SDS-PAGE. The autoradiographs obtained by immunoprecipitation using endemic sera will be compared with the patterns obtained using the respective monoclonal antibodies.

g) Characterization of protective antigen(s) of P. vivax and P. falciparum sporozoites using monoclonal antibodies

This study will be done in collaboration with Drs. E.H. Nardin and R.S. Nussenzweig at New York University Medical Center. Our laboratories at Mahidol University will provide sporozoites to be used in parasite neutralization experiments. In these studies, sporozoites of P. vivax and P. falciparum will be incubated with the respective monoclonal antibodies, recently obtained by Dr. Nardin, and then injected into chimpanzees for determination of loss of infectivity. Gel electrophoresis, using radiolabeled parasite extracts precipitated by the protective monoclonal antibodies, will allow identification of the protective antigens of these parasites.

Facilities available

1. Department of Clinical Tropical Medicine and the Hospital for Tropical Diseases

The Hospital for Tropical Diseases has a total of 150 beds. The number of patients hospitalized with malaria at any given time is approximately fifty. Most patients have falciparum malaria. A number of these patients also have a concomitant infection with P. vivax. As soon as infections are diagnosed the appropriate antimalarials are immediately administered. The Hospital for Tropical Diseases will provide the sera for serological studies and the gametocyte-containing blood (+ 1 ml) for membrane feeding of mosquitoes for sporozoite production.

2. Department of Microbiology and Immunology

The existing facilities include two -70° C freezers for serum storage, one cold room, two refrigerated centrifuges, one Zeiss fluorescent microscope, an Eppendorf microcentrifuge, chromatography and electrophoresis kits for purification of antigen, ultracentrifuges and a set up for maintaining erythrocytic stages of P. falciparum in culture.

3. Department of Medical Entomology

This Department has an insectorium for the rearing and housing of mosquitoes. A small colony of Anopheles balabacensis together with several other Anopheles species, are presently being maintained in the facility. The mosquito-rearing facilities, upon modification, will be able to accommodate the larger number of A. balabacensis necessary for the proposed project. Facilities are also available for the membrane feeding of mosquitoes for sporozoite production. In addition, there are well trained personnel for the dissection of salivary glands from infected mosquitoes for sporozoite recovery.

PROJECT #5

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Education:

University of Medical Sciences, Bangkok (presently Mahidol University)	M.D.	1959	Medicine
University of Adelaide, Adelaide, So. Australia	Ph.D	1966	Microbiology

Major Research Interest:

Immunology of Parasitic Infections

Research and/or Professional Experience:

1978 - present: Professor and Head, Department of Microbiology and Immunology, Mahidol University, Bangkok.

1973 - 1977: Assistant Professor and Head, Department of Microbiology and Immunology, Mahidol University, Bangkok.

1970 - 1972: Acting Head, Department of Microbiology and Immunology, Mahidol University, Bangkok.

1960 - 1969: Instructor, Department of Tropical Hygiene, Faculty of Tropical Medicine, Bangkok.

Honors:

Mahidol University Award for Research, 1977
Gold Medal Award (for top student throughout the medical course), 1959

Publications:

(more than 20 publications from 1964-1974 in parasitic infections)

Savanat Tharavanij, Chaicumpa, V. 1975. Mechanisms of immunity to cholera. J. Med. Ass. (Thailand) 58: 59.

Jatinandana, V. and Tharavanij, S. 1975. Antigenicity of subcellular components of Entamoeba histolytica. Southeast Asian J, Trop. Med. Publ. Hlth. 6: 40.

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University of Medical Sciences, Bangkok	M.D.	1944	(Medicine)
School of Tropical Medicine, Liverpool, G.B.	D.T.M&H	1953	

Professional Society Membership(s):

National

President, 1975-77; 1977-79), Parasitology and Tropical Medicine of Thailand
Vice President (1975-77; 1977-79), The Thai Medical Women Association
Chairman, FUND Raising Committee (1972-74), The Thai Medical Women Association
Adviser, Family Planning, The Thai Medical Women Association
Vice President (1974-76), Medical Association of Thailand
President (1969-71, 1971-73; 1973-75), The Thai Medical Women Association
Member, Committee on Medical Ethics, Medical Council of Thailand
Member of 3 sub-committees of Medical Council of Thailand

International

Member, Scientific Working Group (Malaria). (1967, 1972, 1976, 1978)
Member, Scientific Working Group of WHO, SEARO (1976, 1977, 1978)
Temporary Adviser of WHO, SEARO, November, 1976
Member, WHO Expert Panel of Malaria (1975-present)
Member, WHO TDR Evaluation Advisory Committee, 1978
Editor-in-Chief, "The Southeast Asian Journal of Tropical Medicine and Public Health" since 1973.
Advisor, Roche Far East Research Foundation.
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Special lecturer, TROPMED Postgraduate Courses in SEAMEO member countries.

Past: Vice Rector on Academic Affairs, Mahidol University (1976-78)
Deputy Dean, Faculty of Tropical Medicine, Mahidol University (1968-78)
Director, Bangkok School of Tropical Medicine
Professor, Tropical Medicine, Head, Department of Clinical Tropical
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Director, Hospital for Tropical Diseases (1965-78)
Lecturer, Department of Medicine and Pediatrics, Faculty of Medicine
University of Medical Sciences (Mahidol University)
House Physician, Department of Medicine (including Department of
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Consultant in Medicine, Nakhon Ratchasima Hospital; Somdej Na Sriraj;
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Publications:

Hand Book (in Thai) of Tropical Medicine.

Chemotherapy in Tropical Medicine, Editor.

Papers in Medical Journals: Malaria, Amoebiasis, Chemotherapy of Helthminthic Infections, etc. (over 100 in total)

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Immunity to malaria sporozoites

Research and/or Professional Experience:

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1978 - 1980 International Research Fellow, Department of Immunology,
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Publications:

Sawyer, W.D., Thongthai, C., and Tapchaisri, P. 1973. Gonococci and
Gonorrhoea. J. Med. Ass. Thailand 56: 404.

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Tapchaisri, P. and Sirisinha, S. 1976. Serum and secretory antibody res-
ponses to Neisseria gonorrhoeae in patients with gonococcal infections. Brit.
J. Vener. Dis. 52: 374.

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Education:

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1972 Staff Member, Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok

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Publications:

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Chulalongkorn University, Thailand	B.Sc.	1969	(Biology)
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Purdue University, Lafayette, Ind.	M. Sc.	1974	(Entomology)
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Professional Society Membership(s):

Gamma Sigma Delta, The Honor Society of Agriculture (USA)
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Major Research Interest:

Characterization of the antibody response to Plasmodium falciparum sporozoites in Thailand, and identification of the protective antigen(s).

Research and/or Professional Experience:

1981 - present: Research Assistant, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

1978-1979: Instructor, Department of Biology, Faculty of Science, Prince of Songkla University, Thailand

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Publications:

Asavanich, A.P. and Gallun, R.L. 1979. Duration of feeding by larvae of the Hessian fly and growth of susceptible wheat seedlings. Ann. Entomol. Soc. Am. 72: 218-221.

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Education:

Mahidol University, Bangkok, Thailand	B.Sc. (X-ray Technology)	1977
Mahidol University, Bangkok, Thailand	M.Sc. (Tropical Medicine)	1979

Research and/or Professional Experience:

1980 - present: Research Assistant, Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University.

Publications:

Areekul, S., Subcharoen, A., Cheeramakara, C., Srisrakawot, K. and Limsuwan, S. 1980. Studies on the effect of folic acid supplement on folate and vitamin B12 status in children. Southeast Asian J. Trop. Med. Pub. Hlth. 11:81.

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SUMMARY
OF CORE BUDGET
RESEARCH PROJECTS #1-5 OF PROGRAM PROJECT

Proposal	Admin. & Core	Project #1	Project #2	Project #3	Project #4	Project #5	Total
Principal Investigator	Nussenzweig	Nussenzweig	Cochrane	Ferreira	Nardin	Savanat	
PERSONNEL	82,100.	42,015.	37,550.	34,183.	30,564.	12,860.	239,272.
FRINGE BENEFITS	18,472.	9,455.	8,450.	7,691.	6,877.	-	50,945.
CONSULTANTS	1,750.	-	-	-	-	-	1,750.
EQUIPMENT	72,695.	5,800.	4,050.	7,700.	3,000.	1,000. ¹	94,245.
SUPPLIES	23,520.	7,775.	8,275.	15,000.	8,250.	12,274.	75,094.
TRAVEL	14,990.	2,000.	650.	1,300.	1,300.	5,000.	25,240.
OTHER EXPENSES	60,540.	8,054.	15,573.	7,925.	25,956.	4,000.	122,048.
1st 12 Months	274,067.	75,099.	74,548.	73,799.	75,947.	35,134.	608,594.
3 Year Total	765,292.	238,149.	235,584.	239,713.	247,470.	122,187.	1,848,395.

¹ Additional equipment for Project #5 is included in year one of the Core Budget.

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CORE BUDGET
First Year
 January 1, 1982-December 31, 1982

Personnel

R.S. Nussenzweig, Professor Project Director	40%	25,056.	
Victor Nussenzweig, Professor Project Co-Director	-	-	
R. Altszuler Laboratory Supervisor	100%	21,950.	
A. Warner, Laboratory Aide	50%	9,389	
S. Hecht-Ponger Administrative Assistant	90%	17,870.	
Secretary (to be appointed)	50%	7,835.	\$ 82,100.

Fringe Benefits (22.5%) 18,472.

Refurbishment of existing laboratory space
 converted to Hybridoma Facility (preparative work) 31,000. 31,000.

Equipment (Hybridoma Facility)

Inverted Phase Microscope with accessories	2,000.	
Binocular Microscope with accessories	3,415.	
Baker Laminar Flow Hood	5,200.	
CO ₂ Double Chamber Incubator	5,130	
-20° Refrigerator (double door)	1,050	
Tabletop Refrigerated Centrifuge with accessories	6,000.	22,795.

Equipment (General)

Air conditioner replacement	650.	
Darkroom equipment (i.e., Omega enlarger, copier; grain focuser, easel, etc.)	2,500.	
Miscellaneous equipment (i.e., conductivity meter; pump(s); oven, columns, etc.)	6,500.	9,650.

Equipment (to be purchased on behalf of Mahidol University)

Department of Medical Entomology

Humidifier	1,000.	
Balance, digital	2,000.	
Circulating Bath	750.	
Stereo Dissecting Microscope w/access.	2,000.	5,750.

Department of Microbiology & Immunology

Blower for Hood	1,500.	
Binocular Microscope with accessories (Olympus BH-A)	2,000.	3,500.

-continued-

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(First Year continued)

CORE BUDGET

Maintenance (of)

Animals

Mice (500 animals x 365 days @ .053 per day)	9,675.	
Hamsters (150 animals x 365 days @ .148 per day)	8,103.	
Rats (50 animals x 365 days @ .148 per day)	2,701.	
Rabbits (6 animals x 365 days @ .69 per day)	1,511.	
Primates (28 animals x 365 days @ .995 per day)	10,170.	32,160.
Veterinary and Medical Consultants	1,750.	1,750.
<u>Insectary</u> (includes Insectary Personnel, light traps, Culligan H2O system, cages, etc.)	20,435.	20,435.

Maintenance and Service Contracts (Equipment)

Typewriter and IBM Memory 100	400.	
Compact Picker Gamma Counter (100%)	2,135.	
Beckman Ultracentrifuge (33.3%)	410.	
Scintillation Counter (33.3%)	550.	
Spectrophotometer & Recorder (66.6%)	950.	
Cost of emergency repairs to equipment, exclusive of above service contracts	3,500.	7,945.

Supplies

Radioisotopes, labeled amino acids, fluorescein-conjugated antibodies	10,000.	
Plastics, glassware, chemicals, etc.	7,270.	
General supplies for Mahidol University (unavailable in Bangkok, Thailand)	3,500.	
Office, including photocopy paper, books, journals, charts, etc.	1,250.	
Freight charges for shipment of goods and services to Mahidol University, Bangkok, Thailand	1,500.	23,520.

Travel

Domestic

Approx. (18) trips to Washington, D.C. as part of collaboration with B. Gwadz, NIH.	2,800.	
Approx. (2) trips to collaborative research centers (i.e., Texas, Georgia)	2,000	4,800.

Foreign

Trip for Project Director to Bangkok, Thailand (including accommodations)	3,670.	
Trip for Principal Investigator of one of Projects to Bangkok, Thailand (including accommodations)	3,670.	
Airfare (only) for Thai investigator to visit New York University	2,850.	10,190.

Subtotal

\$274,067.

CORE BUDGETSecond Year

January 1, 1983-December 31, 1983

Personnel

R.S. Nussenzweig, Professor Project Director	40%	25,056.	
V. Nussenzweig, Professor Co-Project Director		-	
R. Altszuler, Laboratory Supervisor	100%	23,706.	
A. Warner, Laboratory Aide	50%	10,140.	
S. Hecht-Ponger, Admin. Assistant	90%	19,300.	
Secretary	50%	8,462.	86,664.

Fringe Benefits (22.5%)

19,499.

Equipment

Refrigerated Chamber Unit (Kelvinator)	2,100.	
-70° Freezer	5,980.	
Air conditioner replacement	750.	
Ice maker	2,200.	
Densitometer DCD-16, Computing (Gelman)	6,550.	
Fluorescent Microscope with accessories	12,500.	
Miscellaneous equipment (refrigerator, columns, etc)	7,450.	37,530.

Maintenance (of)Animals

Mice (500 animals x 365 days @ .056)	10,220.	
Hamsters (150 animals x 365 days @ .163)	8,925.	
Rats (50 animals X 365 days @ .163)	2,975.	
Rabbits (6 animals x 365 days @ .72)	1,580.	
Primates (28 animals x 365 days @ 1.04)	10,630.	
Veterinary and Medical Consultants	1,750.	36,080.
<u>Insectary</u> (includes Insectary Personnel, light traps, Culligan H ₂ O system, cages, etc.)	21,457.	21,457.

Maintenance and Service Contracts (Equipment)

Typewriter and IBM Memory 100	420.	
Compact Picker Gamma Counter (100%)	2,242.	
Beckman Ultracentrifuge (33.3%)	430.	
Scintillation Counter (33.3%)	578.	
Spectrophotometer & Recorder (66.6)	998.	
Cost of emergency repairs to equipment, exclusive of above service contracts	3,500.	8,168.

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Supplies

Radioisotopes, labeled amino acids, fluorescein- conjugated antibodies	11,500.	
Plastics, glassware, chemicals, etc.	8,360.	
General supplies for Mahidol University (unavailable in Bangkok, Thailand)	4,025.	
Office, including photocopy paper, books, journals charts, etc.	1,440.	
Freight charges for shipment of goods and services to Mahidol University, Bangkok, Thailand	1,575.	26,900.

TravelDomestic

Approx. (18) trips to Washington, D.C. as part of collaboration with B. Gwadz, NIH	2,940.	
Approx. (2) trips to collaborative research centers (i.e., Texas, Georgia)	2,100.	5,040.

Foreign

Trip for Project Director to Bangkok, Thailand (including accommodations)	3,855.	
Airfare (only) for Thai investigator to visit New York University	2,995.	6,850.

Subtotal

\$248,188.

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CORE BUDGET

Third Year

January 1, 1984-December 31, 1984

Personnel

R. S. Nussenzweig, Professor Project Director	40%	25,056.	
V. Nussenzweig, Professor Co-Project Director		-	
R. Altszuler, Laboratory Supervisor	100%	25,602.	
A. Warner, Laboratory Aide	50%	10,950.	
S. Hecht-Ponger, Admin. Assistant	90%	20,844.	
Secretary	50%	9,140.	91,592.

Fringe Benefits (22.5%)

20,608.

Equipment

Bausch Lomb Spectrophotometer	4,300.	
Mouse rack with accessories	2,400.	
LKB Fraction Collector with accessories	6,000.	
Miscellaneous equipment (i.e., minigel, autoclave, dishwashing machine, etc.)	8,600.	21,300.

Maintenance (of)

Mice (500 animals x 365 days @ .059)	10,770.	
Hamsters (150 x 365 days @ .179)	9,800.	
Rats (50 animals x 365 days @ .179)	3,270.	
Rabbits (6 animals x 365 days @ .76)	1,655.	
Primates (28 animals x 365 days @ 1.10)	11,245.	
Veterinary and Medical Consultants	1,750.	38,490.
<u>Insectary</u> (includes Insectary Personnel, light traps, Culligan H ₂ O sytem, cages, etc.)	22,530.	22,530.

Maintenance and Service Contracts (Equipment) ..

Typewriter and IBM Memory 100	440.	
Compact Picker Gamma Counter (100%)	2,354.	
Beckman Ultracentrifuge (33.3%)	450.	
Scintillation Counter (33.3%)	606.	
Spectrophotometer & Recorder (66.6%)	1,047.	
Cost of emergency repairs to equipment, exclusive of above service contracts	3,500.	8,397.

-continued-

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Supplies

Radioisotopes, labeled amino acids, fluorescein-conjugated antibodies	13,225.	
Plastics, glassware, chemicals, etc.	9,615.	
General supplies for Mahidol University (unavailable to Mahidol University, Bangkok, Thailand)	4,630.	
Office, including photocopy paper, books, journals, charts, etc.	1,655.	
Freight charges for shipment of goods and services to Mahidol University, Bangkok, Thailand	1,655.	30,780.

Travel

Domestic

Approx. (18) trips to Washington, D.C. as part of collaboration with B. Gwadz, NIH	3,090.	
Approx. (2) trips to collaborative research centers (i.e., Texas, Georgia)	2,205.	5,295.

Foreign

Trip for Project Director to Bangkok, Thailand (including accommodations)	4,045.	4,045.
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Subtotal

\$243,037.

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Budget Justification

Core

First Year

Personnel

The Program Director, R.S. Nussenzweig's salary, is requested in accordance with maximal salary guidelines of the AID's regulations.

All other salary increments in years 2 and 3 are based on estimated percentages including merit and inflationary factors. Percentages applied vary dependent upon job description, time and grade, etc. for each employee. Actual salaries to be paid in future years must be in compliance with official wage and salary programs consistently applied at New York University Medical Center.

Refurbishment and Equipment

The ongoing research involving a large number of monoclonal antibodies makes it necessary to refurbish existing space into a Hybridoma Facility. The space to be converted for this purpose is presently a conference room, without any laboratory facilities or equipment. We hope to remodel the space and acquire the necessary equipment so that it can serve adequately the various investigators of Projects #1, #2 and #4.

Our general equipment requirements for the first year are to replace an air conditioner over 10 years old; purchase much needed darkroom equipment in order to more easily process our autoradiographies and other general photographic needs.

Several pieces of major equipment are being requested on the Core Budget, for Mahidol University. Our purpose in doing so is to facilitate the purchase so that the needed equipment is made available more promptly to our collaborators in Project #5. The Department of Entomology's equipment request is basically derived from the need to upgrade the Insectorium for the rearing of A. balabacensis. While the needs for the Department of Microbiology and Immunology are related to the preparation of monoclonal antibodies reacting with P. falciparum and P. vivax as well as labeling of parasite preparations.

Maintenance

Animals and Insectary: Since 1979, we established a Parasitology Animal and Insectary Service fund. All salaries of the animal facility and insectary personnel, as well as all goods, services, food, bedding, repairs, etc. are prorated for each species of animal and/or insectary use, based on usage by each investigator. The per diem rates are given in the Core Budget for the estimated number of animals and insectary needs to be used in the overall Program Project for the course of each year.

Budget Justification

Core

Maintenance (continued)

Maintenance contracts and service agreements are for the general shared equipment used by the various investigators in Projects #1-4.

Supplies

The supplies listed in the Core are essential for our hybridoma work, and will be dispersed over the entire Program Project.

Travel

Domestic trips are related to collaborative work with other laboratories.

Foreign travel requested is one trip for the Program Director, Dr. R.S. Nussenzweig, and one Principal Investigator (of Projects #1-4) in order to be in a position to collaborate effectively with Drs. Harinasuta, Savanat and others.

A trip for Dr. Pramuan (transportation) is included herein (1st year). The remainder of his expenses will be absorbed from other funds. The investigator will spend approximately 2-3 weeks at the Division of Parasitology and Department of Pathology, in order to learn various methods and procedures. This training is invaluable for the overall scope of the collaborative project.

Second Year

Personnel

See explanatory note in first year.

Equipment

Equipment requested for this year includes items that will either replace overutilized pieces, and/or help in the growing Division's scope of research. Our Cold room, for example, shared by all the Division's faculty, is overcrowded, and as we cannot foresee having the space for another, we wish to utilize the alternative of a Cold Chamber; the need for a -70°C freezer in the second year is due to the overcrowding of the existing freezer in the Division. A second fluorescent microscope is much needed because the single available Zeiss microscope is insufficient for all the investigators and research; consequently overextending the one available. More than 7 investigators are using it presently, and with the increased workload, the availability of another microscope is essential.

Travel

A trip for Dr. Chomcharn is included for the second year. As explained above, Dr. Chomcharn's other expenses will be absorbed from other funds.

Budget Justification

Core

Third Year

Personnel

See explanatory note in first year.

Equipment

A Spectrophotometer is necessary for the general use of the Program Project investigators in Projects #1, #2 and #4. The spectrophotometer presently available in the Division is shared with several members of the Division faculty, and will not serve our expanding needs. A fraction collector is imperative in view of purification of antibodies and F(ab) fragments required for all Projects conducted in the Division of Parasitology.

Travel

See explanatory note in first year.

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PROJECT #1 BUDGET
First Year
 January 1, 1982-December 31, 1982

Personnel

R.S. Nussenzweig, Professor Principal Investigator	15%	-	-
E. Ojo-Amaize, Ph.D. Postdoctoral Fellow	100%	1,800.	
F. Zavala, M.D., Postdoctoral Fellow	20%	3,500.	
L. Shozo Ozaki, Ph.D. Postdoctoral Fellow	100%	20,500.	
M. Maracic, M.S., Sr. Res. Tech.	40%	7,910.	
Res. Tech. I (to be appointed)	50%	8,305.	42,015.

Fringe Benefits (22.5%)

9,455.

Equipment

Isoelectric Focusing Power Supply	1,500.	
Mettler Precision Balance	2,000.	
Sonicator	2,300.	5,800.

Animals (Purchase)

A/J mice (1,200 animals @ \$3.29 each)	3,948.	
Hamsters (375 animals @ \$3.75 each)	1,406.	
Rabbits (20 animals @ \$27.50)	550.	5,904.

Supplies (glassware, plastics, chemicals, radioisotopes, etc.)

7,775. 7,775.

Travel

Attendance at scientific meetings of professional personnel	2,000.	2,000.
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Other

Photocopying, chart preparation, etc.	300.	
Publication costs	600.	
Cost of emergency repairs to equipment, exclusive of maintenance agreements	1,000.	
Books and Journals	250.	2,150.

Subtotal

\$75,099.

757

PROJECT #1 BUDGET

Second Year

January 1, 1983-December 31, 1983

Personnel

R.S. Nussenzweig, Professor Principal Investigator	15%		
E. Ojo-Amaize, Ph.D. Postdoctoral Fellow	100%	1,950.	
F. Zavala, M.D., Postdoctoral Fellow	20%	3,780.	
L. Shozo Ozaki, Ph.D. Postdoctoral Fellow	100%	22,140.	
M. Maracic, M.S., Sr. Res. Tech.	40%	8,545.	
Res. Tech. I (to be appointed)	50%	8,970.	45,385.

Fringe Benefits (22.5%)

10,212.

Equipment

Refrigerated Circulator	1,850.	
Microfuge I (Beckman)	1,950.	3,800.

Animals (Purchases)

A/J mice (1,200 animals @ \$3.62)	4,344.	
Hamsters (375 animals @ \$4.13)	1,548.	
Rabbits (20 animals @ \$30.25)	605.	6,497.

Supplies (glassware, plastics, chemicals, radioisotopes,
etc.)

8,940. 8,940.

Travel

Attendance at scientific meetings of professional personnel	2,100.	2,100.
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Other

Photocopying, chart preparation, etc.	300.	
Publication costs	600.	
Cost of emergency repairs to equipment, exclusive of maintenance agreements	1,000.	
Books and Journals	250.	2,150.

Subtotal

\$79,084.

PROJECT #1 BUDGET
Third Year
 January 1, 1984-December 31, 1984

Personnel

R.S. Nussenzweig, Professor Principal Investigator	15%	-	
E. Ojo-Amaize, Ph.D. Postdoctoral Fellow	100%	2,100.	
F. Zavala, M.D, Postdoctoral Fellow	20%	4,085.	
L. Shozo Ozaki, Ph.D. Postdoctoral Fellow	100%	23,912.	
M. Maracic, M.S., Sr. Tech. Tech.	40%	9,226.	
Res. Tech. I (to be appointed)	50%	9,687.	49,010

Fringe Benefits (22.5%) 11,027.

Equipment

Savant Concentrator-Evaporator	2,000.	2,000.
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Animals (Purchases)

A/J mice (1,200 animals @ \$3.98)	4,776.	
Hamsters (375 animals @ \$4.53)	1,698.	
Rabbits (20 animals @ \$33.25)	665.	7,139.

<u>Supplies</u> (glassware, plastics, chemicals, radio-isotopes, etc.)	10,285.	10,285.
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Travel

Attendance at scientific meetings of professional personnel	2,205	2,205.
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Other

Photocopying, chart preparation, etc.	325.	
Publication costs	650.	
Cost of emergency repairs to equipment, exclusive of maintenance agreements	1,050.	
Books and Journals	275.	2,300.

Subtotal \$83,966.

759

Budget Justification

Project #1

First Year

Personnel

Dr. R.S. Nussenzweig, Program Director, and Principal Investigator of Project #1, will have her salary covered in the Core Budget.

E. Ojo-Amaize and F. Zavala will have partial support from this Project. The balance of their salaries is derived from other sources.

All other personnel, please see explanatory note in Core Budget Justification.

Equipment

The sonicator will be used to disperse the gel containing purified antigen for immunization purposes. The power supply for electrophoresis needs to be added to the existing electrophoresis apparatus. The precision balance is for general laboratory use.

Travel

In this Project, the travel will be used for the 3 postdoctoral fellows, namely, Drs. Ojo-Amaize, F. Zavala and L.S. Ozaki.

Second Year

Personnel

See explanation first year.

Equipment

The cooling device is necessary for some electrophoresis procedures; Microfuge will be for general laboratory use.

Travel

See explanation first year.

Third Year

Personnel

See explanation first year.

Equipment

The concentrator/evaporator will "concentrate" serum samples as well as antigen eluates.

Travel

See explanation first year.

760

PROJECT #2 BUDGET

First Year

January 1, 1982-December 31, 1982

Personnel

A.H. Cochrane, Ph.D. Associate Professor Principal Investigator	70%	25,680.	
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V. Nussenzweig, M.D., Ph.D. Professor, Co-Investigator		-	
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M. Maracic, M.S. Senior Research Technician	60%	11,870.	37,550.
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Fringe Benefits (22.5%)

8,450.

Equipment

Electrophoresis Chamber and Power Supply		1,050.	
ph meter		650.	
Beckman Microfuge I		1,700.	
Air conditioner replacement		650.	4,050.

Animals (Purchase)

Macaca mulatta (10 animals @ \$900. each)		9,000.	
Freight charges for the shipment of <u>M. mulatta</u>		1,500.	
SW mice (150 animals @ \$.87)		130.	
Balb/c mice (150 animals @ 4.55 each)		683.	
CD ₂ F ₁ mice (600 animals @ \$3.42)		2,060.	13,373.

Supplies (glassware, plastics, chemicals, general materials, isotopes, amino acids, tissue culture medium, etc.)

8,275. 8,275.

Travel

Attendance at scientific meetings of professional personnel		650.	650.
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Other

Photocopying, chart preparation, slides, etc.		350.	
Publication costs		500.	
Cost of emergency repairs to equipment, exclusive of maintenance agreements		1,000.	
Books and Journals		350.	2,200.

Subtotal

\$74,548.

PROJECT #2 BUDGET
 Second Year
 January 1, 1983-December 31, 1983

Personnel

A.H. Cochrane, Ph.D. Associate Professor Principal Investigator	70%	27,735.	
V. Nussenzweig, M.D., Ph.D. Professor; Co-Investigator		-	
M. Maracic, M.S. Senior Research Technician	60%	12,815.	40,550.

Fringe Benefits (22.5) 9,124.

Equipment

Entomologic microscope with accessories	1,100	1,100.
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Animals (Purchases)

<u>Macaca mulatta</u> (10 animals @ \$900. each)	9,000.	
Freight charges for the shipment of <u>M. mulatta</u>	1,600.	
SW mice (150 animals @ \$.96 each)	147.	
Balb/c mice (150 animals @ \$5.00 each)	750.	
CD ₂ F ₁ mice (600 animals @ \$3.76 each)	2,256.	13,753.

Supplies (glassware, plastics, chemicals, general materials, isotopes, amino acids, tissue culture medium, etc.) 9,520. 9,520.

Travel

Attendance at scientific meetings of professional personnel	685.	685.
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Other

Photocopying, chart preparation, slides, etc.	350.	
Publication costs	500.	
Cost of emergency repairs to equipment, exclusive of maintenance agreements	1,000.	
Books and Journals	350.	2,200.

Subtotal \$76,932.

762

PROJECT #2 BUDGET
 Third Year
 January 1, 1984-December 31, 1984

Personnel

A.H. Cochrane, Ph.D. Associate Professor Principal Investigator	70%	29,954.	
V. Nussenzweig, M.D, Ph.D. Professor; Co-investigator		-	
M. Maracic, M.S. Senior Research Technician	60%	13,840.	43,794

Fringe Benefits (22.5%)

9,854.

Equipment

Inverted Phase Microscope with accessories	2,300
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Animals (Purchases)

Macaca mulatta (10 animals @ \$900. each)	9,000.	
Freight charges for the shipment of <u>M. mulatta</u>	1,700.	
SW mice (150 animals @ \$1.05 each)	157.	
Balb/c mice (150 animals @ 5.50 each)	825.	
CD2F1 mice (600 animals @ \$4.14 each)	2,484.	14,166.

Supplies (glassware, plastics, chemicals, general materials, isotopes, amino acids, tissue culture medium, etc.)	10,940.	10,940.
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Travel..

Attendance at scientific meetings of professional personnel	725.
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Other

Photocopying, chart preparation, slides, etc.	375.	
Publication costs	525.	
Cost of emergency repairs to equipment, exclusive of maintenance agreements	1,050.	
Books and Journals	375.	2,325.

Subtotal

\$84,104.

763

Budget Justification

Project #2

First Year

Personnel

Dr. A.H. Cochrane, Principal Investigator of Project #2 receives partial support from another source; and is also a Co-Investigator on Project #3 for partial support.

All other personnel, see explanation in Core Budget Justification.

Equipment

The requested equipment is needed for the efficient performance of this project.

Second Year

Personnel

See explanation first year.

Equipment

See explanation first year.

Third Year

Personnel

See explanation first year.

Equipment

See explanation first year.

PROJECT #3 BUDGET
First Year
 January 1, 1982-December 31, 1982

Personnel

A. Ferreira, Ph.D. Assistant Professor Principal Investigator	20%	7,390.	
V. Nussenzweig, M.D., Ph.D. Professor; Co-investigator		-	
A. Cochrane, Ph.D. Associate Professor; Co-investigator	10%	3,670.	
F. Santoro, Ph.D. Postdoctoral Fellow	50%	5,950.	
Research Technician I (to be appointed)	50%	9,400.	
P. Altszuler, Asst. Res. Tech.	40%	5,975.	
J. Josephs, Secretary	10%	1,798.	34,183.

Fringe Benefits (22.5%) 7,691.

Equipment

HPLC Pump	6,500.	
Radial Compressor for HPLC Pump	1,200.	7,700.

Animals (Purchase)

Hamsters (375 animals @ \$3.75 each)	1,425.	1,425.
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Supplies (radioactive amino acids, I¹²⁵; scintillation fluids and vials, solvents for HPLC columns and filters for HPLC; material for SDS-PAGE AND RIA) 15,000. 15,000.

Travel

Attendance at scientific meetings of professional personnel	1,300.	1,300.
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Other

Maintenance and Service Contract for HPLC and Scintillation Counter	5,000.	
Publication costs (including graphs, charts)	1,000.	
General supplies (i.e., duplicating, laundry, books and journals, etc.)	500.	6,500.

Subtotal \$73,799.

PROJECT #3 BUDGET
Second Year
 January 1, 1983-December 31, 1983

Personnel

A. Ferreira, Ph.D. Assistant Professor Principal Investigator	20%	7,982.	
V. Nussenzweig, M.D., Ph.D. Professor; Co-Investigator		-	
A.H. Cochrane, Ph.D. Associate Professor Co-Investigator	10%	3,965.	
F. Santoro, Ph.D. Postdoctoral Fellow	50%	6,425.	
Research Technician I (to be appointed)	50%	10,152.	
P. Altzszuler, Asst. Res. Tech.	40%	6,453.	
J. Josepfs, Secretary	10%	1,942.	36,919.

Fringe Benefits (22.5%) 8,307.

Equipment

Gamma counter (tabletop)	10,000.	10,000.
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Animals (Purchases)

Hamsters (375 animals @ \$4.13)	1,548.	1,548.
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<u>Supplies</u> (radioactive amino acids, I ¹²⁵ ; scintillation fluids & vials, solvents for HPLC columns and filters for HPLC; material for SDS-PAGE and RIA)	17,250.	17,250.
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Travel

Attendance at scientific meetings of professional personnel	1,375.	1,375.
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Other

Maintenance and Service Contract for HPLC and Scintillation Counter	5,250.	
Publication costs (including graphs, charts)	1,000.	
General supplies (i.e., duplicating, laundry, books and journals, etc.)	575.	6,825.

<u>Subtotal</u>		\$82,224.
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PROJECT #3 BUDGET
Third Year
 January 1, 1984-December 31, 1984

Personnel

A. Ferreira, Ph.D. Assistant Professor Principal Investigator	20%	8,620.	
V. Nussenzweig, M.D., Ph.D. Professor; Co-Investigator	-	-	
A.H. Cochrane, Ph.D. Associate Professor; Co-Investigator	10%	4,280.	
F. Santoro, Ph.D. Postdoctoral Fellow	50%	6,940.	
Research Technician I (to be appointed)	50%	10,965.	
P. Altszuler, Asst. Res. Tech.	40%	6,670.	
J. Josephs, Secretary	10%	2,098.	39,573.
<u>Fringe Benefits (22.5%)</u>			8,904.
<u>Equipment (miscellaneous pumps, columns, oven, etc.)</u>		5,000.	5,000.
<u>Animals (Purchases)</u>			
Hamsters (375 animals @ \$4.53)		1,698.	1,698.
<u>Supplies (radioactive amino acids, I¹²⁵; scintillation fluids & vials, solvents for HPLC columns and filters for HPLC; material for SDS-PAGE and RIA</u>		19,840.	19,840.
<u>Travel</u>			
Attendance at scientific meetings for professional personnel		1,445.	1,445.
<u>Other</u>			
Maintenance and Service Contract for HPLC and Scintillation Counter		5,515.	
Publication costs (including graphs, charts)		1,050.	
General supplies (i.e., duplicating, laundry, books and journals, etc.)		665.	7,230.
<u>Subtotal</u>			\$83,690.

Budget Justification

Project #3

First Year

Personnel

A. Ferreira, Principal Investigator for Project #3, will receive partial support for his time effort. The balance of his salary is derived from other sources.

A.H. Cochrane, Principal Investigator of Project #2, is a Co-Investigator on Project #3, and will receive partial support for his time effort.

F. Santoro, will be receiving partial support for his time effort. Support for the balance of his salary is derived from his home-leave salary.

Equipment

The HPLC apparatus is presently shared with another investigator in the Department of Pathology. This investigator, Dr. M. Lamm, is leaving New York University to become chairman of the Department of Pathology at Case Western Reserve University and he will take with him some of the parts of the HPLC, including a pump, Model 600A and a variable length UV detector. We have funds from other sources to buy the UV detector.

Second Year

Personnel

See explanation above. Also see explanation in Core for personnel practice policies applicable to all Projects.

Equipment

During the second year of the contract, we expect to perform a large number of radioimmunoassays. We have only one old gamma counter in the laboratory, and it is not only overutilized, but is frequently broken down. It is, therefore, essential to buy an additional counter, which should cost about \$20,000. We are asking that this contract contributes 50% of its cost.

Third Year

Personnel

See explanation above in first year.

Equipment

We anticipate several miscellaneous pieces of equipment, such as additional pumps, minigels, and other general needs for this third year.

PROJECT #4 BUDGET
First Year
 January 1, 1982-December 31, 1981

Personnel

E.H. Nardin, Ph.D. Instructor Principal Investigator	50%	10,500.	
R.S. Nussenzweig, M.D., Ph.D. Professor; Co-Investigator			
J. Gysin, M.D. Postdoctoral Fellow	80%	3,500.	
L. Caiati, Res. Tech. I	50%	8,304.	
Res. Tech I (to be appointed)	50%	8,260.	30,564.

Fringe Benefits (22.5%) 6,877.

Equipment

Cages for squirrel monkeys (each holds 14 animals)	3,000.	3,000.
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Animals (Purchases)

Squirrel monkeys (20 animals @ \$250. each)	5,000.	
Freight charges for delivery of squirrel monkeys from French Guyana (via Miami, Fla.)	1,400.	
SW mice (150 animals @ \$.87 each)	130.	
Balb/c mice (150 animals @ \$4.55 each)	683.	
CD ₂ F ₁ mice (600 animals @ \$3.42 each)	2,060.	
Rats (50 animals @ \$2.86)	145.	9,418.

Chimpanzee Project (2 experiments, 6 animals each)

Per diem rate (6 animals x 45 days x \$7.20) x 2 expers.	3,888.	
Splenectomy (6 animals x \$500 each) x 2 experiments	6,000.	
Anesthesia, blood smears (+ 15 withdrawals per animal x \$17. each) x 2 experiments	3,060.	
Drug administration (6 animals x 3 x \$15. each) x 2 experiments	540.	
Express Mail of samples (15 samples x \$30) x two experiments	900.	14,388.

<u>Supplies (Filters for culture, glassware, plastics, radioisotopes, reagents, chemicals, columns, tissue culture medium, serum and blood materials)</u>	8,250.	8,250.
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(First Year continued)

PROJECT #4 BUDGET

Travel

Attendance at scientific meetings of professional personnel	1,300.	1,300.
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Other

Photocopying, chart preparation, etc.	250.	
Publication costs	650.	
Cost of emergency repairs to equipment, exclusive of maintenance agreements	1,000.	
Books and Journals	250.	2,150.

Subtotal

\$75,947.

PROJECT #4 BUDGET
Second Year
 January 1, 1983-December 31, 1983

Personnel

E.H. Nardin, Ph.D. Instructor Principal Investigator	50%		
R.S. Nussenzweig, M.D., Ph.D. Professor; Co-Investigator			
J. Gysin, M.D. Postdoctoral Fellow	80%	3,780.	
L. Caiati, Res. Tech. I.	50%	8,967.	
Res. Tech. I (to be appointed)	50%	8,832.	32,919.

Fringe Benefits (22.5%) 7,407.

Equipment

Binocular Microscope with accessories	3,400.	
Squirrel monkey cages	3,500.	6,900.

Animals

Squirrel monkeys (20 animals @ \$250. each)	5,000.	
Freight charges for delivery of squirrel monkeys from French Guyana (via Miami, Fla.)	1,470.	
SW mice (150 animals @ \$.96 each)	147.	
Balb/c mice (150 animals @ \$5.00 each)	750.	
CD ₂ F ₁ mice (600 animals @ \$3.76)	2,256.	
Fats (50 animals @ \$3.15 each)	158.	9,781.

Chimpanzee Project (2 experiments, 6 animals)

Per diem rate (6 animals x 45 days x 7.92)	4,277.	
Splenectomy (6 animals x \$550. each) x 2 experiments	6,600.	
Anesthesia, blood smears (+ 15 withdrawals x \$18.70 ea.) x 2 experiments	3,366.	
Drug administration (6 animals X 3 x \$16.50 x 2 experiments)	595.	
Express Mail of Samples (15 samples x 31.50 x 2 experiments)	950.	15,788.

<u>Supplies</u> (Filters for culture, glassware, plastic radioisotopes, reagents, chemicals, columns, tissue culture medium, serum and blood sample material)	9,485.	9,485.
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Travel

Attendance at scientific meetings of professional
personnel

1,375.

1,375.

Other

Photocopying, chart preparation, etc.

250.

Publication costs

650.

Cost of emergency repair to equipment, exclusive
of maintenance agreements

1,000.

Books and Journals

250.

2,150.

Subtotal

\$85,805.

772

PROJECT #4 BUDGET
Third Year
 January 1, 1984-December 31, 1984

Personnel

E.H. Nardin, Ph.D. Instructor Principal Investigator	50%	12,247.	
R.S. Nussenzweig, M.D., Ph.D. Professor; Co-Investigator			
J. Gysin, M.D. Postdoctoral Fellow	80%	4,085	
L. Caiati, Res. Tech. I.	50%	9,685.	
Res. Tech. I (to be appointed)	50%	9,540.	35,557.
<u>Fringe Benefits (22.5%)</u>			8,000.

Animals (Purchases)

Squirrel monkeys (20 animals @ \$250. each)	5,000.	
Freight charges for delivery of squirrel monkeys from French Guyana (via Miami, Fla.)	1,545.	
SW mice (150 animals @ \$1.05 each)	157.	
Balb/c mice (150 animals @ \$5.50 each)	825.	
CD ₂ F ₁ mice (600 animals @ \$4.14 each)	2,484.	
Rats (50 animals @ \$3.47)	175.	10,186.

Chimpanzee Project (2 experiments, 6 animals)

Per diem rate (6 animals x 45 days x \$8.71) x 2 expers.	4,705.	
Splenectomy (6 animals x \$605. each) x 2 experiments	7,260.	
Anesthesia, blood smears (+ 15 withdrawals per animal) x 2 \$20.57 each) x 2 experiments	3,705.	
Drug administration (6 animals x 3 x \$18.15 each) x 2 experiments	655.	
Express Mail of Samples (15 samples x \$33.50) x 2 experiments	995.	17,320.

<u>Supplies</u> (Filters for culture, glassware, plastics, radioisotopes, reagents, chemicals, columns, tissue culture medium, serum and blood sample materials)	10,910.	10,910.
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(Third Year continued)

PROJECT #4 BUDGET

Travel

Attendance at scientific meetings of professional personnel	1,445.	1,445.
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Other

Photocopying, chart preparation, etc.	275.	
Publication costs	700.	
Cost of emergency repair of equipments, exclusive of maintenance agreements	,050.	
Books and Journals	275.	2,300.

Subtotal

\$85,718.

Budget Justification

Project #4

First Year

Personnel

Dr. R.S. Nussenzweig, Program Director, and Co-Investigator, will have her salary covered in the Core Budget.

Dr. E.H. Nardin, Principal Investigator, will receive 50% of her salary support for her time effort in this project; the balance of her salary is derived from other funds.

Dr. J. Gysin, will receive partial support for his time effort. The balance of his salary is derived from his home-leave salary.

Equipment

The requested equipment is needed for the efficient performance of this Project.

Chimpanzee Project

Our prior experience using the Aotus monkey model for sporozoite induced infection in neutralization experiments has shown that these animals are poorly susceptible to P. vivax sporozoites. These experiments result in very long prepatent periods (greater than 30 days) and erratic results. We therefore feel it is essential to use chimpanzees for these experiments since they are very susceptible to human malaria sporozoites. Chimpanzees are at present the only reliable model for sporozoite induced infection, i.e. for the evaluation of the functional activity of monoclonal antibodies.

Squirrel monkeys, on the other hand, may or may not in the future replace chimpanzees for this purpose, but too little is known about these animals to date. The squirrel monkeys might also be used as a source of infected gametocytes for mosquito feedings.

Second Year

Personnel

See explanation first year.

Equipment

See explanation first year.

Chimpanzee Project

See explanation first year.

Third Year

Personnel

See explanation first year.

Equipment

See explanation first year.

Chimpanzee Project

See explanation first year.

PROJECT #5 BUDGET
First Year
 January 1, 1982-December 31, 1982

Personnel

Savanat Tharavanij, M.D.	100%	-	
Professor, Principal Investigator			
K. Tranakchit Harinasuta, M.D.	100%	-	
Professor, Principal Co-Investigator			
Pramuan Tapchaisri, M.D.	100%	-	
Co-Investigator			
Y. Chomcharn, Co-Investigator	100%	-	
A. Asavanich, Co-Investigator	100%	4,200.	
S. Limsuan, Co-Investigator	100%	3,000.	
Karnchana, Technical Staff	100%	2,160.	
Overtime (for investigative staff)		3,500.	12,860.

Equipment

Air conditioner, 24,000 BTUs, 220 volts	1,000.	1,000.
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Supplies (Department of Medical Entomology)

Slides, cover slips, beakers, pasteur pipettes, etc.	1,000.	
Insectary supplies: membranes for feeding, diet, cages, trays, shelving for trays, mosquito rearing materials	5,400.	6,400.

Supplies (Department of Microbiology & Immunology)

Slides, cover slips, fluorescence free vials for serum storage, plastic centrifuge tubes, screw capped centrifuge tubes, serological pipettes	1,724.	
Oxford pipettes (triple range), pipette tips, pH meter electrodes, beakers	650.	
FITC-labeled anti-immunoglobulin sera, reagents		
production of monoclonal antibody against <u>P. falciparum</u> and <u>P. vivax</u>	2,000.	
Radioisotopes (I^{125} and S^{35} -methionine)	1,000.	
Miscellaneous chemical reagents	500.	5,874.

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(First Year continued)

PROJECT #5 BUDGET

Travel

Three visits per annum to two different endemic sites in order to collect blood samples from patients (Khao KanRiang, Kanchanaburi, West Thailand; Nong Rhee, Kanchanaburi, West Thailand, and/or Chanthaburi, East Thailand

5,000. 5,000.

Other

Fixed per annum charge for patient-use at the Hospital for Tropical Diseases, incurred by the Department of Microbiology & Immunology

3,000. 3,000.

Freight charges for materials to be shipped between Bangkok, and New York; telephone and postal communication between Bangkok and New York

1,000. 1,000.

Subtotal

\$35,134.

777

PROJECT #5 BUDGET
Second Year
 January 1, 1983-December 31, 1983

Personnel

Savanat Tharavanij, M.D. Professor Principal Investigator	100%	-	
K. Tranakchit Harinsasuta, M.D. Professor and Principal Co- Investigator	100%	-	
Pramuan Tapchaisri, M.D. Co-Investigator	100%	-	
Y. Chomcharn, Co-Investigator	100%	-	
A. Asavanich, Co-Investigator	100%	4,830.	
S. Limsuwan, Co-Investigator	100%	3,450.	
Karnchana, Technical Staff	100%	2,480.	
Overtime (for investigative staff)		4,025.	14,785.

Supplies (Department of Entomology)

Slides, cover slips, beakers, pasteur pipettes, etc.	1,150.	
Insectary supplies: membrane feeders, diet, cages, trays, shelving for trays, mosquito rearing, etc.	6,210.	7,360.

Supplies (Department of Microbiology & Immunology)

Slides, cover slips, fluorescence free vials for serum storage, plastic centrifuge tubes, etc.	1,985.	
Oxford pipettes, pipette tips, ph meter electrodes	780.	
FITC labeled anti-immunoglobulin sera, reagents for production of monoclonal antibody against <u>P. falciparum</u> and <u>P. vivax</u>	2,300.	
Radioisotopes (I^{125} and S^{35} -methionine)	1,150.	
Miscellaneous chemical reagents	575.	6,790.

Travel

Three visits per annum to two different endemic sites to collect blood samples from patients (Khao KanRiang, Kanchanaburi, West Thailand; Nong Rhee, Kanchanaburi, West Thailand; and/or Chanthaburi, East Thailand).	5,500.	5,500.
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718

(Second Year continued)

PROJECT #5 BUDGET

Other

Fixed per annum charge for patient-use at the Hospital for Tropical Diseases, incurred by the Department of Microbiology & Immunology

3,900.

Freight charges for materials to be shipped between Bangkok, Thailand and New York; telephone and postal communication between Bangkok, Thailand, and New York

1,300.

Maintenance and Service Agreements of Equipment

1,000.

6,200.

Subtotal

\$40,635.

719

PROJECT #5 BUDGET
 Third Year
 January 1, 1984-December 31, 1984

Personnel

Savanat Tharavanij, M.D. Professor Principal Investigator	100%	-	
K. Tranakchit Harinasuta, M.D. Professor Principal Co-Investigator	100%	-	
Pramuan Tapchaisri, M.D. Co-Investigator	100%	-	
Y. Chomcharn, Co-Investigator	100%	-	
A. Asavanich, Co-Investigator	100%	5,554.	
S. Limsuan, Co-Investigator	100%	3,967.	
Karnchana, Technical Staff	100%	2,852.	
Overtime (for investigative staff)		4,628.	17,001.

Supplies (Department of Entomology)

Slides, cover slips, beakers, pasteur pipettes, etc.	1,325.	
Insectary supplies: membrane feeders, diet, cages, trays, shelving for trays, mosquito rearing materials	7,145.	8,470.

Supplies (Department of Microbiology & Immunology)

Slides, cover slips, fluorescence free vials for serum storage, plastic centrifuge tubes, screw capped centrifuge tubes, serological pipettes Oxford pipettes, pipette tips, Ph meter electrodes	2,285.	
FITC-labeled anti-immunoglobulin sera, reagents for production of monoclonal antibody against <u>P. falciparum</u> and <u>P. vivax</u>	897.	
Radioisotopes (I^{125} and S^{35} -methionine)	2,645.	
Miscellaneous chemical reagents	1,325.	
	665.	7,817.

Travel

Three visits per annum to two different endemic sites to collect blood samples from patients (Khao KanRiang, Kanchanaburi, West Thailand; Nong Rhee, Kanchanaburi, West Thailand; and/or Chanthaburi, East Thailand)	6,000.	6,000.
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280

Other

Fixed per annum charge for patient-use at the Hospital for Tropical Diseases, incurred by the Department of Microbiology & Immunology	4,485.	
Freight charges for materials to be shipped between Bangkok, Thailand and New York; telephone and postal communication between Bangkok, Thailand and York	1,645.	
Maintenance and Service Agreements for Equipment	1,000.	7,130.
<u>Subtotal</u>		\$46,418.

781

Budget Justification

Project #5

We believe that all categories are self-explanatory in this Project.

The budget was essentially prepared by Dr. Savanat in Bangkok, and submitted for inclusion in our overall Program Project. The salaries of the co-investigators, Dr. Savanat, Harinasuta, Pramuan and Chomcharn are provided by other sources.

Most of the equipment will be purchased in the United States and aircargoed to Bangkok, Thailand for expeditious purposes.

Travel within the interior of Thailand will be bi-annual and is necessary for sample collection and constitutes an essential component of our collaborative work in P. falciparum and/or P. vivax malaria. Reports of progress will be included in our future Progress Reports.

NEW YORK UNIVERSITY PROJECT REVIEW**I. Substantive Review****A. Project Description and Background**

The original specific research goals of this project were:

- 1) determination of optional conditions for immunization of rhesus monkeys with Plasmodium knowlesi sporozoites;
- 2) immunization with sporozoites of P. knowlesi of its natural host; i.e. Macaca fascicularis.
- 3) characterization of surface antigen(s) of sporozoites; and
- 4) study of sporozoite-host cell interaction.

In addition, because of rapid progress in the area of hybridoma methodology, and not foreseen in the original application, a supplement was recently granted to identify the protective antigens of rodent malaria sporozoites.

B. Progress to date**1. Evaluation of past performance against the specific project objectives:****a) immunization of monkeys;**

- (i) an initial objective was to examine the variables involved in the induction of immunity in Rhesus monkeys to P. knowlesi by inoculation of irradiated sporozoites. While three animals showed complete protection after intravenous inoculation of sporozoites, nine other animals were non-reactive or only partially protected. A high level of circumsporozoite precipitating antibody correlated with the presence of protection. These irregular results suggested that the Rhesus monkey was a poor simian model, and the original plan to explore the essential variables was not pursued.
- (ii) It was planned to examine the response of a natural host of P. knowlesi to immunization with irradiated sporozoites. To be done in collaboration with Dr. R. Gwadz at the National Institutes of Health, this phase of the work with M. fascicularis is only now

getting underway. The delay has been caused by the dual constraints of lack of sporozoites and of space to house monkeys.

b) The second major objective was to characterize the surface antigens of sporozoites.

This objective has been approached broadly, with ingenuity, and with success. A monoclonal antibody which neutralizes P. berghei sporozoites in vitro and which in minute amounts (10 µg) passively protects mice has been shown to react with a surface protein having a molecular weight of 44,000. In collaboration with M. Aikawa, this monoclonal antibody locates the protective antigen (Ag) on the surface of mature sporozoites; the same material is present only in limited amounts in oocyst sporozoites. One surface Ag can be labeled with ³⁵S-methionine, and yields comparably immunoprecipitation patterns. This work, originally done with P. berghei sporozoites has been extended in ongoing studies to P. knowlesi, P. falciparum, P. cynomolgi and P. vivax with comparable preliminary results.

c) The third major objective was to investigate sporozoite-host cell interactions.

Several technical approaches have been used successfully to study the interaction between sporozoites of P. berghei and of P. knowlesi respectively with mouse and monkey macrophages. The presence of non-immune serum is essential for the sporozoite to penetrate into a macrophage. In contrast, in the presence of immune serum, sporozoites are phagocytized and destroyed by macrophages. These phenomena were species specific, in that the serum and macrophages had to derive from a susceptible host. Currently, collaborative studies with a malaria - network participant, Dr. M. Hollingdale at the Rockville Laboratory, indicate that the presence of the protective monoclonal antibody (mentioned above) blocks penetration and/or development of sporozoites within host cells in-vitro.

d) Supplemental contract objective

In mid-1980, a request was approved for a supplement in the amount of \$156,000. to expedite the breakthrough achieved by the preparation of a protective monoclonal antibody

against P. berghei. As outlined above, this objective has been achieved. Several new monoclonal antibodies of differing activities have been prepared against P. knowlesi, P. vivax, and P. faciparum.

2. Evaluation of results, interpretation of data, significance of findings.

The research that has been accomplished under the present contract has answered and/or met many of the expected objectives in varying degrees. Foremost among the significant results are:

- a) Rhesus monkeys can be completely protected from infection with knowlesi malaria by vaccination with irradiated sporozoites.
- b) There is a positive correlation between circumsporozoite precipitation test titer and complete protection on challenge.
- c) Sera from completely protected vaccinated monkeys neutralize sporozoite infectivity suggesting the vaccination procedures indeed stimulated total protective immunity.
- d) Duration of complete protective immunity was 3 months.

The current contract research activities have shown the feasibility of immunization against a malaria of lower primates using irradiated sporozoites just as had been shown for rodent malaria. This demonstration should permit the generalization that the same should obtain with the human falciparum parasite.

Progress under the objectives of characterization of surface antigens of sporozoites has been rapid especially with the advent of hybridoma technology.

The group has:

- a) Identified the protective antigen of P. berghei which is uniformly distributed over the surface of the sporozoite. It has a molecular weight of 44,000. The monoclonal antibody which has been developed against this Pb-44 surface antigen has been shown to be protective since it neutralizes sporozoite infectivity in-vitro and protects

125

mice upon passive transfer of as little as 10 µg of purified antibody. Biosynthesis studies indicate intracellular precursor(s) of the protective surface antigen.

- b) Applying monoclonal antibody technology to P. knowlesi, 2 surface antigens have been identified. The surface antigen with a M.W. of 42,000 has been identified as the protective antigen. Antibodies to Pb 42 neutralize sporozoites and produce a CSP reaction.
- c) One surface antigen has been identified for human falciparum sporozoites with a M.W. of 72,000. Antibodies to this antigen neutralize sporozoites and produce CSP reaction.

The significant findings produced under this contract indicate that continued support at an equal or greater level is appropriate for attaining the primary USAID goal of a protective malarial vaccine.

3. Use of results

The significant findings generated through this contract have been published in national and international journals. See attached list (taken from Project Progress Report 1/79 - 9/80 and Site Visit Report). Dissemination was obtained by seminars, site visits, presentation of papers at scientific national and international meetings.

4. Evaluation of research methodology, and overall quality of experimental design.

Using extracts of ³⁵S-methionine labelled sporozoites and SDS polyacrylamide gel electrophoresis, those surface antigens of sporozoites of P. berghei (Pb-44) P. knowlesi and P. falciparum which are associated with protection, were identified using sera from immunized animals and monoclonal antibodies. The research methodologies appear to be well suited to the characterization of these surface antigens. The methodologies used for the production of monoclonal antibodies to sporozoite antigens and the demonstration of their sporozoite-neutralization activity are significant findings towards the development of a sporozoite vaccine against malaria.

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A major problem appears to be the lack of an abundant supply of sporozoites of the different species of Plasmodium. This problem is being approached by the significantly increased insectary capacity and gametocyte culture studies with falciparum malaria. The immunization trials in primates have shown that protective immunity can be induced in at least some individuals and, is significantly directly associated with CSP reactivity

This finding that the potential effectiveness of a vaccine can be predicted without challenge has significant potential. Analytical research design in the primate model will be hampered by the lack of adequate numbers of primates. However, the direct correlation of the CSP titer to protection suggests that other test animals, primates or non-primates, may be used to assess the effectiveness of manipulative procedures, adjuvants, dose requirements, etc. and that only those procedures associated with maximum CSP reaction need then be tested in monkeys. At present, the nature of the data is such that they do not lend themselves readily to statistical analysis. However, the newer analytic tests, such as, the RIA test for the idio type and anti-idio type reaction may be open to statistical analysis.

5. Evaluation of the competence of the research personnel and the institutional environment.

The research staff, a strong mixture of parasitologists and immunologist with visiting fellows and graduate students appears to be highly qualified to perform and complete the project. The academic environment appears excellent for support of the project.

6. Evaluation of the physical facilities.

The current and proposed laboratory and library facilities are adequate to complete the present project. Many of the rooms are small and crowded and therefore do not allow for expansion of the effort. However, unused space is available in the building and could be upgraded and equipped for expansion. The insectaries and animal quarters are clean, modern and appear adequate.

7. The project is unique in that post graduate participants from LDC institutions (Thailand, Chile, Brazil, China, etc.) and additional collaborative studies with LDC contribute to the scientific insitutions on a worldwide basis.

C. Significance to A.I.D. Objective

The objectives of this project are considered high priority within the research guidelines developed in January 1981 at the Malaria Immunology Workshop held in Bethesda, Maryland.

D. Relation to existing knowledge

The project has contributed significant new knowledge, notably in the identification of sporozoite antigens associated with protection.

E. Relation to other research

This project appears to be unique among the USAID malaria vaccine networks projects. The new procedures however are in part directly applicable to other projects using merozoites vaccine.

II. Fiscal and Administrative Review

A. Evaluation of past and current work plan budget. Monthly payment vouchers indicate that the project is essentially on target with actual and estimated operational costs.

B. Property Accountability

An updated equipment inventory was presented to this review team and is on file with A.I.D. project managers.

C. Examination of Equipment

A.I.D. purchased equipment was inspected and found to be in fully operational order and under rather heavy demand by the scientific staff. Hybridoma facilities were generally small for for the types of research being conducted in this area.

D. Staffing Pattern

Dr. Nussenzweig has succeeded in bringing together an excellent mix of dedicated young investigators from both developed and developing countries. The mix of professionals and technical support staff

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appears to be fully adequate for the conduct of the proposed research.

E. Travel

Travel funds have been expended to maximally capitalize on research progress in other laboratories. Both senior and junior professional staff have the opportunity to present findings at professional meetings as well as visit other laboratories working the field.

F. Reports

Progress reports are generally well prepared, indepth and provide an excellent overview of research progress. They are, however, sometimes submitted later than is required by the contract.

This site visit of New York University was conducted September 21 - 22, 1981, in New York City. Team members included:

William Collins, Ph.D.

Peter Contacos, Ph.D., M.D.

Thomas Weller, M.D.

Sporozoite-Induced Immunity in Simian Malaria -
New York University Medical Center

The report of Dr. Weller, chairman of the subcommittee comprised of Drs. Carter, Schweigert and Wishik is summarized below:

I. SUBCOMMITTEE REPORT

An on-site review of the project had been carried out a few weeks prior to the RAC meeting. Dr. Weller summarized the findings of the review team.

Dr. Nussenzweig has been one of the more productive researchers in the malaria network. Her work is funded from several sources. However, funding has not been available for an insectary and a few additional laboratory supplies and equipment which the principal investigator feels are important. Several of these items were considered when the project was last reviewed by RAC but the request for support of this type was set aside until the on-site review was completed. The present proposal requested the additional support so that work on several lines of research could be started early to take advantage of the development of a new research technique.

Dr. Weller described the new technique developed by Dr. Nussenzweig to produce an unlimited supply of antigens for laboratory experiments. The technique, the hybridoma technique, is an important contribution to malaria research that will allow Dr. Nussenzweig to proceed with her research much more rapidly than was planned.

During the on-site visit, the review team went over Dr. Nussenzweig needs very carefully and concluded that total support of about \$55,000 exclusive of overhead was required. Dr. Nussenzweig's present proposal was considerably greater than what was discussed during the site visit. The original amount should be quite an adequate level of AID support for her work. Dr. Weller felt the researchers request for the addition of a senior resource person to the project and a contingency budget item should not be supported by AID.

II. SUBCOMMITTEE COMMENTS

Dr. Carter recommended that the additional support be provided since the amount was small and the researcher's record of achievement was excellent.

Dr. Schweigert noted that the researcher's work had high scientific value but agreed with Dr. Weller's suggestion to limit the level of support to about \$55,000.

Dr. Wishik pointed out that AID had been supporting the researcher for several years. She often changed directions in her work, usually for good reasons. Dr. Nussenzweig is the only researcher working on the sporozoite stage of malaria. Many of her developments will be applicable to other types of malaria research. He also recommended providing all the financial support requested in the proposal.

Dr. Connell asked why NIH funding was dropped. The total money allocated to malaria is large. If \$40,000 can be saved it may be possible to support an additional researcher in other areas of work on malaria.

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Dr. Weller suggested that RAC could recommend support for the additional staff member for Dr. Nussenzweig but that the \$19,000 budget item that is essentially a contingency item could be dropped.

Dr. Erickson remarked that NIH support was dropped because of the general feeling that AID is sponsoring malaria research. He described the total for work on the malaria vaccine and pointed out that extra money that became available at the end of the fiscal year for the two previous years would not be available this fiscal year. Funding for the vaccine research was expected to be tight.

III. ACTION

Motion: That the request for supplementation of current support for sporozoite vaccine research be approved to permit exploitation of new discoveries stemming from application of the hybridoma technique. Specifically, it is recommended that the additional funding at an annual level of \$83,945 plus overhead be provided. This deletes the \$15,000 requested for replacement of NIH insectary services - a contingency that may not materialize.

Vote: Unanimous approval.

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Sporozoite-Induced Immunity in Simian
Malaria - New York University

Dr. Wishik, Chairman of the RAC subcommittee, introduced this project by indicating that past funding of this unit in the malaria research network has totalled \$700,000. Support is now proposed for a three-year period to the amount of almost another million.

As will be recalled, the overall strategy for attacking the problem of a malaria vaccine gave lower priority to the sporozoite line of attack. Nevertheless, it was generally agreed that it was too early to be sure that this line should not be kept open. Recent dramatic progress with merozoite work increases, rather than decreases the importance of maintaining parallel investigations. The possibility exists that eventually a dual type vaccine may be needed. The NYU work is now almost unique in its focus on sporozoites.

In the earlier NYU work on rodent malaria, a search was included for some acceptable adjuvant that would enhance the weak response, requiring immunizing injections of tremendous numbers of sporozoites. Those efforts were not successful. Work by others showing successful immunization without adjuvants and very recent reports of use of an adjuvant developed in Japan further discourages emphasis on an adjuvant search at present.

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With past AID support, Dr. Nussenzweig has made a number of accomplishments:...

1) In harvesting sporozoites from mosquitoes, a greater yield in purer form has been achieved. Part of this results from the interesting finding by other investigators that a prior injection with a different species of malaria parasite intensifies growth in the test animal of the second type of parasite introduced, 2) She has demonstrated that immunity can be conferred with sporozoites in the model used (rodent malaria), 3) Information has been gathered concerning the degree of antigenic specificity of plasmodium species and strains, 4) With the recent shift from rodent to monkey work (Rhesus), previous knowledge concerning the lack of cross-immunizing relationship between erythrocytic stages and sporozoites has been further refined to indicate that rather exact stages of maturation of sporozoites modify the immunity conferred, 5) More detailed information is being collected concerning immunizing procedures - dosage, number of injections, routes, etc., 6) Presence of an antibody type mechanism has been demonstrated, 7) With electron microscopy, antigen has been located on the membrane wall of the parasite, 8) The parasites have two types of antigens during the sporozoite stage etc.; one seems to be present throughout the sporozoite maturation; another develops during that maturation. Only the latter seems to confer

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immunity, 9) With gel electrophoresis, beginnings have been made in the effort to characterize the antigen. This aims toward further chemical definition of the antigen and its possible fraction and concentration. Of course, ultimate synthesis is the hope of all investigators in order to obviate complete dependency on mass tissue culture, and 10) Work has been done with live cells and macrophages.

In the present proposal, some of the same and other lines of investigation would be followed, including:

- 1) Moving from past parasite-animal models to use of a monkey which is naturally injected by a particular malaria parasite. This is the Macaca fascicularis and P. knowlesi;
- 2) More precise determination of most effective immunization procedure - dosage, etc.,
- 3) Further efforts to characterize antigens and antibodies and to determine ultra-structural localization,
- 4) Studies of the sporozoite behavior in relation to liver and other host cells, and
- 5) Development of in-vitro tests and assays to correlate with animal work. The one most advanced up to now is an immuno-fluorescence test for a P. falciparum antibody.

It is planned to do a field study to learn the diagnostic value of this test in a P. falciparum endemic area in The Gambia.

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The other members of the subcommittee have submitted statements and may wish to comment, especially Dr. Weller who participated in the site visit in November, 1977.

Drs. Carter, Connell, Heady, and Schweigert fully concurred with the chairman's report. Dr. Connell indicated that several clinical issues may come up in a year or so. Dr. Schweigert felt the budget was rather generous and should be carefully examined by AID staff.

Dr. Weller was concerned with the budget, especially because of three NIH grants along similar lines to the principal investigator. He was also concerned with the proposed work in The Gambia. The epidemiological study is not really part of our malaria immunity and vaccination program at this time.

Mr. Smith suggested that when the Agency first funded this investigator, she was receiving funds from five agencies. The present NIH grants are focused on rodent malaria and ultra-structure, both of which do not overlap to any degree with our contract, as we fund research on simian malaria. She also has a WHO grant, but it is primarily for training LDC students. The budget is relatively high but facilities at NYU require updating. The Gambia trip is a very small component of the project and is to confirm laboratory results in the field.

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Dr. Wishik read the motion to the RAC as follows:

Motion: That AID staff give careful attention to the appropriateness of a moderate reduction of the budget, especially with respect to equipment costs; and that, otherwise, the proposal be approved as submitted.

The RAC unanimously approved the subcommittee motion.

Sporozoite-Induced Immunity in Simian
Malaria - New York University

Dr. Wishik, Chairman of the Subcommittee composed of Drs. Carter, Merrill, and Schweigert, reported as follows:

There have been tremendous progress and accomplishments in the malaria program. Commendation was given to Ed Smith, TA/H, Manager of the program. The November 5-6, 1975 meeting of the RAC endorsed the malaria program with a request for shift in emphasis of the work at New York University. The subject proposal is fully responsive to the RAC request. The proposal is a solid document which covers both of the aspects requested. It is regarded as outstanding. The subcommittee will recommend approval of the staff request for two years and \$259,836.

Dr. Carter stated that Dr. Ruth Nussenzweig is highly respected in the field and that he believed this to be an excellent proposal. Drs. Merrill and Schweigert concurred.

Motion: That the proposal be approved as requested to include both sporozoite work and adjuvant research with a shift in emphasis to the former.

Moved by Wishik, seconded by Schweigert.

Vote: Unanimous approval.

Dr. Wishik stated his belief in the importance of parallel lines of research. The advance made now supports parallel work on sporozoite immunity.

Dr. Wishik introduced a request from the University of Hawaii, by Dr. Siddiqui, for approval of additional funds to cover biochemical

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services in the approved project on in vitro production of antigens.

This request is consistent with the Subcommittee's recommendations.

Drs. Carter and Merrill concurred.

Motion: That the project be expanded to include a biochemical component as requested.

Moved by Wishik, seconded by Schweigert.

Vote: Unanimous approval.

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AGENCY FOR INTERNATIONAL DEVELOPMENT
RESEARCH ADVISORY COMMITTEE

Minutes of the Fifty-first Meeting
November 5-6, 1975

Dr. Ralph Smuckler, Chairman opened the meeting and asked for comments on minutes of the 50th meeting held on June 27-28, 1975. Dr. Whitney moved and Dr. Schweigert seconded acceptance; approved unanimously. Dr. Erven J. Long extended greetings and introduced Dr. James Sentz who recently joined the Research Office staff of TAB.

Project Review

Health

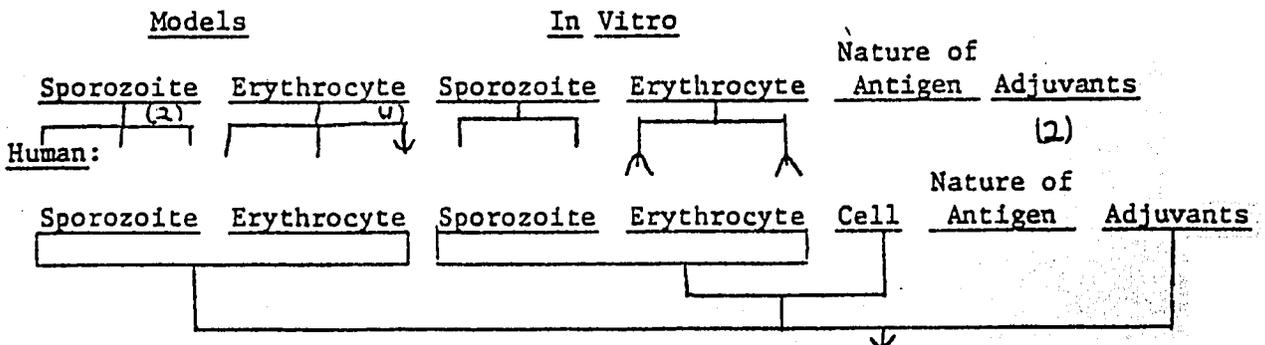
Malaria Immunity and Vaccination
Parke, Davis and Company, University of
New Mexico, New York State University,
Pan American Health Organization and Other
Contractors to be selected

Subcommittee: Dr. Samuel Wishik, chairman, Dr. James Carter, Dr. Malcolm Merrill and Dr. B.S. Schweigert.

This proposal is for expansion of present research, but as a collaborating network, with a funding level of \$2,915,000 for a three-year period.

Dr. Wishik reporting for the subcommittee, complimented AID for having supported and kept the project alive at a time when results were not very promising but the necessity for continuation was critical. With the use of the following diagram of animal and human system models, he reviewed the philosophy and rationale for the proposed research network. Within this diagram the encircled numbers refer to research, either proposed or approved, by institution as listed in the table showing proposed network associations.

Animal:



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The essence of the mosquito's life cycle in malaria transmission was clarified and Dr. Wishik indicated that, though immunization could be effected by controlling one phase of this cycle, present efforts are being directed toward sporozoite and erythrocyte control. Because there are two major and two auxiliary components for control in animal systems, parallel lines of investigation are justified with highest priority on the erythrocyte stage indicated by a heavy arrow. The in vitro work represents an attempt to grow malaria parasites and the antigen in the laboratory; the nature of the antigen needs to be better understood; and adjuvants are important because reactions to date are not sufficiently strong and need to be amplified.

Dr. Wishik summarized project studies to date as a basis for evaluating the overall proposal:

Item 1 - The University of New Mexico project under Dr. Silverman is in its last year and is to be continued through Dr. Reickmann's proposal.

Item 2a - New York University project under Dr. Nussenzweig approved by previous RAC for funding at \$60,000 per year for three years is now authorized for \$275,000 per three years including adjuvant work.

Item 2b - A five months extension at \$44,000 is proposed for Dr. Nussenzweig to carry on biological materials for support of future sporozoite stage work; otherwise termination of project in February 1976 will result in loss of valuable material. The subcommittee proposes that Dr. Nussenzweig submit in early 1976 a revised proposal with emphasis on sporozoitic stage control.

The general recommendation of advisory groups is that adjuvants are a less important approach to immunity at present.

Item 3 - Parke, Davis and Company thrust is to evaluate different materials for in vitro propagation of the malaria parasite for antigen production. Malignant cells appear to have promise for this purpose but it is not known if the vaccine produced by the method will be safe for human use.

Items 5-7 - A previous RAC meeting recommended contracts in the amount of \$200,000 be developed for critical research and several such projects have been negotiated. Rockefeller University is working on in vitro methodology for antigen production; University of Hawaii is engaged in in vitro cultivation of erythrocytic stage using red blood cells, however the primary problem is that these have not been replicated; New York University is also working on in vitro antigen production and is attempting sporozoite stage growth on mosquito cells and liver cells.

Item 8 - Because availability of the Aotus monkey is decreasing with increasing cost, the PAHO contract will (a) transfer work to Latin America where the monkeys are available, (b) develop institutional cooperation in Latin America where malaria is prevalent, and (c) thereby establish a base for evaluation of vaccine.

In summary Dr. Wishik indicated some advisors believe the in vitro approach in column 2 by University of New Mexico is too ambitious with too many things in one place at one time. To more realistically pursue his objectives, Dr. Wishik suggested that Dr. Rieckmann add a highly qualified immunologist to his staff. The subcommittee reasoned that Parke, Davis and Company has had experience with tissue culture work but never with parasites and therefore suggested they obtain consultation in this area. Concern was expressed about the overhead included in the proposal. For the Rockefeller University the subcommittee suggested that either a cell biologist or tissue culture specialist be added to the project. Favorable comment was made on the value of introducing and training new workers in the field and it was indicated that Dr. Traeger would do this at Rockefeller University. Dr. Wishik noted that Dr. Siddiqui at the University of Hawaii who has worked in the field for 15 years will concentrate on antigen studies and also train new workers.

The philosophy of the Pan American Health Organization (PAHO) to move the monkey model work to Colombia to be near the source of Aotus monkeys was discussed briefly and concern expressed that the Government of Colombia be formally involved. In addition RAC expressed concern that Dr. Reickmann's involvement with this project would overcommit his time and it was later confirmed that these services would be on a very short term consultant basis.

The subcommittee further suggested that workers on the various projects in the network should meet with advisory groups regularly and as often as possible so that AID will have advantage of being kept up to date on information.

Dr. Rechcigl noted the Parke, Davis and Company proposal is for \$200,000 per year and will need to be acted upon by RAC. Dr. Carter indicated he was pleased with progress to date and summarized several recommendations made at the September 3-4 review meeting in Washington.

- (1) Principal investigators should personally present projects to peer groups,
- (2) Reconfirmation should be made of new findings through replication of work if possible,
- (3) Peer group evaluation of work for funding,
- (4) AID should include funding to train new workers (graduate students),

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(5) The impact of the shortage of Aotus monkey exports from Colombia was noted. Dr. Carter was favorable to Dr. Reickmann's proposal taking over the Silverman work at University of New Mexico and suggested that the focus of his objectives be sharpened by presentation before a peer group, also that he add an immunologist to his group for this work.

He noted that Dr. Nussenzweig's work was concentrated on adjuvants and agreed emphasis should be redirected to sporozoite immunity. Dr. Carter acknowledged the difficulty in locating organizations to conduct the screening type of effort Parke, Davis and Company would assume and supported their adding an immunologist for this work. He further suggested that the use of cancer cells be set aside as the FDA could not be expected to approve their use.

Dr. Carter also supported PAHO's need for a cell biologist rather than a parasitologist. He expressed concern that this project did not define the work that would be done and agreed that the Government of Colombia should be actively involved rather than for the U.S. contractor to drift back into an imperialistic form of exploitation. Dr. Carter requested AID followup on these items for later approval.

Dr. Merrill emphasized two points in support of Dr. Wishik's report. (1) Dr. Reickmann's involvement with the Colombian project would be advantageous but the dilution of his time is a matter of concern; (2) he would prefer to consider the principal investigator's defense before a peer group as consultation in order to preserve the scientific independence of investigators and avoid dilution of initiative.

Dr. Schweigert commended the quantum jump forward in network planning following the New Mexico visit.

Dr. Anderson noted work is entirely in the western hemisphere and asked if there was merit in extending the network.

Dr. Montgomery noted an important element in this work is a standing group of advisors and asked who is or will be the network center, AID or some other?

Dr. Howard made two general points. First he noted that young people were not being trained in this area in the U.S. since loss of emphasis on malaria after World War II and only four institutions were now growing malaria parasites in the U.S. Consequently AID was spearheading this effort by default. He noted the best advice available on this work was to support the best investigators and to have them consult with peer groups. Secondly he noted the University of New Mexico project activities are unique and not being repeated elsewhere, hence the application to primates is important in the network.

Mr. Edgar Smith, TA/H, spoke for the project in support of the four proposals. He specifically noted that in supporting Dr. Nussenzweig,

AID was taking advantage of some work previously supported by others. He reported that AID did have an official request from PAHO for support of this work and there was official involvement and approval of Colombia. Dr. Espinal, who had trained at New York University, would be the Colombian worker. He noted the PAHO work plan is in the proposal and Dr. Reickmann would assist only in planning activities. Mr. Smith pointed out that in addition to the Aotus, a monkey available in the Philippines is also usable. He noted that the necessary expertise is concentrated in the U.S.; the only other location of importance is Guy's Hospital in London.

Dr. Wishik moved that RAC recommend the following:

- Motion: (a) That RAC endorse the authorizations of funds for the following projects:
- Dr. Trager at Rockefeller University for \$231,000 over 3 years;
 - Dr. Siddiqui at University of Hawaii for \$102,000 over 2 years;
 - Dr. Jahiel at New York University for \$82,000 over 2 years.
- (b) That RAC approve support for the work of Dr. Nussenzweig at New York University as follows and for the purposes indicated:
- (1) An additional amount of \$44,000 for the current fiscal year;
 - (2) continued use during the current fiscal year of funds from the \$275,000 already authorized;
 - (3) merger of the two areas of activity--sporozoite immunization and adjuvant development, with a shift in emphasis to the former;
 - (4) submission of a new proposal for a revised program of work that would be done after this fiscal year.
- (c) That RAC recommend awarding the contract for in vitro antigen development by Dr. Brackett at Parke-Davis to the amount of \$426,000 over a two-year period.
- (d) That RAC recommend awarding the contract for work under Dr. Reickmann at the University of New Mexico for the amount of \$1,402,000 over a three-year period starting about April, 1976 and that the work be subjected to frequent periodic review by consultant experts concerning scope and direction.

- (e) That RAC recommend the development of research work on malaria vaccine in Latin America by arrangement with PAHO to the amount of \$313,000 over a three-year period and that A.I.D. Staff and PAHO participate together in closely coordinating such work in collaboration with respective governments.
- (f) That RAC recommend that A.I.D. seek additional investigators on in vitro antigen development and related studies to the amount of \$774,000 over a three-year period and that expert consultants and the RAC subcommittee on malaria be involved as appropriate in such developments.

Considerable discussion developed around the approval of open contracts proposed in the amount of \$774,000 and how they would be developed in relation to RAC. Dr. Wishik questioned that they should come back to RAC for approval. Dr. Schweigert noted it would be unusual to approve a blank 3/4 million check. Dr. Carter suggested inclusion of a RAC subcommittee member in peer review. Dr. Merrill questioned AID staff if it would interfere with administrative procedure to bring the proposals before RAC for final approval with only a short delay. Dr. Montgomery suggested two options (a) present to RAC if it is timely for approval but (b) refer to subcommittee if need to move ahead quickly.

Mr. Farrar noted that open flexibility has appeal, but formal RAC review is somewhat like an iceberg requiring extensive review within AID, however this would be expedited if follow present precedents.

Dr. Wishik moved RAC resolution on AID policy as follows:

Whereas the goal is to combine tracks of research etc., be it resolved that: (1) RAC endorse AID's continuing a major effort toward securing effective means for immunization against malaria; (2) RAC praise the work AID staff has done toward developing a network of institutions to work into new directions and terminate when either the goal has been reached or the payoff declines; (3) personal contact between investigators and advisors with involvement of RAC be continued; (4) AID continue interagency involvement.

Dr. Anderson questioned the appropriateness for RAC to make a commitment on first clause. Dr. Peterson suggested this had been covered in June RAC and Dr. Smuckler acknowledged that malaria is critical in the area of world health.

Dr. Montgomery asked if AID was a sponsor of last resort and what was the implication if AID should continue work which Army has dropped? Dr. Long queried if there were ways to increase the priority of this work to the Army and other agencies. Dr. Howard indicated that in effect AID was funding on a last resort basis, but noted that AID was not permitted to provide funds in areas where other funds persist. Dr. Long noted that since RAC is not a programming body these resolutions would be appropriate as advisory. Dr. Merrill asked for RAC concurrence on this resolution. There was no disagreement.

DRAFT

ON-SITE REVIEW OF ROCKEFELLER UNIVERSITY PROJECT

I. Substantive Review

A. Introduction and Background:

This is the third contract at Rockefeller University for work on malaria vaccines under the leadership of Dr. William Trager. Previous work by Dr. Trager resulted in the first cultivation of malaria parasites in vitro, a breakthrough that revolutionized approaches to achieving a successful vaccine. Achievement of the objectives of the third contract has been adversely influenced by administrative actions at Rockefeller University. For a period, the impression was widespread that malaria research at Rockefeller would be phased-out. As a consequence, the four senior members of the research team sought jobs elsewhere and left prior to the termination of the contract. It is a credit to A.I.D., and to Dr. Trager, that each of the four moved to a senior staff position elsewhere, where each has already initiated an active program of research related to malaria vaccine development. Concurrent with the departure of Drs. Reese, Scheibel, Jensen and Langreth, the junior scientists and post doctoral fellows also departed. Currently, the climate for malaria research at the Rockefeller University appears more favorable. As the climate for malaria research has waxed and waned at Rockefeller University, it is to Dr. Trager's credit that he continues to personally pursue the objectives of the current contract with skill, perservance, and a high degree of personal involvement. However, circumstances beyond the control of the principal investigator, resulted in the first phase of the contract reflecting the combined efforts of a highly productive team, whereas the current situation reflects the work of Dr. Trager with a small supporting staff.

B. Progress to date

1. Evaluation of results, interpretation of data, significance of findings.

The project has not achieved targeted objectives for a multiplicity of reasons. In part this has been due to exploitation of a new marker on infected red blood cells, i.e. the presence of "knobs" and the

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culturation of knobby and of knobless lines. A major factor for the project not being on target is the fact that the research scientific staff departed during the second year of the contract - resulting in a logical end for the project.

The contract lists a series of objectives, deserving of comment:

1) Additional immunization experiments with merozoite vaccines in Aotus monkeys were proposed, with emphasis on different antigenic materials and adjuvants:

a) One experiment was completed in which 6 Aotus monkeys were employed; the results showed little protection. This work was then postponed at the suggestion of the Network Advisory Committee and in view of the shortage of Aotus monkeys. Regardless, the materials and protocols have been developed for a second experiment employing nine monkeys scheduled to start in two weeks.

b) Separation and identification of parasite antigens possessing protective activity:

This work was carried out by Dr. Reese; a paper is in press describing the results. In brief, Aotus and human immune sera were used that inhibited parasite growth in vitro. Using these sera in different immunoelectrophoretic procedures, a parasite component was identified that has a molecular weight of 47,000.

c) Cultivation of large numbers of parasites and their separation by stages and from uninfected red cells. Many of the methods previously used have been discontinued. Parasite production methods have not much been improved. We are probably at maximum in vitro production levels with the methods currently being used within the network.

d) Determination of factors in the medium affecting growth in vitro. No significant findings have resulted from studies on medium constituent substitutions.

- e) Biochemical studies of normal and infected erythrocytes and of free parasites.

While studying the carbohydrate/energy metabolism of P. falciparum and the roles of O₂ and of cytochrome oxidase using chelators, it was observed that some of the chelators had good antimalarial activity. This is an example of unexpected "spin-off" which relates to broad USAID interests in malaria control. Nothing was done with proposed ATP biochemical studies. Dr. Scheibel left Rockefeller University toward the end of the second year.

- f) Synchronization of cultures -
None of the proposed studies on synchronization were attempted.

- g) Extracellular development of P. falciparum in vitro -
Nothing was done on this objective.

- h) Electron microscopy of P. falciparum -
Much of the proposed studies under this objective were transferred to the USUHS project with Dr. Susan Langreth who had been associated with this project. Dr. Langreth collaborates with this project in determining the composition and quality of parasite preparations used in the vaccination trials mentioned above.

This project has always effected a high degree of collaboration and cooperation with other network laboratories.

2. Evaluation of results, interpretation of data, significance of findings

Some of the objectives of the project have been approached, others modified by the findings of other workers and others ignored. Overall, the productivity, given the administrative and personnel difficulties, has been significant. However, the productivity does not reflect the level of expected productivity of this research group.

The research was not able to attain "large scale" production of the merozoites for use in the conduct of immunization trials. Methods were developed, however, which expanded the production to approximately 4×10^9 merozoites per day - a significant improvement, but not large enough for expanded studies. Immunization trials using culture derived parasites were minimal and the results inconclusive.

One of the major findings was the identification of a protective antigen using antigen derived from cultures of P. falciparum and immune sera. Using SDS polyacrylamide gel electrophoresis, an antigen, shared by parasites from different geographic areas was identified (MW - 47,000 Daltons). Sera were shown to inhibit in vitro cultures and inhibition was directly related to the presence of this particular antigen.

A number of findings were made which, although not directly related to the contract, were significant to an understanding of the biology of the malaria parasite. The identification of parasites without "knobs" and the cloning of lines of parasites (K+ and K-) which provided new, previously undescribed markers for P. falciparum. The studies with chloroquine sensitivity of P. falciparum cultured parasites led to the development of improved field culture techniques to monitor drug resistance. Studies of the effects of aromatic chelators on cultures led to investigations of possible new antimalarials. Significantly, the senior staff, under Dr. Trager, has been trained and stimulated so that they are pursuing important aspects of the network effort in their new locations.

In general, the studies approach a number of different broad-range programs; this makes generalizations difficult. As a whole, the contract resulted in a number of significant findings even though certain aspects of the contract were neither completed or even attempted.

3. Use of the results

The results of the research on the objective listed in the contract have been published in a variety of journals - see attached bibliography. Research results have also been disseminated through progress reports, presentations at scientific meetings, seminars, WHO and other workshops, etc.

4. Evaluation of research methodology, including analytic research design and the overall quality of experimental work.

There have been some improvements in the application of newer methodologies available to the field of malarial immunology such as the addition of red blood cell extract for maintaining optional merozoite integrity. Generally speaking, the methods and experimental procedures are currently effective in terms of the goals of the study.

5. Evaluation of the competence of the research personnel and the institutional environment.

There can be no questions as to the competence of Dr. William Trager (Principal Investigator and the majority of the research staff). Unfortunately, because of the general understanding that malaria research at Rockefeller University would basically terminate with the termination of the current U.S.A.I.D. contract, 4 of the key scientists left Rockefeller University for positions at other universities or foundations 1 to 2 years before termination of the contract. Presently, only one junior research scientist and Dr. Trager are working on this project.

6. Evaluation of the physical facilities

The physical facilities remain generally unchanged from the previous 2 contracts. The laboratory space and equipment is adequate indeed above average. Use of equipment in other departments is available as required. Library facilities at the University are excellent. There were no experimental field sites specifically required for this project although trips have been made to the field for collection of new strains and/or serum samples for project studies.

C. Significance to A.I.D. objectives

The project as originally stated related directly to key problems in the U.S.A.I.D. malaria vaccine research network. Even though the priorities of the research network have changed since the present contract was negotiated, the project still is concerned with some priority needs of the research network.

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D. Relation to existing knowledge

The project adequately uses and takes advantage of previous research results and knowledge. The project has minimally contributed new knowledge of significant value specifically in comparison to the monumental contribution which obtained during their first U.S.A.I.D. contract. Regardless, some of the new knowledge will certainly have useful application in eventually attaining the USAID. goal of a protective malarial vaccine. Moreover, some of the project studies have resulted in potentially valuable "spin-offs" to the field of malaria chemotherapy (work of Scheibel on chelating agents).

E. Relation to other research

There is a minimal overlap of primary objectives between other projects within or outside the network. When duplication does exist, it is considered "desirable replication" rather than "classical duplication." Coordination between projects is primarily through USAID/Washington but is also voluntary through one or more principal investigators.

II. Fiscal and Administrative Review

A. Evaluation of past and current work plan budget

Approximately one year ago the University requested a supplemental grant of approximately \$100,000 to cover a cost overrun of about 25% of the projects third year requirements. An audit, conducted in New York by the Department of Health and Human Services, uncovered several budget areas where funds were misused and indicated deficiencies in contract fiscal management by the University's Financial Office Staff and the Principal Investigator.

The monies were supplemented upon the stipulation that a much more detailed accounting system be adopted by the parties involved.

B. Property Accountiability

The university has not yet provided a detailed equipment list to A.I.D. for this project as well as the two previous contracts of Dr. Trager. Mr. Lyons, University Comptroller, has assured A.I.D. staff that a detailed list

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of all equipment purchased under Dr. Trager's three contracts will be forthcoming before November 1981.

C. Examination of Equipment

The laboratory is very well equipped and is generally in good operating condition. All equipment is however, generally underutilized because of the lack of professional staff.

D. Staffing Pattern

For reasons already covered in this report, the project presently has only one junior scientist to support Dr. Trager's program.

- E. Reporting requirements have not been generally within the timing requirements of the contract language, nor have the progress reports provided an in depth analysis of current results.

III. Analysis of future work plans

- A. The expected output of the project will not be met primarily because of the changing policy in regard to the termination vs. renewal of the project. This in turn resulted in the significant loss of the senior scientific research staff as early as 1 year and as late as 2 years into the project.

B. Recommendations of future work plans

Due to the reduced scientific personnel and the University's vacillating support and/or interest, contracts of this scope or magnitude should not be considered. Rather, proposals with a limited number of highly defined objectives within a small budget could be considered.

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This site visit report of Rockefeller University was conducted September 23 - 24, 1981, in New York City.

Team members included:

William Collins, Ph.D.

Peter Contacos, Ph.D., M.D.

Thomas Weller, M.D.

Contract Submitted to
AGENCY FOR INTERNATIONAL DEVELOPMENT

A. NAME & ADDRESS OF ORGANIZATION

The Rockefeller University
1230 York Avenue
New York, New York 10021

DATE OF PREPARATION OR SUBMISSION 14 September 1981

PERIOD FOR WHICH PROPOSAL IS VALID
6 months

B. TYPE OF ORGANIZATION

Educational

C. TITLE OF PROPOSED RESEARCH

Studies on axenic (extracellular) cultivation of
Plasmodium falciparum and on P. vivax in vitro.

D. DESIRED STARTING DATE

1 January 1982

DURATION OF WORK

3 years

Principal Investigator:

William Trager

W. Trager 9/14/81
Signature Date

Approved for the Rockefeller University:

Rodney W. Nichols
Executive Vice President

Rodney W. Nichols 9/17/81
Signature Date

E. RESEARCH PLAN

I. Background

The continuous culture of P. falciparum in vitro, first obtained in 1976 (1), opened the way to a multipronged attack on the problems of identifying and obtaining antigens of this parasite that might serve as a protective vaccine. It was soon shown (2) that crude parasite material rich in merozoites, obtained from culture, would induce some immunity in Aotus trivirgatus monkeys when injected with an appropriate adjuvant. Two lines were then open for further work: 1) purification of a whole organism preparation, preferably of merozoites; 2) identification of individual immunogenic parasite constituents. Much progress has been made along both of these lines in this laboratory and elsewhere (see (3) and attached Progress Report).

There is now basis for hoping that once the immunogenic constituents have been identified, it might be possible to prepare them by application of the techniques of recombinant DNA. On the other hand, these methods might not work, and it may well be too difficult to separate parasite material completely from host cell material when cultures grown in their host erythrocytes are used. If the parasites could be grown, even for a limited time, extracellularly and hence under axenic conditions, their separation from host cell material would be facilitated. Furthermore, such axenic cultures would provide information on the nutritional requirements of the parasites and their biochemical relationships with their host erythrocytes not otherwise obtainable. We propose, therefore, to attempt the axenic cultivation of P. falciparum.

Secondly, we propose work on the cultivation of P. vivax. This species is now the principal form of malaria in India and parts of

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South America. It is a cause of great economic disruption and it has not been adequately controlled. Successful cultivation would bring this parasite into the laboratory (as has been the case with P. falciparum), where biochemical and physiological studies would provide new approaches to its control.

II. Specific Proposals

1. Axenic cultivation of P. falciparum

The only obligate intracellular protozoan parasite with which limited extracellular development in vitro has been obtained is the bird malaria P. lophurae (4). Young trophozoites of this species removed from their host erythrocytes will develop into schizonts extracellularly in a medium that must include duck erythrocyte extract, adenosine triphosphate, coenzyme A, glutathione, nicotinic acid amide in addition to gelatin and a complete nutrient mixture. Preliminary experiments with P. falciparum, done before in vitro cultures had been obtained using ring stages from infected Aotus monkeys, showed that some extracellular development of this species would occur (5).

We are now in an infinitely better position to attempt this problem. This is so not only for purely technical reasons, as the much greater ease of obtaining parasite material of desired stage from cultures than from monkeys, but also because so much has already been learned in the last 5 years about the parasite-host cell complex. This last, combined with the fact that more is known about the human erythrocyte than any other kind of cell, provides a basis for the possibility that we may have greater success with axenic culture of

P. falciparum than we could achieve with P. lophurae.

The experiments will be done in the following way. Two different isolates of P. falciparum will be maintained as stock cultures in continuous flow vessels (6). These will be synchronized every 4 weeks. Synchronized parasites at each desired stage will be freed from their host cells by lysis with a potent immune serum with complement or by agglutination with a lectin and subsequent fragmentation, as developed recently by Heidrich (7). The freed parasites will be suspended directly in culture media under test, ranging from modified RPMI 1640 to various kinds of red cell extracts as used previously with P. lophurae. They will be incubated in an atmosphere of 3% CO₂, 2% O₂, 95% N₂ under a variety of physical conditions. Survival and development will be assessed on the basis of light and electron microscopic morphology and on the extent of incorporation of labeled substrates added to the cultures during the last four hours of an 18 hr or longer incubation period.

In the early work with P. lophurae (8), it was noted that living parasites in good condition could be recognized by their bright appearance under phase contrast microscopy. The condition of parasites can also be recognized by their appearance after fixation and staining. These criteria will be equally applicable to P. falciparum. Much additional information was provided by electron microscopy (9,10). This is indispensable for recognition of the condition of the membranes and for demonstration that the parasite is indeed free from the red cell plasma membrane. Finally, the extent of incorporation of labeled precursors provides an additional objective criterion of parasite activity. It is important to use several amino acid tracers since

they are incorporated to a different degree into different parasite proteins. We would probably use at least methionine, isoleucine, proline and histidine as the amino acids and hypoxanthine as nucleic acid precursor. The information already available from the work with P. lophurae will of course be applied, but not to the exclusion of entirely new approaches. Of special interest would be experiments begun with merozoites rather than young trophozoites. Whereas the trophozoite, when removed from its host cell is surrounded by the parasitophorous membrane derived originally from the red cell but quickly modified by the parasite, the merozoite is naked except for its surface coat. We know that the merozoite must interact with receptors on the host erythrocyte and that this interaction initiates its change into a ring. Since work now under way in several laboratories is likely to tell us the nature of the receptors involved, this substance could be supplied in the medium to initiate extracellular development.

II, 2. Cultivation of P. vivax

Although we have not seriously attempted the cultivation of this organism, it would seem from personal reports from others that the methods used for P. falciparum which have worked also for P. knowlesi (11), P. fragile (12), P. inui (13), possibly for P. malariae (14), and most recently for P. cynomolgi (15), will not do for P. vivax. The cultivation of the vivax-like parasite of rhesus monkeys P. cynomolgi in particular has made it difficult to understand why P. vivax has not been grown.

Very recently, however, French workers have reported (16) cultivation of one strain of P. vivax essentially by the same methods used

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for P. falciparum except for an increase in glucose concentration, 3 x daily change of medium and provision of fresh red cells every 2nd day. It is of interest that the human blood used to start the culture had the relatively high parasitemia of 2.5%. At the end of the 43-day culture period, parasitemia was only 0.25%. We know that others have used the same small modification in culture method without success. Hence this report is at the same time encouraging and indicative of the need for a more systematic approach using a dependably available source of parasites.

We plan to have such a source in Aotus trivirgatus monkeys. Monkeys recovered from and immune to P. falciparum, as well as newly acquired unused monkeys, would be infected one at a time with a strain of P. vivax that gives infections with 3 to 4% parasitemia. Dr. W.E. Collins has agreed to provide us with starting material of this strain (see attached letter).

A 3-4% parasitemia in Aotus would permit cultures to be set up with human erythrocytes at 10 x the number of monkey erythrocytes and still provide a readily detectable initial parasitemia of about 0.3%. Experiments will however also be done with Aotus blood since this contains a relatively high proportion of young cells. Human blood will be tested for Duffy factor to exclude Duffy negative ones (17). After the removal of white cells the proportion of young red cells will be enhanced by centrifugation in a density gradient (as Percoll) and use of the upper layers of cells. In addition, through the hematology departments of several of the New York hospitals we hope to obtain small samples of blood from individuals in remission from anemia and hence rich in reticulocytes and young erythrocytes. Comparative

experiments with such cells and normal mature red cells should tell us just how important for cultures of P. vivax it is to have young red cells.

A number of tissue culture media and combinations of them will be tried. It may be of special interest to try media successfully used for long-term propagation of human bone marrow cells (12). All will be obtained as powders or mixed in the laboratory since we found in early work with P. falciparum that the stated storage life of media purchased in solution is not reliable. Cultures will be tried with different gas mixtures and with continuous or intermittent gentle mixing of the cells. Medium will be changed manually or by the continuous flow method.

It will be of special interest to try the new gas-permeable tissue culture dishes which would permit direct access of the gas mixture to the settled layer of infected red cells.

IV. References

1. Trager, W. and Jensen, J.B. (1976) Human Malaria Parasites in Continuous Culture. *Science* 193, 673-675.
2. Reese, R.T., Trager, W., Jensen, J.B., Miller, D.R. and Tantrafahi, R. (1978) Immunization against Malaria with Antigen from in vitro Cultivated Plasmodium falciparum. *Proc. Natl. Acad. Sci. USA* 75, 5665-5669.
3. Trager, W. (1980) Cultivation of Malaria Parasites in vitro: Its Application to Chemotherapy, Immunology and the Study of Parasite-Host Interactions. The Host Invader Interplay, H. Van den Bossche, ed., Janssen Research Foundation, 537-548.

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4. Trager, W. (1971) Malaria Parasites (Plasmodium lophurae) Developing Extracellularly in vitro: Incorporation of Labeled Precursors. J. Protozool. 18, 392-399.
5. Trager, W. (1974) Initial Extracellular Development in vitro of Plasmodium falciparum. Proc. 3rd Int. Congr. Parasitol. Vol. 1, p. 132.
6. Trager, W. (1979) Plasmodium falciparum in Culture: Improved Continuous Flow Method. J. Protozool. 26, No. 1, 125-169.
7. Heidrich, H.G., Russmann, L. Bayer, B. & Jung. A. (1979) Free Flow Electrophoresis for the Separation of Malaria-Infected and Uninfected Mouse Erythrocytes and for the Isolation of Free Parasites (Plasmodium vinckei): A New Rapid Technique for the Liberation of Malaria Parasites from their Host Cells. Z. Parasitenkd. 58, 151-159.
8. Trager, W. (1950) Studies on the Extracellular Cultivation of an Intracellular Parasite (Avian Malaria). I. Development of the Organisms in Erythrocyte Extracts and the Favoring Effect of Adenosine-Triphosphate. J. Exptl. Med. 92, 349-386.
9. Trager, W., Langreth, S.G. & Platzer, E.G. (1972) Viability and Fine Structure of Extracellular Plasmodium lophurae Prepared by Different Methods. Proc. Helminthol. Soc. Washington (Basic Research in Malaria, Special Issue), 39, 220-230.
10. Langreth, S.G. & Trager, W. (1973) Fine Structure of the Malaria Parasite Plasmodium lophurae Developing Extracellularly in vitro. J. Protozool. 20, 606-613.
11. Chen Zhengren, Gao Minxin, Li Yuhua Han Shumin & Zhang Nailin (1980) Studies on the Cultivation of Erythrocytic Stage Plasmodium in vitro. Chinese Med. J. 93, 31-35.

12. Chin, W., Moss, De Z., & Collins, W.E. (1979) The Continuous Cultivation of Plasmodium fragile by the Method of Trager-Jensen. Amer. J. Trop. Med. Hyg. 28, 591-592.
13. Nguyen-Dinh, P., Campbell, C.C. & Collins, W.E. (1980) Cultivation in vitro of the Quartan Malaria Parasite Plasmodium inui. Science 209, 1249-1251.
14. Rai Chowdhury, A.U., Chowdhury, D.S. & Regis, M.L. (1979) Simultaneous Propagation of P. malariae and P. falciparum in a Continuous Culture. Indian J. Med. Res. 70 (Suppl.) 72-78.
15. Nguyen-Dinh, P., Gardner, A.L., Campbell, C.C., Skinner, J.C. & Collins, W.E. (1981) Cultivation in vitro of the Vivax-Type Malaria Parasite Plasmodium cynomolgi. Science 212, 1146-1148.
16. Larrouy, G., Magnavol, J-F., Moro, F. (1981) A propos de l'obtention par culture in vitro de formes intra-erythrocytaires de Plasmodium vivax. C.R. Acad. Sci., Paris 292, 929-930.
17. Miller, L.H., Mason, S.J., Clyde, D.F. & McGinniss, M.H. (1975) The Resistance Factor to Plasmodium vivax in Blacks. The Duffy-Blood-Group Genotype FyFy. New England J. Med. 295, 302-304.
18. Gartner, S. & Kaplan, H.S. (1980) Long-Term Culture of Human Bone Marrow Cells. Proc. Natl. Acad. Sci. U.S. 77, 4756-4759.

F. Available Facilities and Major Items of Equipment

The laboratory has the facilities for the proposed work, including access to a scintillation counter.

G. Personnel (Faculty)

Principal Investigator William Trager (Professor Emeritus) will devote 50% of his time to the specific purposes of the project. He will have

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working with him Dr. Norbert Lanners, Assistant Professor (see attached C.V.). Dr. Lanners has a strong background in protozoology, cell biology and experimental physiology, as well as in parasitology. He will participate in all phases of the work, axenic culture of P. falciparum as well as the experiments with P. vivax. He will also be responsible for electron microscopy to be done in connection with extra-cellular cultivation of P. falciparum.

Budget Justification

The Aotus monkeys are required as hosts for P. vivax. Since one infection will remain at a sufficiently high level to permit only 2 or 3 uses for experiment, it is necessary to have a number of monkeys in which the parasites can be sequentially passed. In between experiments, it should be possible to store infected blood frozen in liquid nitrogen. We will have on hand a sufficient number of monkeys for this purpose from the work with P. falciparum, but we will need board for them,

The estimated amounts of blood and culture media are needed for maintenance of P. falciparum stock cultures and for preparation of experimental media for both axenic culture of P. falciparum and for cultivation of P. vivax.

The budget includes salaries for 2 technicians and 1 helper. This is a minimal technical staff. The Rockefeller University will provide salary for a secretary who will devote about half of his time to the work of this project.

The proposal is for a 3-year period. In the light of present knowledge the axenic culture of P. falciparum has to be regarded as a relatively long term proposition. Results with P. vivax should however be definitive within 2 years. Accordingly, the budget for the 3rd year does not include funds for monkey board, since these are needed only for the work with P. vivax.

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J. Budget for 1st Year: January 1, 1982 - December 31, 1982

1. Salaries

Faculty	% Effort	Salary
William Trager, Professor Emeritus	50	22,200
Norbert Lanners, Assistant Professor	100	25,000
Non-Faculty		
2 Assistants for Research at 21,000	100	42,000
1 Helper at 13,300	100	13,300
		<u>102,500</u>
Fringe Benefits 22%		<u>22,550</u>
		125,050

2. Consultant Fees None

3. Equipment

Glassblowing	1,000
Instrument Shop	<u>2,000</u>

3,000

4. Expendable

Animal Board, 12 monkeys at \$1.826/Day	8,000
Blood, 100 Units at 50/Unit	5,000
Glassware, Wrapping & Sterilization 600/mo.	7,200
Culture Media, Chemicals, Syringes	25,000
Misc. Operating Costs	<u>2,500</u>

47,700

5. Service Contract for Phillips
E.M. 4 of 5,600

2,800

6. Travel for 2 at 700 each

1,400

7. Publication Costs

2,000

181,950

53.8% MTDC DHHS negotiated rate, March 31, 1981

96,275

278,225

Note:

Implementation of Circular A-21 requires the University to incorporate indirect costs into animal board rates. This change in the rate structure will be accomplished over a three-year period beginning 7/1/81. Animal board rates for the second year of this proposal have been increased by 29½% -- 20% to implement A-21 and 9½% for an annual cost increase. This represents the final increment to accomplish fully burdened rates. For subsequent years, the increase is calculated at 9½%.

BUDGET FOR ALL 3 YEARS

	1st	2nd	3rd
Personnel	125,050	136,930	149,938
Consultant	0	0	0
Equipment	3,000	0	0
Expendables			
Animal Board	8,000	10,360	0
Other	39,700	43,472	47,602
Service Contract	2,800	3,066	3,357
Travel	1,400	1,533	1,678
Publication	2,000	2,190	2,398
Total Direct Costs	181,950	197,551	204,973
53.8% MTDC	96,275	106,282	110,275
Total Cost	278,225	303,833	315,248
			TOTAL <u>\$897,306</u>

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H. NORBERT LANNERS, Dr. rer. nat. (PhD)

The Rockefeller University
New York, N.Y. 10021
(212) 360-1658

1480 York Ave. (Apt. 3-I)
New York, N.Y. 10021
(212) 794-9253

Dr. rer. nat. (PhD) in Zoology (magna cum laude). Experience in Biological/Biomedical Research with Emphasis on Developmental Cell Biology of Protozoa. Experience in all aspects of Light Microscopy, UV Microbeam Irradiation and Micro-manipulation of cell nuclei, and in Microinjection of drugs into cells (protozoa). Experience in Electron Microscopy.

PROFESSIONAL EXPERIENCE

September 1980 to Present: Rockefeller University, New York, N.Y.

Assistant Professor - Department of Parasitology.

Research on antigenicity of gametocytes and infected erythrocytes in Plasmodium falciparum malaria.

Cooperative research in cultivation of human malaria parasites and their use in developing a protective vaccine. Cultivation of P. falciparum gametocytes.

September 1976 to August 1980: Cornell University Medical College, New York, N.Y.

Research Associate - Laboratory of Dr. Bernice Grafstein, Dept. of Physiology. Foundation supported work was concerned with Axonal Regeneration in the goldfish optic system. Methods include Transmission Electron Microscopy and Light Microscopy in conjunction with HRP Tracing techniques.

Participated in TEACHING of Neuroanatomy to Medical Students, Neurosurgery Residents, and Surgeon's Assistants.

May 1976 to May 1979: Rockefeller University, New York, N.Y.

Adjunct Research Associate - Laboratory of Dr. William Trager, Dept. of Parasitology.

May 1976 to August 1976: University of Puerto Rico, Rio Piedras, P.R.

Visiting Scientist - Agricultural Experiment Station.

Invited to set up an Electron Microscopy Laboratory for investigation of virus infected plant material. Trained Professional and Technical Staff in techniques for Transmission Electron Microscopy and in the operation and maintenance of a Siemens Elmiskop Ia electron microscope.

September 1973 to April 1976: Rockefeller University, New York, N.Y.

Postdoctoral Fellow - Laboratory of Dr. Maria A. Rudzinska, Dept. of Parasitology. Research on suctorian protozoa (Heliophrya erhardi), with emphasis on the mechanism of nuclear migration during sexual reproduction, and on sexual differentiation of gametic nuclei.

November 1972 to August 1973: University of Tübingen, Germany.

Scientific Assistant - Laboratory of Dr. Karl G. Grell, Zoologisches Institut. Primarily responsible for TEACHING of Protozoology and Comparative Invertebrate Zoology (Grosspraktikum) to biology majors and graduate students.

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H. Norbert Lanners, Dr. rer. nat.

EDUCATION

Dr. rer. nat. (PhD) in Zoology (magna cum laude), University of Tübingen, 2/73
Germany. Thesis: Nuclear differentiation in Heliophrya erhardi.
Advisor: Dr. Karl G. Grell

University of Tübingen (Germany); Zoology, Botany, Organic Chemistry 9/66 - 11/72

University of Heidelberg (Germany); Biology, Chemistry, Physics 9/63 - 8/66

Extracurricular Studies in Theology and Philosophy
Universities of Wuppertal, Heidelberg and Tübingen

HONORS AND AWARDS

June 1977 Co-Chairman, Fifth International Congress of Protozoology,
New York; during session: "Cytology and Cytochemistry of
Free-Living Protozoa".

1977 - 1978 Fellowship Award for Research in the Neurosciences by the
National Paraplegia Foundation.

1978 - 1979 Research Grant Award by the Technology and Research Foundation
of the Paralyzed Veterans of America.

May 1979 Invited participant, Fifth Biennial Conference on Regeneration
in the Central Nervous System: Cellular Mechanism for Recovery
from Nervous System Injury (Fort Lauderdale, Florida).

January 1980 Invited speaker and panelist, First International Symposium on
Spinal Cord Reconstruction (Las Vegas, Nevada).

SOCIETIES

Society of Protozoologists

European Cell Biology Organization

Electron Microscopy Society of America

PERSONAL

Married, excellent Health;

Fluent in German (native language), English, reading knowledge of French.

B:

- COLBY, R.H. and H.N. LANNERS. Effect of cytochalasin B on Amoeba proteus.
Abstract of Papers, 11th Annual Meeting, Society for Cell Biology, 1971,
p. 60.
- LANNERS, H.N. Untersuchungen zur Kerndifferenzierung bei Heliophrya (Cyclophrya) erhardi (RIEDER) MATTHES, Ciliata, Suctoria. Thesis, Fachbereich Biologie, Eberhard-Karls-Universität Tübingen, Germany, 1973.
- LANNERS, H.N. Beobachtungen zur Konjugation von Heliophrya (Cyclophrya) erhardi (RIEDER) MATTHES, Ciliata, Suctoria.
Archiv für Protistenkunde 115: 370-385, 1973.
- LANNERS, H.N. Experimente zur Differenzierung der Makronucleusanlage von Heliophrya (Cyclophrya) erhardi.
Archiv für Protistenkunde 115: 386-402, 1973.
- LANNERS, H.N. and M.A. RUDZINSKA. Nuclear exchange during conjugation in the suctor Heliophrya erhardi: An electron microscope study.
Journal of Protozoology 22: 30A, 1975.
- LANNERS, H.N. and M.A. RUDZINSKA. Ultrastructure of the contact area of conjugants in the suctor Heliophrya erhardi.
Journal of Protozoology 22: 41A, 1975.
- LANNERS, H.N. and M.A. RUDZINSKA. Microtubules and filaments during fertilization in Heliophrya erhardi (Ciliata, Suctoria).
Journal of Ultrastructure Research 52: 134, 1975.
- LANNERS, H.N. Mechanism of the nuclear exchange during conjugation in the suctor Heliophrya erhardi.
Abstract of Papers, 5th International Congress on Protozoology, New York, 1977, p. 349.
- LANNERS, H.N. Ultrastructure of the fusion area during conjugation in the suctor Heliophrya erhardi (Rieder) Matthes.
Journal of Protozoology 25: 453-460, 1978.
- LANNERS, H.N. The attachment of the suctor Heliophrya erhardi to the substrate: Homologies to other suctoria.
Protistologica 14: 467-474, 1978.
- LANNERS, H.N. and B. GRAFSTEIN. Early stages of regeneration in goldfish optic axons: electron microscopy.
Abstract of Papers, 92nd Annual Meeting, American Association of Anatomists, Miami, 1979, p. 598.
- LANNERS, H.N. and B. GRAFSTEIN. Early stages of axonal regeneration in the goldfish optic tract: An electron microscopic study.
Journal of Neurocytology 9: 733-751, 1980.

- LANNERS, H.N. and B. GRAFSTEIN. Effect of a conditioning lesion on regeneration of goldfish optic axons: Ultrastructural evidence for enhanced outgrowth and pinocytosis.
Brain Research 196: 547-553, 1980.
- LANNERS, H.N. Pronuclei of Heliophrya erhardi Matthes during conjugation and their differential association with coated and uncoated microtubules.
Journal of Cell Science 45: 245-255, 1980.
- LANNERS, H.N. The exchange of pronuclei during conjugation in the suctor Heliophrya erhardi: Mechanism of movement.
In preparation.
- LANNERS, H.N. and B. GRAFSTEIN. Uptake and retrograde transport of horseradish peroxidase by regenerating goldfish optic axons in vivo: The effect of a conditioning lesion.
In preparation.
- LANNERS, H.N. Microtubules, chromatin and nucleoli during macronuclear changes in the course of conjugation of the suctor Heliophrya erhardi.
In preparation.
- TRAGER, W., M. TERSHAKOVEC, L. LYANDVERT, H. STANLEY and H.N. LANNERS. Clones of Plasmodium falciparum obtained by microscopic selection; their characterization with regard to knobs, chloroquine sensitivity, and formation of gametocytes. Processing of the National Academy of Sciences, USA, in print.

Large Scale Cultures of Human Malaria
(Plasmodium falciparum) and Their Use
for Immunization and for Antigenic
Analysis of the Parasites - The Rocke-
feller University

Dr. Carter, Chairman of the RAC subcommittee, introduced this proposal by indicating that it would add over one million dollars over a three year period to the present budget. The research plan presented would extend current efforts to improve culture methods in such a way that large scale production of malaria parasites was feasible. In addition, Dr. Trager would focus on the identification, characterization and purification of the antigen. A team, which included Dr. Weller, made a site visit to Rockefeller (August 1978). The several recommendations of that team review have been provided to RAC, and the subcommittee concurs with them.

The subcommittee in general notes the remarkable progress of Dr. Trager and his staff and recommends approval of the project. Dr. Carter noted that Dr. Trager is scheduled to retire in two years. He suggested that the Agency contact Rockefeller University to indicate to them the very key nature of this project and the importance of Dr. Trager.

Drs. Balck and Schweigert agreed with Dr. Carter's report. Dr. Black suggested that, from his contacts, a vaccine may be ten years off. He was interested in the karyotype studies with the Aotus monkeys.

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Dr. Weller felt he had little to add in support of the project. He did wish to share some of the highlights of the site visit. Dr. Trager has accumulated an excellent group of assistant professors and their future, after Dr. Trager retires, is somewhat in doubt. Dr. Weller felt AID should convey the importance of the team to the President of Rockefeller University and indicate the desirability of keeping the research team as an intact unit. Research objectives are being rapidly achieved, yet Dr. Weller wondered why some of the biochemical studies were included in the project, considering their academic nature.

Dr. Wishik concurred with the subcommittee comments. He also wanted to mention the recent site visit. He noticed scientific personnel involved with routine laboratory work which could be done by support staff. Lastly, Dr. Wishik felt project investigators should be given some latitude to pursue peripheral subjects since many times it is such activities that lead to major breakthroughs.

Mr. Smith indicated that Dr. Trager has another year on the old proposal but has come in with a new proposal because of his progress. Dr. Trager is hesitant to ask for liberal amounts of money and as you recall was under-budgeted last year.

Dr. Carter presented the following motion:

Motion: That the Rockefeller University project be approved at the level of funding requested, with the proviso that additional monies can be provided if the principal investigator deems this necessary.

Prior to the vote, Dr. Weller indicated that there was an implied commitment from the University that Dr. Trager could stay for the entire three year period. Dr. Weller and Mr. Simpson discussed the most effective route to convey RAC's commendation to Rockefeller University. More consideration of this would be explored in the future. Drs. Montgomery and Schweigert cautioned RAC on advising the University on internal matters.

The RAC voted their unanimous approval of the motion.

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Minutes of the March 30-31, 1978 RAC Meeting

Cultivation of Human Malaria Parasites
Rockefeller University; Additional Funds
Requested \$60,582

Dr. Carter presented, for RAC consideration, a request from Dr. Trager at Rockefeller University for supplemental funds in the amount of approximately \$61,000. The possibility of this request was mentioned at the last RAC meeting.

Dr. Weller indicated that Dr. Trager was not the type of individual who would ask for additional funds unless they were critically needed. A number of activities in his lab have supposedly progressed more rapidly than anticipated. He would recommend the increased funding but that this be a funding plateau until the whole RAC committee sees a report on the status of all activities in Dr. Trager's lab. The procedure is important here, but the project manager, Mr. Smith (DS/HEA), indicated that delays would occur if we waited until the July RAC meeting to consider this request.

Dr. Schweigert said that as policy RAC should have a project paper in hand to review before considering funding recommendations.

Mr. Smith indicated that Dr. Trager has proceeded much faster than anticipated and must cut back on several important experiments if he doesn't receive additional funds. The last time RAC reviewed the project area, seven areas for additional work were suggested and Dr. Trager is making significant progress in four of these.

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An intensive review of this project is now scheduled for August 1978.

Dr. Black suggested that if one has breakthroughs one should concentrate their resources in the area to best capitalize on the breakthroughs. Dr. Anderson wondered if the review date would be altered if RAC did not recommend this funding supplement?

Dr. Carter suggested a motion.

Motion:

That supplementary funds in the amount of \$60,582 be provided as requested.

Dr. Weller offered a second.

The motion was approved by the Committee with a single negative vote.

Dr. Carter suggested that AID hold annual meetings with other interested parties such as WHO in this very important research area. Mr. Smith indicated that AID has in the past supported such activities and will, in October of this year, sponsor a meeting on the immunology of malaria in Washington to include representatives of various interested organizations.



Cultivation of Human Malaria (*Plasmodium falciparum*) and the Use of the Culture for Experimental Immunization of Monkeys -
Rockefeller University

Dr. Wishik, Chairman of the Subcommittee composed of Drs. Carter, Schweigert, and Weller, reported as follows. There is unanimous approval of this project by the subcommittee members. The RAC has participated in lengthy discussions on malaria immunization over the past several years. Some of this history was reviewed for new members.

More than 10 years ago, the WHO launched an effort to eradicate malaria. After a promising start, the situation has worsened because of malaria parasite resistance to medications and mosquito resistance to pesticides. Malaria today is on the increase. The situation is not comparable to that of smallpox, whose eradication depends exclusively on eliminating human infectious cases by immunization.

The other approaches to control of widespread vector diseases besides drugs and pesticides include environmental change and immunization. The latter seemed almost out of the question for malaria since parasitic vaccines, in contrast to bacterial and viral vaccines, had not been developed. On the basis of some epidemiologic and laboratory findings, AID decided to take the gamble and make a significant investment in the possibility of achieving a vaccine for immunization of humans against malaria.

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Under the leadership of the Health Division under Dr. Lee Howard and steered skillfully and diligently by Mr. Edgar Smith, also with the on-going strong interest and active participation of RAC, a world strategy was developed. In this, AID encouraged involvement of academic institutions, country governments, international agencies, and others; supported and participated in prompt exchange of information on new developments; and set up its own multi-pronged research support program. The scheme established priorities and favored avenues of attack with respect to mosquito vector, parasite species, and animal host; stages of the plasmodium life cycle; in vitro culture cells from mosquito, blood, and other tissues; adjuvants for strengthening immune response; among other investigations. Separate contracts were sought to encourage tissue culture work which could move toward mass production if a parasite could be grown successfully. A world network of authorities in the field was called upon for advice and participation.

A breakthrough has occurred sooner than expected. Dr. Trager of Rockefeller University, the Principal Investigator of this proposal is now growing a human malaria parasite in the laboratory in vitro. Mr. Smith will explain what is proposed and how the Trager achievements modify the emphasis and timing of the other components of the master strategy of AID on malaria immunization.

Mr. Smith, TA/H, reviewed the AID role in advertising for interested contractors; of the 7 responses 4 were relevant and led to 4 contracts. The 6 month effort of Dr. Trager has now maintained the living parasite for over 120 days on a laboratory scale. A WHO meeting in Geneva in July 1976 referred to this as posing "new horizons" for antigen production. The work has been replicated in several laboratories. The purpose of the revised project is to detail the conditions for optimal production. The University of New Mexico and Guys Hospital in London are collaborating in vaccine testing; a 3 month study report is due by January 1, 1977. Earlier estimates had indicated this achievement as requiring possibly more than 25 years. There is now agreement on the possibilities of early practical application.

Dr. Carter endorsed the continuation and expansion of Dr. Trager's work. He pointed out that there had been somewhat of an adversary role in the formulation of the program, inevitable but productive.

Dr. Schweigert was in full support of the project.

Dr. Weller commented that this finding indicates that there may be a vaccine for other parasitic diseases. He knows that Dr. Trager has worked on this problem for over 25 years. Dr. Weller has been trying to grow schistosomes, but use of the Trager method has been unsuccessful.

Dr. D. Peterson asked if the need for human blood posed a problem. He was advised by Mr. Smith that the method utilizes discarded human blood which has become overage in the blood bank, and it is readily available. Future use of the Aotus monkey will pose a problem;

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consideration is being given to the establishment of a laboratory under PAHO grant in Colombia.

Dr. Anderson asked about vaccine availability for early use. Mr. Smith commented that the WHO estimate in July was overoptimistic, and that from 3-5 years will be required for human testing. Dr. Trager estimates that 3 years will be required for the specification of optimal conditions of vaccine production. Human testing requires adjuvant development. The monkey model does not fit humans. As in the present discovery, accurate prediction is not possible. Dr. Wishik stated that there are many steps in purification of a safe and efficient vaccine. Other supportive projects are now ongoing.

Motion: That the proposal be approved.

Moved by Dr. Wishik; seconded by Dr. Schweigert

Vote: Unanimous approval.

MEMORANDUM

October 2, 1981

TO: RAC Malaria Subcommittee
FROM: S&T/HEA, C. Pease
SUBJECT: Reorientation of Project 931-0453.12 -
Biomedical Research Institute

In February 1981, researchers at the Biomedical Research Institute submitted an expanded proposal to begin conducting research on the culture of the exoerythrocytic stages of P. vivax and P. falciparum. The RAC subcommittee meeting with AID staff on March 2, 1981, felt the request for reorientation was premature and that such work should be deferred until the rodent model is more thoroughly studied.

As pointed out in the letter of March 31, 1981, from the Biomedical Research Institute, the main objective of their present contract was to develop methods for the in vitro cultivation of the EE stages of P. berghei. Determination of the vaccine potential was a lower priority and was proposed for years 2 and 3. The revised budget provided by the contractors would allow both the in vitro cultivation studies on human malaria as well as to pursue the vaccine potential in the rodent model.

In addition, the researchers have some excellent studies on the mechanism of entry of P. berghei sporozoites into cultured cells (in collaboration with Dr. Nussenzweig).

The original RAC recommendation predicated 3rd year funding for the project on satisfactory progress made on contract objectives. Since significant progress has been made against the major primary contract objectives we would request that the RAC subcommittee recommend that the 3rd year funding be approved and that the project should be reoriented to include initial research activities on the in vitro cultivation of studies to determine the vaccine potential in the P. berghei model.

BIOMEDICAL RESEARCH INSTITUTE

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Area Code: 301

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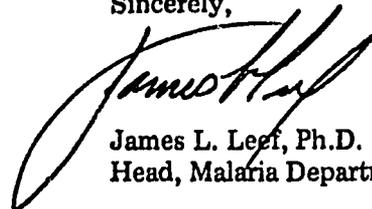
28 September 1981

Dr. James Erickson
Senior Research Officer
Vector Born Diseases
Office of Health
DS/HEA
USAID
Washington, D. C. 20523

Dear Dr. Erickson:

Please find enclosed copies of our original proposal to A.I.D. regarding tissue culture of exoerythrocytic stages of malarial organisms. Also included is the amended proposal following the suggestions of the RAC committee followed by the proposal submitted by us in May of 1981 along with pertinent correspondence.

Sincerely,



James L. Leef, Ph.D.
Head, Malaria Department

JLL/rmh

Enclosures

Original Proposal

ORIGINAL

Research Contract Proposal

entitled

IN VITRO CELL CULTURE OF THE EXO-ERYTHROCYTIC STAGE OF

MALARIAL PARASITES

Submitted to

**Office of Health (DS/HEA)
Room 307 RPE
Development Support Bureau
United States Agency for
International Development
Washington, D. C. 20523**

Submitted by

**Biomedical Research Institute
12111 Parklawn Drive
Rockville, Maryland 20852**

Michael R. Hollingsworth

Principal Investigator

Vernon P. Perry

**Vernon P. Perry
Director**

Project Length 3 Years

A. Relevance of Proposed Work to AID

Malaria continues to be one of the most widespread and devastating diseases of man in tropical and subtropical areas of the world.

Despite early successes in the global effort mounted by USAID and WHO to eradicate this disease during the past 25 years, more than a billion people remain at risk (1). This campaign was based initially on a time limited approach with interruption of transmission by selective destruction of vectors, the primary weapon. Later, the campaign was given an added dimension through mass prophylactic drug administration by distribution of chloroquinized table salt and through intensive programs of case detection and treatment. Although these efforts produced spectacular successes, malaria continued unabated in many areas of the world and there has been a rapid resurgence of the disease during the last decade in geographic areas where malaria had ceased to be considered a major problem. A few examples suffice to make the point. During the 1950's when the eradication campaign began, there were approximately 75 million cases of malaria each year reported from India alone. One decade later, in 1963, this number had dwindled to about 150,000. However, by 1977, the attack rate had climbed once more to 6.8 million diagnosed cases and present estimates are between 10 and 40 million cases for 1978. This trend has been repeated in many other areas where malaria was thought to be eradicated or under control. Sri Lanka once considered to have been in the surveillance phase of eradication recently reported over a million cases, and Haiti reported a record high 70,000 cases in 1978, an increase of 40% over the previous year. Likewise in the Magdalena Valley of Colombia where malaria control measures had been operating effectively for more than 20 years, epidemics of faciparum and vivax malaria once again took a major toll. The same experience is being repeated in Turkey where a full scale epidemic of vivax malaria is in progress. In Africa south of the Sahara, malaria has never relinquished its top priority as the most prevalent and important disease of man.

In short, the malaria eradication campaigns have effectively collapsed especially in tropical areas of the underdeveloped world. As prevalence of the disease abated because of the success achieved by these campaigns, malaria lost its top priority and workers and funds were diverted to other programs. While responsibility for the collapse cannot be assigned to any one single factor, this loss of priority certainly played a dominant role.

By the time the diversion of funds and personnel from malaria eradication campaigns to other social programs was well underway in many countries, the gravity of this mistake was beginning to be recognized and two additional complications became apparent. One of these was insecticide resistance in the anopheline vectors and the other was drug resistance in the parasites. These two major difficulties combined to increase the urgency of developing new approaches to management of the disease.

Recognizing this necessity as early as 1965, USAID launched a research program into the feasibility of developing a vaccine against malaria. The Agency has expanded this effort into a collaborative network of at least ten laboratories working on various aspects of malaria immunity. Noteworthy among the accomplishments of this network has been the continuous culture of the parasite responsible for pernicious tertian malaria, Plasmodium falciparum (2). This breakthrough provided the technology for in vitro production of antigens in quantities sufficient to support a research program aimed at development of a vaccine against this species based on the erythrocytic cycle of the parasite. This, in turn, has enabled several laboratories to successfully immunize monkeys against P. falciparum. On the other hand, questions have been raised relative to whether the amount of antigen produced would be sufficient to actually produce the quantities of vaccine which would be required. (WIIO/SWG/Imm. Report of December 1979.) Furthermore, although several attempts have been made this technology has not been successfully applied to the second important human species of malaria parasite Plasmodium vivax, which causes benign tertian malaria (Palmer - Pers. Comm).

The most likely alternative to cultivation of the P. vivax erythrocytic cycle would be the cultivation of the exo-erythrocytic stage. Although the first continuous culture system of malaria parasites was achieved with the exo-erythrocytic stages of avian plasmodia, it was not until recently that the exo-erythrocytic stage of a mammalian parasite was successfully established in cultured cells (3). This success was particularly encouraging because it demonstrated that sporozoites were capable of developing in a wide variety of cells, although development did not proceed to completion of the cycle. The objective of this proposal is to complete development to exo-erythrocytic merozoites using the rodent malaria parasite P. berghei and to extend the system to culture the exo-erythrocytic stages of the human parasites P. vivax and P. falciparum. This would provide a system for producing antigens to be used for vaccine development against P. vivax as well as an additional system for producing antigens of P. falciparum.

B. Scientific Aspects of Proposed Work

1. Specific Objectives

The ultimate objective of this project is to achieve the full in vitro cycle of development of the exo-erythrocytic stage of Plasmodium berghei in a suitable cell culture system and to apply this to culture of the exo-erythrocytic stages of Plasmodium vivax and Plasmodium falciparum.

- a. To attempt the culture of the exo-erythrocytic stages of P. vivax and P. falciparum, using techniques already successful with P. berghei.
- b. To determine the vaccine potential of the antigens produced in cultures of EE forms of P. berghei.
- c. To evaluate sporozoite purification techniques in order to standardize the methods of establishing the exo-erythrocytic stage of P. berghei in cultured rat embryonic brain cells.
- d. To evaluate selected cell lines and culture media combinations in order to achieve full maturation of the P. berghei exo-erythrocytic stage.
- e. To study sporozoite - host cell interactions by scanning and transmission electron microscopy.
- f. To characterize the developing exo-erythrocytic stages of P. berghei by immunofluorescence, electron microscopy and animal infectivity studies.

2. Rationale and Literature Review

The USAID initiated a program 15 years ago to develop an effective vaccine which would protect man against malaria. Noteworthy among the accomplishments of research laboratories in the USAID malaria vaccine network was the continuous culture of the parasite responsible for pernicious tertian malaria, Plasmodium falciparum (2). This breakthrough provided the technology for in vitro production of antigen in quantities sufficient to support development of a vaccine based on the erythrocytic cycle of this parasite. In addition, USAID supports vaccine development based on an alternative stage of the parasite life cycle, the sporozoite (4). In the latter case the antigen is obtained from an infected mosquito. Both antigens, although available in relatively large quantities, must be prepared in purified form before they can be administered safely to humans (5).

The third alternative antigen source for vaccine development the exo-erythrocytic (EE) stage grown in fixed cell culture has thus far been neglected. The advantages of the EE approach over other methods of producing antigen have been recently reviewed by Beaudoin, 1977 (6). Briefly, these advantages include adequate amounts of immunogenic parasites as free as possible from microbial and host-cell contamination, capability of subculture and indefinite maintenance with changes of nutrient media, and yield of parasites with little disturbance to the cultures themselves. The continuous in vitro cultivation of several species of avian malaria

was achieved as early as 1966 (7). Although the EE cycle in the liver of many mammalian malaria parasites appears to be self limiting in vivo (8), adaptation of these stages to tissue culture conditions similar to those used for avian parasites could result in their continuous culture in vitro, especially in the case of P. vivax. This parasite is a relapsing species and may well experience multiple schizogonic generations in the liver. To date, attempts to cultivate this important parasite of man have been unsuccessful using the P. falciparum continuous cultivation methods of Trager and Jensen.

The principal investigator of the present proposal (C. V. attached) joined the laboratory of Professor William Trager at the Rockefeller University early in 1978. There, he established the EE stage of Plasmodium lophurae in embryonic turkey brain cell culture and developed a method of harvesting large numbers of purified EE merozoites almost completely free of contaminating material using ion exchange columns (9). The availability of purified merozoite preparations made possible biochemical and immunological characterization of surface and internal antigens of EE merozoites and permitted a comparison of these forms with erythrocytic merozoites isolated from infected duck blood (Kilejian and Hollingdale, in preparation). This culture system was easily adapted for electron microscopy investigations and resulted in the only detailed study to date on the mechanism of entry of EE merozoites into turkey brain cells. This likewise made possible a comparison of penetration of EE merozoites with that erythrocytic merozoites (10).

Little is known regarding the antigenicity of EE forms. Prior studies using avian malaria have suggested that vaccination of birds with EE stages will produce protective immunity against challenge and that this can be obtained without use of adjuvants (11, 12).

Until recently, the culture of mammalian EE stages has received little attention although its study was recommended as long ago as 1975 by the WHO Scientific Working Group on malaria (13). A necessary prerequisite for such work is a continuous supply of infected mosquitoes. The Biomedical Research Institute (BRI), where experiments outlined in this proposal will be performed, possesses three large insectaries and animal quarters where the entire life cycle of Plasmodium berghei is maintained. This continuous availability of P. berghei-infected Anopheles stephensi mosquitoes has allowed an initial study of the in vitro culture of P. berghei EE stages. The first successful infections of rat embryonic liver and brain cells with salivary gland sporozoites was achieved in 1979 using techniques derived from the avian system (3). EE stages were observed that had developed to a large multinucleate schizont, although production and release of merozoites has not yet been achieved. Unfortunately the levels of infectivity were low (maximum of 4 EE stages/culture) and erratic (3% of liver and 1.4% of brain cultures became infected). It was also found that centrifugation of the sporozoites onto the cells at 1,600 x g was a prerequisite to initiate infection. This suggested the possibility that the

mechanism of sporozoite entry into cells in this system may have been different than the in vivo mechanism. Once inside the cell, however, the sporozoite was still able to initiate the EE cycle. This study also showed that cells other than hepatocytes from the rat would support EE growth. This suggests that growth requirements of the EE forms in culture are not as specific as originally believed.

Since joining the malaria research group at BRI in July 1979, the principal investigator has continued the work on in vitro culture of P. berghei. A system based on an established line of cultured human embryonic lung (WI38) cells, grown in RPMI 1640 and supplemented with 10% fetal bovine serum, has been successfully infected with salivary gland sporozoites of P. berghei. This has yielded a 10 fold increase in the numbers of parasites per culture, with 60% of the cultures infected. Centrifugation is not required for sporozoite penetration in this system, and therefore the infection may more closely resemble an in vivo infection.

In the proposed study, completion of the EE cycle of Plasmodium berghei in vitro will be attempted. The technology established for this mammalian parasite will be adapted to culture of EE stages of the human parasites, P. vivax and P. falciparum. The availability of EE parasites, produced in vitro, will make detailed studies of mammalian forms possible for the first time. These would include biochemical, behavioral and immunological investigations.

3. Experimental Design

The proposed research will be in the following areas:

- a. Purification of Plasmodium berghei sporozoites, freed from mosquito cell and tissue debris and microbial contaminants, while retaining high infectivity in vivo to mice and in vitro to rat embryonic cells. This will permit cell cultures to be reproducibly infected with sporozoites and will allow quantitative comparison between different culture conditions.
- b. Examination of further selected cell lines, derived from rodent and other species, for increased susceptibility to sporozoite infectivity. Emphasis will be on those cell lines for which infectivity does not require centrifugation of sporozoites with cell monolayers. The advantage would be to more closely approximate the normal mechanism of cell entry in the in vivo system.
- c. Suitable cell lines will be tested in combination with various culture media to optimize the system permitting the full maturation of the P. berghei EE stage with subsequent release of merozoites. The ultimate goal would be achievement of the continuous culture of the EE stage.

- d. Examination of the interaction between sporozoites and susceptible cells to determine mechanism of attachment and penetration.
- e. Characterization of the developing exo-erythrocytic stages by 1) immunofluorescence using anti-sporozoite and anti-red blood cell stage sera and 2) electron microscopy.
- f. Determination of the infectivity of exo-erythrocytic stages during cultivation as measured by the appearance of a subsequent red blood cell infection.
- g. Preparation and testing of candidate vaccines prepared from products of EE cultures.
- h. Application of the culture procedures to P. vivax and P. falciparum.

4. Procedures and Methods

The complete cycle of Plasmodium berghei will be maintained in vivo in mice and Anopheles stephensi mosquitoes.

a. Purification techniques of sporozoites infective for cell monolayers.

The present system utilizes infected salivary glands which are obtained by dissection. The glands are pooled and ruptured to release sporozoites. The complete mixture is then added to a cell monolayer, centrifuged at 1,600 x g for 30 min, and incubated in 5% CO₂ in air (3). The major disadvantages of this approach are that an unknown number of sporozoites are added to the culture, and large amounts of mosquito tissue and microbial contaminants are present. This has resulted in low numbers of EE stages per culture (a maximum of 4). Attempts to increase numbers by using more salivary glands results in destruction of the cell monolayers and/or an overwhelming microbial contamination. Published methods of purifying sporozoites using discontinuous density gradients (14) and ion exchange columns (15, 16) will be evaluated for their ability to remove mosquito debris and microbial contaminants and will be monitored by phase microscopy and confirmed by electron microscopy and serology. The number of sporozoites in the inoculum and their infectivity can also be ascertained. Success of a procedure will be determined by sporozoite infectivity in cell cultures. Sporozoites purified from whole mosquito bodies, separated thoraces and abdomens, and salivary glands will be examined. A dose response curve describing the relationship between the number of sporozoites added and numbers of developing EE stages observed will be constructed. Reproducibility with high numbers of developing EE stages will allow quantitative experiments to be designed to compare different culture procedures and conditions.

- b. Examination of selected cell lines for increased susceptibility to sporozoite infection. The original publication on successful development of EE stages in culture by Strome et al. (1979) suggested that rat embryonic brain cells were the most susceptible to sporozoite infection. Cell lines of rat embryonic liver and turkey embryonic brain and liver were also infected, though success was less than that obtained with rat embryonic brain cells. These results showed that cell lines other than those from rat could support EE growth of P. berghei. This agrees with the demonstration that EE stages of the avian Plasmodium lophurae will grow in embryonic mouse liver cells as well as embryonic turkey brain cells (17). Therefore, it is proposed to examine other cell lines for infectivity with purified sporozoites. The success in using both human embryonic liver and human embryonic lung (WI38) cells suggests that emphasis should be given to other cultures of human embryonic lines. These would be of particular relevance in the attempt to culture P. vivax and P. falciparum. Rodent and other mammalian cell lines, particularly those from monkey, will also be examined. Cell monolayers will be monitored to enumerate numbers of developing EE stages and to determine their maturation and release of merozoites.

Different culture media will also be examined in an attempt to identify those growth factors important in initial development and subsequent maturation of the EE stages. The close association of the EE stage with the host cell nucleus and its occasional development within the nucleus suggests that co-factors involved in nucleic acid metabolism may be required. The requirement of P. lophurae for folic acid (7) supports such an hypothesis. All culture systems currently in use have been supplemented with heat inactivated fetal bovine serum. It is proposed that sera from other species also be evaluated.

- c. Sporozoite host cell interaction and penetration.

Centrifugation of sporozoites onto monolayers of human embryonic lung (WI38) cells is not required to initiate EE stage development. This indicates that this cell system may be a suitable in vitro model to study the initial interaction and attachment of a sporozoite to a susceptible cell and its penetration into the cell. While penetration of host cells by EE (10) and erythrocytic (18) merozoites has been reported, little is known of the mechanism of sporozoite entry. It is proposed to use electron microscopy to study sporozoite penetration of WI38 and other cell types. Sporozoites will be added to cell monolayers which will be fixed in glutaraldehyde periodically during the 48 hr cycle. With the large number of developing EE stages we have achieved in this system, phase microscopy can be used to localize EE stages for further examination.

by electron microscopy following the procedures of Beaudoin and Strome (19). It would also be of great interest to compare these results to those in the rat embryonic brain system to determine whether the penetration mechanisms do indeed differ in these two cell systems.

Factors influencing attachment and penetration of sporozoites will also be examined. Cytochalasin B has been reported to inhibit penetration of Plasmodium falciparum erythrocytic merozoites into red blood cells although attachment of the merozoites to the red blood cell does still occur (20). It is proposed that similar experiments be performed using P. berghei sporozoites and WI38 cells. Centrifugation is required to initiate infection in rat embryonic cells but sporozoite attachment may still occur. The role of centrifugation will also be further examined both in rat embryonic brain cells and WI38 cells: (1) Cell monolayers will be centrifuged at 1,600 x g for 30 min and then purified sporozoites added, (2) cell monolayers and sporozoites will be centrifuged together at 1,600 x g for 30 min; (3) sporozoites will be added to cell monolayers without centrifugation. Gluteraldehyde-fixed monolayers will be examined by phase and electron microscopy and developing EE stages will be counted. Finally, the ability of sporozoites from irradiated mosquitoes to attach and penetrate susceptible cell lines will be examined.

d. Characterization of the developing EE stages by immunofluorescence and animal infectivity.

To date, all observations of developing EE stages have been by light microscopy of Giemsa-stained cell monolayers. We propose to use immunofluorescent antibody (IFA) techniques to positively identify bodies seen in stained monolayers as EE stages of Plasmodium berghei. Recent work (21) has suggested that during in vivo development of P. berghei in rat liver, the EE stages show maximal fluorescence with anti-sporozoite serum during the first 24 hrs of infection, and this then declines. By 48 hrs, maximal fluorescence is given with anti-red blood cell stage serum. It is proposed that this cycle of reactivity be studied using our in vitro model. Anti-sporozoite and anti-RBC stage sera will be produced in mice and rabbits. Infected monolayers will be tested from the time of sporozoite infection to the maximum EE stage development at 48 hr. Correlation with in vivo results would suggest at which stage during the EE cycle further development halts and whether the in vitro stage at 48 hr has acquired RBC stage antigens. This would also be of considerable aid in examining other cell lines in an attempt to achieve full maturation of the EE stage with subsequent release of merozoites. A cell line with EE stages showing increased RBC stage reactivity would be a likely candidate for further examination. It is proposed that selected cell lines, both human embryonic and other lines, be examined for EE development first by Giemsa-staining then by IFA techniques. Development with or without centrifugation would

be studied. Those lines showing either high infectivity or fuller maturation would then be further examined in selected culture media. It is thought that this approach will lead to the establishment of the full EE cycle in vitro.

In addition, the functional viability of infected cell monolayers will be tested. Large numbers of monolayers will be infected with purified sporozoites. Monolayers will be removed periodically from coverslips, pooled and infected into mice, and the appearance of RBC infections monitored. Thus, infectivity to mice of infected cell monolayers would be a further criterion for the study of suitable culture conditions required to achieve full cycle development. This criterion was used to measure successful maintenance of P. berghei in vitro. In this case rats were infected with sporozoites and their livers removed after varying periods of time, and placed in organ culture. Infections then resulted when pieces of these organs were reinjected into rats (22).

e. Antigenicity and protective immunity of EE stages.

If development of P. berghei EE stages can be completed in culture, it will be possible to collect merozoites for immunological and biochemical studies. Thus P. berghei would be unique of all malarials in that the infectious units of all stages would be available for comparative study including sporozoites, exo-erythrocytic merozoites and erythrocytic merozoites. Selected mouse strains will be injected with exo-erythrocytic merozoites attenuated by irradiation, freezing, formalin or other agents, with and without adjuvants. Such mice will be challenged with sporozoites, exo-erythrocytic merozoites or blood stages, and protective immunity measured. Also, using rabbit antisera to each of these three stages, antigenic cross-reactivity can be determined using, for example, immunofluorescence or ferritin labelling of surface coats. It should also be possible to label merozoites with isotopes and to compare each stage by polyacrylamide gel electrophoresis techniques.

f. Cultivation of the EE stages of P. vivax and P. falciparum.

Following the avian experience which was directly applicable to cultivation of EE stages of P. berghei, techniques successful with P. berghei will be applied to the cultivation of human malaria EE stages. A regular supply of P. vivax and P. falciparum sporozoites in Anopheles freeborni, A. maculatus and A. balabacensis has been offered by Dr. W. Collins, CDC, Atlanta, Georgia. These mosquitoes are infected from P. vivax or P. falciparum infections in monkeys, and will be used to initiate EE studies of these species in tissue culture. Dr. Collins has indicated that he is willing to supply BRL with these mosquitoes routinely on an as available basis. Other studies in progress at BRL involve the feeding of mosquitoes on P. falciparum-infected red blood cell cultures

containing gametocytes. At the meeting of the WHO Scientific Working Group on the Immunology of Malaria, held in Geneva in December 1979, it was reported that mosquitoes have been successfully infected with gametocytes of P. falciparum produced in vitro. These infections developed into sporozoites. (Meuwissen, 1979, unpublished). This report has led to an intensification of efforts at BRI and when successful, such mosquitoes will provide a continual and adequate source of P. falciparum sporozoites for initiating cultures of EE stages of this parasite.

Regular shipment of infected mosquitoes by CDC will be sent to BRI by air. The facility at BRI already has a high security insectary and laboratory, designed to contain human malaria infected mosquitoes, and trained laboratory personnel to run the facility. No additional insectary facilities would be required. BRI has convened a human pathogen committee to set safety guidelines and monitor the use of human malaria. The experimental procedure for P. vivax and P. falciparum infected mosquitoes will parallel procedures used for the P. berghei WI38 cell system, but may be modified later. Essentially, sets of salivary glands from five mosquitoes will be isolated in human group A serum or sera from other appropriate species. Each pool of salivary glands will be ruptured and added to 1 cm coverslip cultures of WI38 cells grown in RPMI 1640 + 10% fetal bovine or other appropriate sera. After a 24 hr incubation, the medium will be replaced with fresh medium containing 1% serum which causes the monolayer to remain in a stationary, non-dividing phase. Cultures will be incubated for periods corresponding to the in vivo prepatent period between sporozoite inoculation and the appearance of blood stage infections. This would be 8 days for P. vivax and 6-9 days for P. falciparum (S). Cultures will be stained periodically and examined by light microscopy for the presence of developing EE trophozoites or schizonts. Availability of developing schizonts will enable for the first time direct detailed study of this stage of a human malaria parasite. Evaluation of cultures of human malaria will follow the approaches developed for EE cultures of P. berghei. Immunofluorescent antibody techniques similar to those used on P. berghei will be adopted to confirm light microscopy observations. For this, anti-serum to P. vivax or P. falciparum sporozoites will be produced in rabbits.

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CURRICULUM VITAE

NAME: Michael Richard Hollingdale

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1964 — 1967 University of Liverpool, G.B. B.Sc. (Honors) Bacteriology

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From 1979 —

Associate Scientist, and Principal Investigator, Biomedical Research Institute, Rockville, MD.

Guest Scientist, Navy Medical Research Institute, Bethesda, MD.

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1976 - 1978:

Immunopathological mechanisms in hypersensitivity pneumonitis. Project funded by NIH grant.

Presentation of Papers at the American Society for Microbiology, the American Thoracic Society, and the Maryland Lung Association.

1978 - 1979:

The antigenic and biochemical structure of the exo-erythrocytic stage of avian malarial parasites. Project funded by the Malaria component of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

PUBLICATIONS:

Hollingdale, M. R.: The antigens of *Mycoplasma hominis*. Ph.D. Thesis, University of London, 1971.

Hollingdale, M. R. and Lemcke, R. M. The antigens of *Mycoplasma hominis*. *J. Hygiene, Camb.* 67: 585-602, 1969.

Lemcke, R. M. and Hollingdale, M. R.: The antigens of *Mycoplasma hominis*. Proceedings of the Fifth International Congress of Infectious Diseases. (Vienna), 403-406, 1970.

Hollingdale, M. R. and Lemcke, R. M.: Antigenic differences within the species *Mycoplasma hominis*. *J. Hygiene, Camb.* 68: 496-477, 1970.

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Hollingdale, M. R. and Murray, I. G.: Antibody responses in Farmer's Lung Disease. Proceedings of the Fourth International Congress on Aspergillosis and Farmer's Lung. (Basel) 280-287, 1974.

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Hollingdale, M. R.: Antibody responses in patients with Farmer's Lung Disease to antigens from *Thermoactinomyces vulgaris*. *J. Hygiene, Camb.* 74: 35-41, 1975.

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Herrmann, J. E., Hollingdale, M. R., Collins, M. F., and Vinson, J. W.: Enzyme immunoassay and radioprecipitation test for the detection of antibodies to *Rochalimaea* (*Rickettsia*) *quintana*. Proceedings of the Soc. for Exper. Biol. Med. 154: 285-288, 1977.

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- Hollingdale, M. R.: Lipopolysaccharides in *Micropolyspora faeni*. *J. Allergy Clin. Immunol.* 61: 236-237, 1978.
- Hollingdale, M. R. and Kilejian, A.: Purification of *Plasmodium lophurae* exoerythrocytic merozoites by an ion exchange column. *J. Protozool.* 26: 616-619, 1979.
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- Hollingdale, M. R., Vinson, J. W., and Herrmann, J. E.: Immunochemical and biological properties of the outer membrane associated lipopolysaccharide and protein of *Rochalimaca quintana*. *J. Infect. Dis.* 141: 672-679, 1980.
- Hollingdale, M. R., McCullough, M., Leef, J. L. and Beaudoin, R. L. *In vitro* cultivation of exoerythrocytic stages of *Plasmodium berghei* and *P. yoelii* from sporozoites. *Proceedings Tenth Intl. Mtg. Trop. Med. Malaria.* Manila, Philippines, In Press, 1981.
- Hollingdale, M. R., Leef, J. L., McCullough M. and Beaudoin, R. L. *In vitro* culture of the full development cycle of the exoerythrocytic stage of *Plasmodium berghei*. *Science*, submitted.
- Leef, J. L., Hollingdale, M. R. and Beaudoin, R. L. Principles of cryopreservation of Protozoan parasites and erythrocytes. *Bull. W.H.O.* In Press, 1981.
- Hollingdale, M. R. and McCullough, M. *In vitro* intranuclear development of the exoerythrocytic stage of *Plasmodium berghei*. *J. Parasit.* submitted.
- Hollingdale, M. R., Leef, M. F. and McCullough, M. Immunofluorescent staining of exoerythrocytic stages of *Plasmodium berghei* and *P. yoelii* grown *in vitro* from sporozoites. *J. Protozool.*, submitted.

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Hollingdale, M. R. Endotoxin of *Micropolyspora faeni*, the agent of Farmer's Lung Disease, Amer. Rev. Resp. Dis. 116: 264, 1977.

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Hollingdale, M. R., Danforth, H. D., Leef, J. L., and Beaudoin, R. L.: *In vitro* culture of exoerythrocytic stage of *Plasmodium berghei*. Abstract Ann. Mtg. Soc. Protozool. 86, 1980.

Hollingdale, M. R., McCullough, M., Leef, J. L., and Beaudoin, R. L. *In vitro* Cultivation of exoerythrocytic stages of *Plasmodium berghei* and *P. yoelii* from sporozoites. Abstract, Tenth International Congress on Tropical Medicine and Malaria, Manila, Philippines, 1980.

Leef, J. L., Hollingdale, M. R., and Beaudoin, R. L. Principles of cryopreservation of Protozoan parasites and erythrocytes. Abstract, Consultation on malaria parasite strain characterization, cryopreservation and banking, World Health Organization, Geneva, Switzerland. 1980.

Ramsey, J. M., Hollingdale, M. R. and Beaudoin, R. L. The infection of tissue culture cells with ⁶⁰Cobalt irradiated malaria sporozoites. Int. Symp. on the use of nuclear techniques in the study of Parasitic Diseases of man and animals. IAEA. Vienna, Austria. 1981. Abstract accepted, manuscript in preparation.

Ramsey, J. M., Beaudoin, R. L. and Hollingdale, M. R. Observations on the attachment and entry of *P. berghei* sporozoites to Tissue Culture cells. VI Intl. Cong. Protozool. Warsaw, Poland, 1981. Submitted.

Hollingdale, M. R., Leef, J. L., and Beaudoin, R. L. Inhibitory effect of primaquine and chloroquine to *in vitro* cultured exoerythrocytic stage of *Plasmodium berghei*. VI Intl. Cong. Protozool. Warsaw, Poland, 1981. Submitted.

CURRICULUM VITAE

James Lewis Leef

PERSONAL:

Date of Birth: 6th March 1937
Place of Birth: San Francisco, California
Nationality: American
Marital Status: Married
Present Position: Head of Department — Malaria Research
Present Address: Biomedical Research Institute
American Foundation for Biological Research
12111 Parklawn Drive
Rockville, Maryland 20852

EDUCATION:

B. A.: University of California at San Francisco, 1967
M. A. Candidate: University of California at San Francisco, 1968 —
1969
Ph. D. University of Tennessee — Oak Ridge Graduate
School of Biomedical Sciences, 1973

GRANTS AND CONTRACTS:

Principal Investigator on three U. S. Navy Contracts concerning various aspects of developing a Malaria vaccine. 1979 to present.

Principal Investigator on A. I. D. Contract to study feasibility of electrophoretic purification of sporozoites of Plasmodium berghei. Concluded 1980.

Investigator on Clark Foundation Grant to develop Schistosomiasis vaccine. 1980 to present.

FELLOWSHIPS & AWARDS:

NIH Fellowship, 1969-1973

APPOINTMENTS:

Research and Teaching Associate
University of Illinois
College of Veterinary Medicine
January 1974 to August 1976

Guest Scientist
Naval Medical Research Institute
Bethesda, Maryland
September 1976 to present

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Consultant W. H. O. Conference on Immunology of Malaria. Bethesda, 1978.

Consultant W. H. O. Meeting regarding Cryopreservation of blood samples taken in the field. Washington, D. C. 1979.

Consultant W. H. O. Meeting on Malaria parasite strain characterization. Cryopreservation and Banking of isolates. Geneva, 1980.

SOCIETIES:

American Association of Tissue Banks,
Tissue Culture Association
Society for Cryobiology
The New York Academy of Sciences

RESEARCH INTERESTS:

The mechanisms of injury to macromolecules, cells and tissues due to freezing. Immunology, malariaology, cryobiology, cell physiology, biochemistry.

MILITARY STATUS:

USNR 1954 — 1961. Hon. Discharge, 1961.

ADDITIONAL TRAINING:

1961 — 1966

Employed as research assistant in cryobiology with the Institute of Medical Sciences, Pacific Presbyterian Medical Center, San Francisco, California.

1966 — 1969

as above except position changed to Research Associate.

1968 — 1969

Independent electron microscope study of the ultrastructural changes in heart valves (human and porcine) following freezing.

1969 — 1971

Research experience in various biochemical laboratories during first two years of graduate school.

1971 — 1974

Development of skills leading to completion of dissertation research. This included extensive training in low temperature biology and the related areas of biophysics, biochemistry and cell biology.

January 1974 to August 1976

Development of immunological skills and techniques to study various immune response parameters of young, compared to adult, and senescent mice on the basis of cellular immunity.

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Preparation and presentation of laboratory courses in immunology, virology, clinical bacteriology and mycology for first year veterinary medical students.

Providing guidance in research for an honors senior biology course. Students were to gain experience in conducting original research projects; area was immunology and cryobiology.

August 1976 to
June, 1979

Training in the areas of parasitology and entomology in order to pool, freeze and store malarial organisms at low temperature for ultimate use in the development of a malaria vaccine. This additional training included learning the procedures necessary to run insectaries where mosquitoes are reared and where the sexual portion of the life cycle is carried out; and, for the animal colonies where the asexual portion of the life cycle of Plasmodial organisms is maintained. This also involved the training of technical personnel for both routine work in the insectaries and animal rooms and the training and supervision of personnel for assistance in the cryobiological research laboratory.

June 1979 to
Present

Responsibility for the Malaria Vaccine research group at the Biomedical Research Institute. This includes supervisory duties as principal investigator for research scientists and technicians in areas of Immunology, Cryobiology, Tissue Culture Methodology and the inherent administrative responsibilities involved in managing several research contracts.

2/6/1

PUBLICATIONS:

Lee, Hun, Victor Richards and James Leef. Effect of X-irradiation on changes in the cell distribution curve of mouse bone marrow as detected by electronic particle counting. *Nature* 205: 820-821 (1965).

Persidsky, M., V. Richards and J. Leef. A viable assay of frozen thawed bone marrow cells by means of the Coulter counter. *Cryobiology* 2: 10 (1965).

Persidsky, M., V. Richards and J. Leef. Cryopreservation of bone marrow with low molecular weight polyvinylpyrrolidone. *Cryobiology* 2: 74-77 (1965).

Persidsky, M., V. Richards and J. Leef. Volume changes in bone marrow and Ehrlich ascites cells after freezing as an index of preservation efficiency. *Cryobiology* 3: 59-67 (1966).

Lee, Hun, Victor Richards, James Leef and Maxim Persidsky. Cell size distribution of mouse bone marrow. *Amer. J. Clin. Path.* 47: 466-471. (1967).

Gaertner, Frank and James L. Leef. Sedimentation properties of anthranilate synthetase from osmotic lysates of wall-less variant of *Neurospora*. *Biochem. Biophys. Res. Comm.* 41: (5), 1192-1197 (1970).

Leef, J. The physiological response to low temperature of conidia of *Neurospora crassa* in the dehydrated, hydrated or germinated state. Ph. D. Thesis. Univ. of Tenn., Oak Ridge Biomedical Graduate School. (1974).

Strome, C. P. A., Tubergen, T. A., Leef, J. L. and Beaudoin, R. L. A quantitative long-term cryobiological study of malarial parasites. *Bull World Health Org.* 55: (2-3): 305-308 (1977).

Leef, J. L. and Mazur, P. Physiological response of *Neurospora* conidia to freezing in the dehydrated, hydrated or germinated state. *Appl. Environ. Microbiol.* 35 (1): 72-83 (1978).

Leef, J. L., and F. H. Gaertner. A cryobiological method for the enrichment of fungal mutants. *J. Gen. Microbiology.* 110: 221-224 (1979).

Leef, J. L., C. P. A. Strome, and R. L. Beaudoin. The low temperature preservation of sporozoites of *Plasmodium berghei*. *Bull. W. H. O.* 57 (Suppl. 1): 87-91 (1979).

Leef, J. L., C. P. A. Strome, C. A. Kroener and R. L. Beaudoin. An apparatus for obtaining controlled cooling rates in freezing biological material. *Cryobiology.* 16: 509-512 (1979).

Strome, C. P. A., P. L. DeSantis, J. L. Leef and R. L. Beaudoin. Large scale isolation of *Plasmodium berghei* sporozoites from infected mosquitoes. *Journal of Tissue Culture Methods.* 6 (1): 13-15 1980.

Strome, C. P. A., P. L. DeSantis, J. L. Leef and R. L. Beaudoin. A convenient technique for the dissection of Mosquito Salivary Glands. *Journal of Tissue Culture Methods.* 6 (1): 9-11 1980.

Strome, C. P. A., P. L. DeSantis, J. L. Leef and R. L. Beaudoin. Centrifugation of *Plasmodium berghei* on to embryonic rat brain and liver cultures. *Journal of Tissue Culture Methods.* 6 (1): 17-19 1980.

Leef, J. L., M. R. Hollingdale and R. L. Beaudoin. Principles of Cryopreservation of Protozoan Parasites and Erythrocytes. *Bull. W. H. O.* In Press.

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Heidrick, H. G., H. D. Danforth, J. L. Leef and R. L. Beaudoin. Purification of Whole Body Plasmodium berghei Sporozoites by free-flow electrophoresis separation. In Preparation 1981.

Heidrick, H. G., J. H. Rieckman, J. L. Leef and R. L. Beaudoin. Separation of Intracellular Plasmodium falciparum Red Blood Cell Stage parasites by free-flow electrophoresis. In Preparation 1981.

Hollingdale, M. R., McCullough, M., Leef, J. L. and Beaudoin, R. L. In vitro cultivation of exoerythrocytic stages of Plasmodium berghei and P. yoelii from sporozoites. Proceedings Tenth Intl. Mtg. Trop. Med. Malaria, Manila, Philippines, In Press. 1981.

Hollingdale, M. R., Leef, J. L., McCullough, M., Beaudoin, R. L. In vitro culture of the full development cycle of the exoerythrocytic stage of Plasmodium berghei. Science, submitted.

ABSTRACTS:

Persidsky, M., V. Richards and J. Leef. Bone marrow cell viability assessment after freezing using the Coulter counter. Abs. 9th Ann. Meeting Biophys. Soc., Feb. 26-28, San Francisco, p. 19 (1965).

Persidsky, M., J. Leef and V. Richards. Dog kidney cryopreservation under thermal gradient-free conditions. *Cryobiology* 4: 264 (1968).

Persidsky, M. J. Leef. Thermal gradient-free cryopreservation of tissue. *Biophys. J.* 9: A-192 (1969).

Persidsky, M. and J. Leef. Cryopreservation under ultrahyperbaric conditions. *Cryobiology* 6: 280 (1969).

Persidsky, M., J. Leef and W. Kerth. Ultrahyperbaric cryopreservation of heart valves. *Cryobiology* 6: 281 (1969).

Leef, J. and Kenneth Floyd. Canavanine-conditional cell division mutants. *Bact. Proc.* 70th Ann. Meeting, p. 70-71.

Bank, H. and J. Leef. A correlative histochemical, electron microscope and freeze-cleaving study of wall-less mutant of Neurospora crassa. *Proc. Amer. Soc. for Cell Biology*. 11th Ann. Meeting, 21 (1971).

Leef, L. and P. Mazur. The physiological response of conidia of Neurospora crassa to low temperature in the dehydrated, hydrated or germinated state. *Cryobiology* 12 (6): 568 (1975).

Leef, J. and F. Gaertner. A cryobiological method for isolating fungal mutants. *Cryobiology* 12 (6): 584 (1975).

Beaudoin, R. L., Tubergen, T. A., Strome, C. P. A. and Leef, J. A quantitative study of the cryopreservation of malaria parasites. *Fifth Int. Cong. Protozoology*: 344 (1977).

Beaudoin, R. L., C. P. A. Strome, J. L. Leef, M. P. Bawden and M. F. Leef. Preservation and storage of sporozoite vaccine preparations. Presented at Fourth Internat. Cong. Parasit. Parasitology (Sec. E): 112-113 (1978).

Leef, J. L., C. P. A. Strome and R. L. Beaudoin. The cryobiological preservation of sporozoites using DMSO and mouse serum. *Fed. Proc.* 37 (3): 682 (1978).

Leef, J. L., C. P. A. Strome, C. A. Kroener and R. L. Beaudoin. An apparatus for obtaining controlled cooling rates between the ranges of 10° to 100°C per minute. *Cryobiology* 15: 684-685 (1978).

Leef, J. L., C. P. A. Strome and R. L. Beaudoin. A comparative study of the preservation of P. berghei sporozoites using both high and low molecular weight compounds. *Cryobiology* 15: 681-682 (1978).

Hollingdale, M. R., Danforth, H. D., Leef, J. L. and Beaudoin, R. L. *In vitro* culture of the exoerythrocytic stage of Plasmodium berghei. *Ann. Mtg. Soc. Protozool.* 86, 1980.

Hollingdale, M. R., McCullough, M., Leef, J. L. and Beaudoin, R. L. *In vitro* cultivation of exoerythrocytic stages of Plasmodium berghei and P. yoelii from sporozoites. Tenth Intl. Congress Trop. Med. Malaria, 290, Manila, Philippines, 1980.

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Leef, J. L., Hollingdale, M. R. and Beaudoin, R. L. Principles of cryopreservation of Protozoan parasites and erythrocytes. Consultation on malaria strain characterization, cryopreservation and banking. W. H. O. Geneva, Switzerland. 1980.

Hollingdale, M. R., Leef, J. L., and Beaudoin, R. L. Infectivity of *In vitro* cultured exo-erythrocytic schizonts and merozoites of Plasmodium berghei. VI Intl. Cong. Protozool. Warsaw, Poland, 1981. Submitted.

Pamela J. Leland: Ms. Leland has been with the Malaria group at the Biomedical Research Institute for approximately two and a half years. She attended Montgomery College and Towson State University and majored in biological sciences. She has been actively engaged in improving methods of increasing our output of infected mosquitoes and increasing the number per mosquito. This involves improvements in rearing the mosquitoes and methods of infecting them including such parameters as optimizing the gametocytemia of the donors and developing methods of handling the infected mosquitoes to minimize losses due to premature death of these infected mosquitoes.

Fred Mitchell: Mr. Mitchell has had twenty years experience with cultivation of the exoerythrocytic stages of malarial parasites while employed as a technician in the Malaria Division of the Naval Medical Research Institute. In addition, he has had extensive experience in all phases of malariology including rearing and infecting anopheline mosquitoes, preparation and evaluation of blood films, purification of sporozoites, animal care and routine lab work. In addition, Mr. Mitchell had co-authored several papers on malaria cultivation, biology and immunology.

Christopher F. Paul: Mr. Paul has been with the Malaria research group at the Biomedical Research Institute approximately two years. He attended Montgomery College and majored in Biological Sciences. He has all the skills necessary for maintenance of the entire P. berghei life cycle. His particular area of expertise developed in this lab is in cryobiology. He has learned both theoretical and practical aspects of low temperature biology and is capable of performing complex experiments with little or no supervision. For the last year, he has been attending night class in computer programming, a skill that could be especially helpful to us, and is currently at the intermediate level.

Biomedical Research Institute:

The malarial unit at BRI is singularly suited for this study. It contains the Insectary, where the entire life cycle of P. berghei is maintained, and the high security Insectary and Laboratory where mosquitoes infected with human parasites will be housed. The Institute also possesses a fully operational tissue culture laboratory including a CO₂ incubator, vertical flow hood, inverted phases microscope and centrifuges. There is also an electron microscope facility with a RCA-3E microscope, and fully equipped dark room. The animal quarters house rat, mice and guinea pigs, and will be moving to a larger facility in 1980. All general laboratory equipment for this study is available including microscope and centrifuges. Trained Laboratory and tissue culture personnel are already present and completely familiar with the P. berghei EE system.

JUSTIFICATION FOR BUDGET ITEMS OTHER THAN PERSONNEL:

Supplies .

- (1) The expenses anticipated for the culture supplies are for media, antibiotics, buffers and other normal reagents necessary for carrying on work of this nature for a period of one year. These costs were estimated from past experience.
- (2) The amount needed for plasticware and other disposable supplies was estimated as above.
- (3) Animals - The number of mice, rats and rabbits along with the cost of their food and bedding was estimated as projected cost of the number of animals needed.

Equipment -

- (1) Incubator, CO₂ (single chamber); This is a necessary piece of equipment required for incubating our cultures since our present capacity must be enlarged to accomodate more cultured material. The price given was for that of Napco Automatic CO₂ Incubator, Model 7341. We obtained several quotations and found this to be the least expensive incubator which would fit our needs.
- (2) Epifluor Attachment for existing AO microscope in our laboratory. This is a necessary facility to add to allow fluorescent staining as part of the IFA work we plan to do.

Publication -

The amount stated was based on our past experience.

Travel -

Part of our study involves the use of mosquitos in-

fected with human malaria. Such mosquitoes cannot be sent via air freight so one of our personnel as a courier will need to make trips to CDC in Atlanta to hand carry the insects. We feel that about 15 such trips (\$200 round trip) will be necessary during the course of one year.

Overhead -

The overhead at this institution is currently equal to 100% of the total cost of the personnel salaries.

Projected 3 Year Budget

	1st Year	2nd Year	3rd Year
Personnel	62.1	68.3	75.1
Overhead	62.1	68.3	75.1
Equipment	8.5	5.0	5.0
Supplies	10.0	12.0	14.0
Travel	4.0	4.0	4.0
Publication Costs	1.0	1.5	2.0
Total Cost/Year	147.7	159.1	175.2
			Grand Total - <u>482.0</u>

AID 1 TISSUE CULTURE OF EE STAGES

PERSONNEL	% TIME	SALARY	
Microbiologist (Tissue Culture) P. I.	100	22.0	
Malariologist	60	15.6	
Insectary technician	50	7.0	
Tissue culture technician	100	14.0	
Lab technician	25	3.5	
		<hr/>	
	Salaries	62.1	<u>62.1</u>
OVERHEAD			
Billed at BRI as 100% of salaries			<u>62.1</u>
EQUIPMENT			<u>8.5</u>
Incubator 5,000			
Analytical balance 1,000			
Epifluor attachment 2,500			
SUPPLIES			<u>10.0</u>
Will include media, plasticware, chemicals, serologic reagents and E/M supplies. The estimate also includes cost of animals and board.			
TRAVEL			<u>4.0</u>
Transportation Atlanta return to obtain infected mosquitoes (see justification attached). Attendance at one national meeting for professionals.			
PUBLICATION COSTS			<u>1.0</u>
	TOTAL COST		<u>147.7</u>

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Amended Proposal
incorporating the RAC Suggestions
This Proposal has been supported by A.I.D. since 1980

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Revised

Research Contract Proposal

entitled

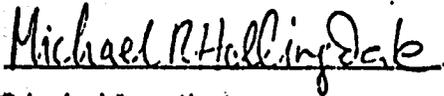
**IN VITRO CELL CULTURE OF THE EXO-ERYTHROCYTIC STAGE OF
MALARIAL PARASITES**

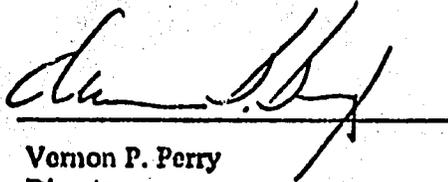
Submitted to

Office of Health (DS/HEA)
Room 307 RPE
Development Support Bureau
United States Agency for
International Development
Washington, D. C. 20523

Submitted by

Biomedical Research Institute
12111 Parklawn Drive
Rockville, Maryland 20852


Principal Investigator


Vernon P. Perry
Director

Project Length 3 Years

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A. Relevance of Proposed Work to AID

Malaria continues to be one of the most widespread and devastating diseases of man in tropical and subtropical areas of the world.

Despite early successes in the global effort mounted by USAID and WHO to eradicate this disease during the past 25 years, more than a billion people remain at risk (1). This campaign was based initially on a time limited approach with interruption of transmission by selective destruction of vectors, the primary weapon. Later, the campaign was given an added dimension through mass prophylactic drug administration by distribution of chloroquinized table salt and through intensive programs of case detection and treatment. Although these efforts produced spectacular successes, malaria continued unabated in many areas of the world and there has been a rapid resurgence of the disease during the last decade in geographic areas where malaria had ceased to be considered a major problem. A few examples suffice to make the point. During the 1950's when the eradication campaign began, there were approximately 75 million cases of malaria each year reported from India alone. One decade later, in 1963, this number had dwindled to about 150,000. However, by 1977, the attack rate had climbed once more to 6.8 million diagnosed cases and present estimates are between 10 and 40 million cases for 1978. This trend has been repeated in many other areas where malaria was thought to be eradicated or under control. Sri Lanka once considered to have been in the surveillance phase of eradication recently reported over a million cases, and Haiti reported a record high 70,000 cases in 1978, an increase of 40% over the previous year. Likewise in the Magdalena Valley of Colombia where malaria control measures had been operating effectively for more than 20 years, epidemics of faciparum and vivax malaria once again took a major toll. The same experience is being repeated in Turkey where a full scale epidemic of vivax malaria is in progress. In Africa south of the Sahara, malaria has never relinquished its top priority as the most prevalent and important disease of man.

In short, the malaria eradication campaigns have effectively collapsed especially in tropical areas of the underdeveloped world. As prevalence of the disease abated because of the success achieved by these campaigns, malaria lost its top priority and workers and funds were diverted to other programs. While responsibility for the collapse cannot be assigned to any one single factor, this loss of priority certainly played a dominant role.

By the time the diversion of funds and personnel from malaria eradication campaigns to other social programs was well underway in many countries, the gravity of this mistake was beginning to be recognized and two additional complications became apparent. One of these was insecticide resistance in the anopheline vectors and the other was drug resistance in the parasites. These two major difficulties combined to increase the urgency of developing new approaches to management of the disease.

Recognizing this necessity as early as 1965, USAID launched a research program into the feasibility of developing a vaccine against malaria. The Agency has expanded this effort into a collaborative network of at least ten laboratories working on various aspects of malaria immunity. Noteworthy among the accomplishments of this network has been the continuous culture of the parasite responsible for pernicious tertian malaria, Plasmodium falciparum (2). This breakthrough provided the technology for in vitro production of antigens in quantities sufficient to support a research program aimed at development of a vaccine against this species based on the erythrocytic cycle of the parasite. This, in turn, has enabled several laboratories to successfully immunize monkeys against P. falciparum. On the other hand, questions have been raised relative to whether the amount of antigen produced would be sufficient to actually produce the quantities of vaccine which would be required. (WHO/SWG/Imm. Report of December 1979.) Furthermore, although several attempts have been made this technology has not been successfully applied to the second important human species of malaria parasite Plasmodium vivax, which causes benign tertian malaria.

The most likely alternative to cultivation of the P. vivax erythrocytic cycle would be the cultivation of the exo-erythrocytic stage. Although the first continuous culture system of malaria parasites was achieved with the exo-erythrocytic stages of avian plasmodia, it was not until recently that the exo-erythrocytic stage of a mammalian parasite was successfully established in cultured cells (3). This success was particularly encouraging because it demonstrated that sporozoites were capable of developing in a wide variety of cells, although development did not proceed to completion of the cycle. The objective of this proposal is to complete development to exo-erythrocytic merozoites using the rodent malaria parasite P. berghei. This may provide an alternative system for producing antigens to be used for vaccine development against P. berghei and potentially allow full biochemical and immunological evaluation and comparison of all stages of the P. berghei cycle.

B. Scientific Aspects of Proposed Work

1. Specific Objectives

The ultimate objective of this project is to achieve the full in vitro cycle of development of the exo-erythrocytic stage of Plasmodium berghei in a suitable cell culture system.

- a. To evaluate sporozoite purification techniques in order to standardize the methods of establishing the exo-erythrocytic stage of P. berghei in cultured rat embryonic brain cells.
- b. To evaluate selected cell lines and culture media combinations in order to achieve full maturation of the P. berghei exo-erythrocytic stage.
- c. To characterize the developing exo-erythrocytic stages of P. berghei by immunofluorescence.
- d. To determine the vaccine potential of the antigens produced in cultures of EE forms of P. berghei.

2. Rationale and Literature Review

The USAID initiated a program 15 years ago to develop an effective vaccine which would protect man against malaria. Noteworthy among the accomplishments of research laboratories in the USAID malaria vaccine network was the continuous culture of the parasite responsible for pernicious tertian malaria, Plasmodium falciparum (2). This breakthrough provided the technology for in vitro production of antigen in quantities sufficient to support development of a vaccine based on the erythrocytic cycle of this parasite. In addition, USAID supports vaccine development based on an alternative stage of the parasite life cycle, the sporozoite (4). In the latter case the antigen is obtained from an infected mosquito. Both antigens, although available in relatively large quantities, must be prepared in purified form before they can be administered safely to humans (5).

The third alternative antigen source for vaccine development, the exo-erythrocytic (EE) stage grown in fixed cell culture has thus far been neglected. The advantages of the EE approach over other methods of producing antigen have been recently reviewed by Beaudoin, 1977 (6). Briefly, these advantages include adequate amounts of immunogenic parasites as free as possible from microbial and host-cell contamination, capability of subculture and indefinite maintenance requiring only replenishment of nutrient media, and routine harvesting of parasites with little disturbance to the cultures themselves. The continuous in vitro cultivation of several species of avian malaria was achieved as early as 1966 (7). Although the EE cycle in the liver of many mammalian malaria parasites appears to be self limiting in vivo (8), adaptation of these stages to tissue culture conditions similar to those used for avian parasites could result in their continuous culture in vitro, especially in the case of P. vivax. This parasite is a

relapsing species and may well experience multiple schizogonic generations in the liver. As already pointed out, attempts to date to cultivate this important parasite of man have been unsuccessful using the P. falciparum continuous cultivation methods of Trager and Jensen.

The principal investigator of the present proposal (C. V. attached) joined the laboratory of Professor William Trager at the Rockefeller University early in 1978. There, he established the EE stage of Plasmodium lophurae in embryonic turkey brain cell culture and developed a method of harvesting large numbers of purified EE merozoites almost completely free of contaminating material using ion exchange columns (9). The availability of purified merozoite preparations made possible biochemical and immunological characterization of surface and internal antigens of EE merozoites and permitted a comparison of these forms with erythrocytic merozoites isolated from infected duck blood (Kilejian and Hollingdale, in preparation). This culture system was easily adapted for electron microscopy investigations and resulted in the only detailed study to date on the mechanism of entry of EE merozoites into turkey brain cells. This likewise made possible a comparison of penetration of EE merozoites with that erythrocytic merozoites (10).

Little is known regarding the antigenicity of EE forms. Prior studies using avian malaria have suggested that vaccination of birds with EE stages will produce protective immunity against challenge and that this can be obtained without use of adjuvants (11, 12).

Until recently, the culture of mammalian EE stages has received little attention although its study was recommended as long ago as 1975 by the WHO Scientific Working Group on malaria (13). A necessary prerequisite for such work is a continuous supply of infected mosquitoes. The Biomedical Research Institute (BRI), where experiments outlined in this proposal will be performed, possesses three large insectaries and adequate animal quarters where the entire life cycle of Plasmodium berghei is maintained. This continuous availability of P. berghei-infected Anopheles stephensi mosquitoes has allowed an initial study of the in vitro culture of P. berghei EE stages. The first successful infections of rat embryonic liver and brain cells with salivary gland sporozoites was achieved in 1979 using techniques derived from the avian system (3). EE stages were observed that had developed to a large multinucleate schizont, although production and release of merozoites has not yet been achieved. Unfortunately the levels of infectivity were low (maximum of 4 EE stages/culture) and erratic (3% of liver and 14% of brain cultures became infected). It was also found that centrifugation of the sporozoites onto the cells at 1,600 x g was a prerequisite to initiate infection. This suggested the possibility that the mechanism of sporozoite entry into cells in this system may have been different than the in vivo mechanism. Once inside the cell, however, the sporozoite was still able to initiate the EE cycle. This study also showed that cells other than hepatocytes from the rat would support EE growth, suggesting that growth requirements of the EE forms in culture are not as specific as originally believed.

Since joining the malaria research group at BIRI in July 1979, the principal investigator has continued the work on *in vitro* culture of *P. berghei*. A system based on an established line of cultured human embryonic lung (WI38) cells, grown in RPMI 1640 and supplemented with 10% fetal bovine serum, has been successfully infected with salivary gland sporozoites of *P. berghei*. This has yielded a 10 fold increase in the numbers of parasites per culture, with 60% of the cultures infected. Centrifugation is not required for sporozoite penetration in this system, and therefore the infection may more closely resemble the *in vivo* situation.

In the proposed study, completion of the EE cycle of *P. berghei in vitro* will be attempted. If successful, it is proposed that preliminary studies be initiated to see if the technology established for this mammalian parasite can be adapted to culture of EE stages of the human parasites, *P. vivax* and *P. falciparum*. The availability of EE parasites, produced *in vitro*, will make detailed studies of mammalian EE forms possible for the first time. These would include biochemical, and immunological investigations.

3. Experimental Design

The proposed research will be in the following areas:

- a. Purification of *Plasmodium berghei* sporozoites, freed from mosquito cell and tissue debris and microbial contaminants, while retaining high infectivity *in vivo* to mice and *in vitro* to rat embryonic cells. This will permit cell cultures to be reproducibly infected with sporozoites and will allow quantitative comparison between different culture conditions.
- b. Examination of further selected cell lines, derived from rodent and other species, for increased susceptibility to sporozoite infectivity. Emphasis will be on those cell lines for which infectivity does not require centrifugation of sporozoites with cell monolayers. The advantage would be to more closely approximate the normal mechanism of cell entry in the *in vivo* system.
- c. Suitable cell lines will be tested in combination with various culture media to optimize the system permitting the full maturation of the *P. berghei* EE stage with subsequent release of merozoites. The ultimate goal would be achievement of the continuous culture of the EE stage.
- d. Characterization of the developing exo-erythrocytic stages by immunofluorescence using anti-sporozoite and anti-red blood cell stage sera.
- e. Determination of the infectivity of exo-erythrocytic stages during cultivation as measured by the appearance of a subsequent red blood cell infection.
- f. Preparation and testing of candidate vaccines prepared from products of EE cultures.

4. Procedures and Methods

The complete cycle of Plasmodium berghei will be maintained in vivo in mice and Anopheles stephensi mosquitoes.

a. Purification techniques of sporozoites infective for cell monolayers.

The present system utilizes infected salivary glands which are obtained by dissection. The glands are pooled and ruptured to release sporozoites. The complete mixture is then added to a cell monolayer, centrifuged at $1,600 \times g$ for 30 min, and incubated in 5% CO₂ in air (3). The major disadvantages of this approach are that an unknown number of sporozoites are added to the culture, and large amounts of mosquito tissue and microbial contaminants are present. This has resulted in low numbers of EE stages per culture (a maximum of 4). Attempts to increase numbers by using more salivary glands results in destruction of the cell monolayers and/or an overwhelming microbial contamination. Published methods of purifying sporozoites using discontinuous density gradients (14) and ion exchange columns (15, 16) will be evaluated for their ability to remove mosquito debris and microbial contaminants and will be monitored by phase microscopy and confirmed by electron microscopy and serology. The number of sporozoites in the inoculum and their infectivity can also be ascertained. Success of a procedure will be determined by sporozoite infectivity in cell cultures. Sporozoites purified from whole mosquito bodies, separated thoraces and abdomens, and salivary glands will be examined. A dose response curve describing the relationship between the number of sporozoites added and numbers of developing EE stages observed will be constructed. Furthermore, the infectivity of sporozoites isolated after different times during the sporogonic cycle will be examined. Similar studies have demonstrated that infectivity of P. berghei sporozoites to mice increases to a constant level from day 17 through day 24 of the sporogonic cycle and then declines (Srnkovsky, unpublished data). Reproducibility with high numbers of developing EE stages will also allow quantitative experiments to be designed to compare different culture procedures and conditions.

b. Examination of Selected Cell Lines and Culture Media for Increased Susceptibility to Sporozoite Infection

The original publication on successful development of EE stages in culture by Strome et al (1979) suggested that rat embryonic brain cells grown in MLM Medium (medium 199 + Basal medium, Eagle + 10% fetal bovine serum + $10^{-5}M$ folinic acid, Grand Island Biological Company, GIBCO, Grand Island, N. Y. 14072) were susceptible to sporozoite infection. Rat embryonic liver and turkey embryonic brain cell lines were also infected, though success was less than in the rat embryonic brain

cells. However, these results showed that cell lines other than those derived for rat tissue would support EE growth of P. berghei. This agrees with the demonstration that EE stages of the avian parasite, P. lophurae, grows in mouse embryonic liver cells as well as in turkey embryonic brain cells (17). Therefore it is proposed to examine other cell lines for greater infectivity with purified sporozoites. The success in using both human embryonic liver and human embryonic lung (WI38) cells suggest that emphasis should be given to cultures of human embryonic lines. Rodent and other mammalian cell lines, particularly those from monkey will also be examined. Cell monolayers will be monitored to enumerate numbers of developing EE stages and to determine their maturation and release of merozoites.

Different culture media will also be examined in an attempt to identify those growth factors important in initial development and subsequent maturation of the EE stages. Preliminary studies using rat embryonic brain cells grown in different culture media have suggested that the culture medium itself also influences infectivity. For example, infectivity was highest in MLM medium, and in NCTC 135 medium but rare in RPMI 1640 medium (both from GIBCO). However, WI38 cells grown in RPMI 1640 gave the higher levels of infectivity than rat embryonic brain cells in MLM. Thus infectivity may be critically related to both cell type and culture medium and evaluation of both together will be required to give optimal infectivity.

The close association of the EE stage with the host cell nucleus (3) suggests that co-factors involved in nucleic acid metabolism may be required. Indeed, occasional intranuclear development of EE stages has been observed on rat embryonic brain cells grown in MLM and NCTC 135, and only intranuclear and not cytoplasmic EE stages were observed in such cells grown in L-15 (Liebovitz) medium (GIBCO). Analysis of constituents of each medium may suggest a relationship between intranuclear development and the presence or absence of certain factors. For example, L-15 is deficient in most nucleic acid derivatives found in MLM or NCTC 135 media. The requirement of P. lophurae for folic acid (7) also supports such an hypothesis. Thus supplementation of growth media with such individual components may lead to either increased infectivity or completion of the EE cycle. All culture systems currently in use have been supplemented with 10% heat inactivated bovine serum. It is proposed to test higher concentrations of this and sera from other species. Finally, all cultures have so far been incubated at 37°C. Because in vivo rodent temperature is higher, in vitro incubation at higher temperatures will also be examined.

c. Characterization of the developing EE stages by immunofluorescence and animal infectivity.

To date, all observations of developing EE stages have been by light microscopy of Giemsa-stained cell monolayers. We propose to use immunofluorescent antibody (IFA) techniques to positively identify and antigenically characterize bodies seen in stained monolayers infected with EE stages of P. berghei. Recent work (18) has suggested that during in vivo development of P. berghei in rat liver, the EE stages show maximal fluorescence with anti-sporozoite serum during the first 24 hrs of infection, and this then declines. By 48 hrs, maximal fluorescence of EE stages is produced with anti-red blood cell stage serum. It is proposed that this cycle of reactivity be studied using our in vitro model. Anti-sporozoite and anti-RBC stage sera will be produced in mice and rabbits. Infected monolayers will be tested from the time of sporozoite infection to the maximum EE stage development at 48 hr. Correlation with in vivo results would suggest at which stage during the EE cycle further development halts and whether the in vitro stage at 48 hr has acquired RBC stage antigens. This would also be of considerable aid in examining other cell lines in an attempt to achieve full maturation of the EE stage with subsequent release of merozoites. A cell line with EE stages showing increased RBC stage reactivity would be a likely candidate for further examination. It is proposed that selected cell lines, both human embryonic and other lines, be examined for EE development first by Giemsa-staining then by IFA techniques. Development with or without centrifugation would be studied. Those lines showing either high infectivity or fuller maturation would then be further examined in selected culture media. It is thought that this approach will facilitate the establishment of the full EE cycle in vitro by providing an assay which would measure development at the immunochemical level.

In addition, the functional viability of infected cell monolayers will be tested. Large numbers of monolayers will be infected with purified sporozoites. Monolayers will be removed periodically from coverslips, pooled and introduced into mice, and the appearance of RBC infections monitored. Thus, infectivity to mice of infected cell monolayers would be a further criterion for the study of suitable culture conditions required to achieve full cycle development. This criterion was used to measure successful maintenance of P. berghei in vitro. In this case rats were infected with sporozoites and their livers removed after varying periods of time, and placed in organ culture. Infections then resulted when pieces of these organs were reinjected into rats (19).

d. Antigenicity and protective immunity of EE stages.

If development of P. berghei EE stages can be completed in culture, it will be possible to collect merozoites for immunological and biochemical studies. Thus P. berghei would be unique of all malaras in that the infectious units of all stages would be available for comparative study including sporozoites, exo-erythrocytic merozoites and erythrocytic merozoites. Selected mouse strains will be injected with exo-erythrocytic merozoites attenuated by irradiation, freezing, formalin or other agents, with and without adjuvants. Such mice will be challenged with sporozoites, exo-erythrocytic merozoites or blood stages, and protective immunity measured. Also, using rabbit antisera to each of these three stages, antigenic cross-reactivity can be determined using, for example, immunofluorescence or ferritin labelling of surface coats. It should also be possible to label merozoites with isotopes and to compare each stage by polyacrylamide gel electrophoresis and autoradiography techniques.

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JUSTIFICATION FOR BUDGET ITEMS OTHER THAN PERSONNEL:

Supplies

- (1) The expenses anticipated for the culture supplies are for media, antibiotics, buffers and other normal reagents necessary for carrying on work of this nature for a period of one year. These costs were estimated from past experience.
- (2) The amount needed for plasticware and other disposable supplies was estimated as above.
- (3) Animals: The number of mice, rats and rabbits along with the cost of their food and bedding was estimated as projected cost of the number of animals needed.

Equipment

- (1) Incubator, CO₂ (single chamber): This is a necessary piece of equipment required for incubating our cultures since our present capacity must be enlarged to accommodate more cultured material. The price given was for that of Napeo Automatic CO₂ Incubator, Model 7341. We obtained several quotations and found this to be the least expensive incubator which would fit our needs.
- (2) Epifluor Attachment for existing AO microscope in our laboratory. This is a necessary attachment to allow fluorescent evaluation as part of the IFA work we plan to do.

Publication

The amount stated was based on our past experience.

Travel

Amount sufficient for attendance at scientific meetings.

Overhead

The overhead at this Institution is currently equal to 100% of the total cost of the personnel salaries.

AID 1 TISSUE CULTURE OF EE STAGES

PERSONNEL	% TIME	SALARY	
Microbiologist (Tissue Culture) P. I.	100	22.0	
Malariaologist	60	15.6	
Insectary technician	50	7.0	
Tissue culture technician	100	14.0	
Lab technician	25	3.5	
		<hr/>	
	Salaries	62.1	<u>62.1</u>
OVERHEAD			
Billed at BRI as 100% of salaries			<u>62.1</u>
EQUIPMENT			<u>6.0</u>
Incubator 3,500			
Epifluor attachment 2,500			
SUPPLIES			<u>7.5</u>
Will include media, plasticware, chemicals and serologic reagents. The estimate also includes cost of animals and board.			
TRAVEL			<u>1.0</u>
Attendance at one national meeting for professionals.			
PUBLICATION COSTS			<u>1.0</u>
	TOTAL COST		<u>139.7</u>

827

Projected 3 Year Budget

	1st Year	2nd Year	3rd Year
Personnel	62.1	68.3	75.1
Overhead	62.1	68.3	75.1
Equipment	6.0	—	—
Supplies	7.5	10.0	12.0
Travel	1.0	1.5	2.0
Publication Costs	1.0	1.5	2.0
Total Cost/Year	139.7	149.6	166.2

Grand Total 455.5

888

**The Amended Proposal
Submitted to A.I.D.
in May of 1981**

889

Research Contract Proposal

Entitled

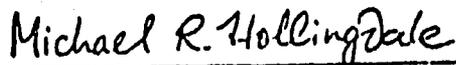
**IN VITRO CULTIVATION OF THE EXOERYTHROCYTIC STAGE OF
PLASMODIUM VIVAX AND PLASMODIUM FALCIPARUM PARASITES**

Submitted to

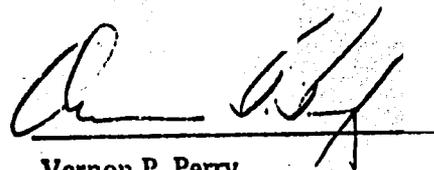
**Office of Health (DS/HEA)
Room 307 RPE
Development Support Bureau
United States Agency for
International Development
Washington, D. C. 20523**

Submitted by

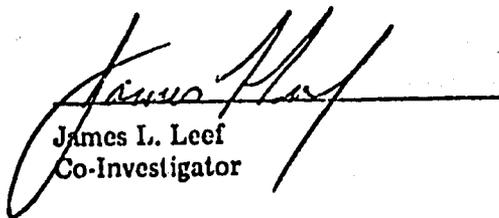
**Biomedical Research Institute
12111 Parklawn Drive
Rockville, Maryland 20852**



**Michael R. Hollingdale
Principal Investigator**



**Vernon P. Perry
Director**



**James L. Lee
Co-Investigator**

Project Length 3 Years

890

A. Progress achieved on initial contract

The principal objective of the present contract (September 1, 1980 to May 31, 1983) was to establish the full *in vitro* development cycle of the exoerythrocytic (EE) stage of *Plasmodium berghei* in a suitable cell culture system. This was to be achieved by research in six proposed areas: purification of sporozoites, evaluation of selected cell lines, evaluation of different culture media, characterization of the developing EE stages by immunofluorescence, determination of the infectivity of cultured EE stages to mice, and preparation and testing of EE antigens as potential vaccines.

It can now be reported that the principal objective of this contract has been achieved. That is, the full *in vitro* development cycle of the EE stage of *P. berghei* has successfully been established in culture. Sporozoites of *P. berghei* enter a human embryonic cell line, WI38, undergo full EE development ending in segmentation and release of merozoites. In addition, during the experiments to complete the cycle, five of the six proposed areas of research have been fulfilled, namely a method of purification of sporozoites has been determined, evaluation of different cell lines cultured in various media allowed for the selection of WI38 cells as a uniquely susceptible host cell, the immunofluorescent antibody (IFA) reactivity of developing EE parasites was shown to be similar to that reported *in vivo*, and *in vitro* cultured EE parasites were infective to mice upon subculture giving rise to a patent blood infection. The last area of research, vaccine potential, has not been determined because of the complexities of producing EE antigens in sufficient quantity for immunization. Furthermore, it is felt that such a project would be more relevant if done using EE antigens from human malarias when available and is, therefore, of less immediate priority than establishing human malarias in culture.

Because of progress in achieving these objectives, this proposal refocuses the experimental goals to apply the experience gained in the cultivation of *P. berghei* to the cultivation of the human malarias, *P. vivax* and *P. falciparum*. What is next described is a detailed summary of results from current research. The primary objectives of this new proposal are listed on page 7, and the detailed application of methods used for *P. berghei* to the experimental design for *P. vivax* and *P. falciparum* is described on pages 8 to 12.

Summary of results

1. Sporozoite purification.

Successful purification of sporozoites used to initiate EE cultures *in vitro* must fulfill three criteria. The sporozoites should be free of microbial contaminants, be as free as possible of mosquito tissue, and be infectious to cultured cells. The procedure adopted meets these criteria. *Anopheles stephensi* mosquitoes are infected by feeding on *P. berghei*

infected mice, and used 21 days later after sporozoite migration to the salivary glands. Either salivary glands or whole thoraces are dissected in Medium 199 + 7% Bovine Serum Albumin (BSA), triturated in a pestle and mortar, and applied to a discontinuous density gradient (1) containing Medium 199, 7% BSA and hypaque (S.G. = 1.115 upper layer, S.G. = 1.147 lower layer). After centrifugation at 16,000 g for 12 min., the semi-purified sporozoites are collected from the interface and applied to a DEAE-cellulose ion exchange column (DE-52, Whatman) equilibrated with Dulbecco's PBS, I = 0.161, pH 8.0 (2) containing 1% glucose and 3% BSA. Purified sporozoites are collected in normal mouse serum and enumerated. Such a procedure yields a sporozoite suspension with much reduced mosquito tissue, and they are usually free of microbial contaminants. The sporozoites retain infectivity *in vivo* to mice, and *in vitro* to WI38 cells. A dose response curve of thoracic derived sporozoites (Fig. 1) indicates about 1 in 5,000 produces 1 EE parasite, however, the infectivity of salivary gland sporozoites is much higher where approximately 1 in 100 produce 1 EE parasite. Sporozoites from the abdomen fail to produce EE parasites. Thus, the *in vitro* reactivity of sporozoites in producing EE parasites is similar to that *in vivo*, that is, salivary gland sporozoites are much more infectious than those from the thoraces, whereas abdominal sporozoites largely lack infectivity.

2. Examination of selected cell lines and culture media.

The original culture system (3) used rat embryonic brain (REB) cells grown in MLM Medium (Medium 199 + BME supplemented with 10% fetal bovine serum + 10^{-5} M folic acid + 0.1 U/ml insulin + 10^{-3} M sodium pyruvate). Our initial results indicated that human embryonic lung cells, WI38, grown in RPMI 1640, supplemented as MLM, were more susceptible to infection than REB cells. Subsequently additional cell types were tested in several culture media (Fig. 2). However, none were as susceptible as WI38 cells. From these experiments, two culture systems were the most successful in terms of range of EE stages per culture, and the average numbers of EE stages that could be reproducibly produced (Table 1). All these experiments were done using direct salivary gland isolations rather than using the purification technique described earlier. It was found that insulin, sodium pyruvate and folic acid could be omitted from culture media, which were only supplemented with 10% fetal bovine serum, penicillin (50 U/ml) and streptomycin (50 μ g/ml). REB cells were most reproducibly susceptible when cultured in NCTC-135, though average numbers of parasites per culture remained at about 8. In one experiment with MEM- and one experiment with Waymouth's medium, 400 and 211 parasites were produced respectively. However, in each case parasites were small and did not develop to large schizonts. Infections in WI38 cells were usually much higher and currently an average of 100-500 parasites

per culture are produced, or 0.25 — 1.25% of all cells are infected. As many as 2000 parasites, representing an infection of 5% of all cells, have been produced. Growth curves of *P. berghei* have been studied in each system (for example see Fig. 3), and showed that EE parasites of *P. berghei* are larger in WI38 cells (30 μ) than in REB cells (20 μ).

During the course of these experiments, a second rodent malaria, *P. yoelii* became available, and like *P. berghei*, EE parasites of *P. yoelii* developed in both REB and WI38 cells, but the maximum schizont size of *P. yoelii* was larger than *P. berghei* (29 μ in REB and up to 40 μ in WI38 cells) reflecting *in vivo* differences. (Table 2). Also as *in vivo*, *P. yoelii* schizonts reached maximum size earlier, 42-45 hr., than *P. berghei*, 48 hr. (Table 2).

Modifying the culture conditions resulted in complete segmentation of *P. berghei* EE parasites with the release of merozoites. Modifications were: the cell monolayer was cultured two to three days, rather than 18-24 hr., before the addition of sporozoites, furthermore, the culture medium was not changed during EE development, folic acid and fungizone which were thought to be inhibitors of nucleic acid metabolism were omitted, and the time of culture was extended beyond the 48-55 hr. previously used. Under these conditions, segmentation started at 60 hr., further nuclear division had occurred and the cytoplasm appeared clumped with deep clefts from the periphery. By 68 hr., segmentation was completed, and free merozoites were seen within the parasitophorous vacuole (Fig. 4). By phase microscopy, segmenters have been observed to rupture, initially by a small opening in the WI38 cell membrane which allowed a slow stream of merozoites to escape, followed by an explosive release of merozoites. Because EE parasites mature over an extended period of time, 65 to 90 hr. culture, it has not been possible to isolate free merozoites and consequently to determine their vaccine potential. In order to do this, techniques will need to be found to induce greater synchrony of development. Furthermore, free merozoites are extremely fragile and have never been observed outside the parasitophorous vacuole by Giemsa staining. The only observations made have been by phase microscopy of unfixed cells.

Factors influencing the entry of sporozoites to REB and WI38 cells have also been studied. It was known that centrifugation of the sporozoite suspension on to the cell monolayer at 1600 g for 30 min. was required to initiate infection in REB cells grown in MLM medium (3). Further experiments (Table 3) have shown that centrifugation is not absolutely required when REB cells are cultured in NCTC-135 as a few EE parasites develop when it is omitted, but more develop when it is used. In fact, REB cells which have been centrifuged at 1600 g for 30 min. prior to the addition of sporozoites develop as many EE parasites as when sporozoites are centrifuged on to the cells. Thus it is suggested that centrifugation alone alters the REB membrane surface allowing sporozoite entry, rather than mechanically forcing the sporozoite into the cell. In contrast *P. yoelii* sporozoites will infect REB cells grown in NCTC-135 to the same degree with or without centrifugation, suggesting

that even though these two malaria species are closely related, subtle differences in their *in vitro* method of entry of cells does occur. For both species, centrifugation of WI38 cells was not required, and may, in fact, inhibit entry.

As with erythrocytic merozoite entry into red blood cells, sporozoite entry of WI38 cells is inhibited by incubation with cytocholasin B (10 μ g/ml). Preliminary experiments (Table 4) have shown that incubation of both WI38 cells and sporozoites with cytocholasin B completely inhibits subsequent EE development. Experiments are underway to determine if the inhibitory effect of cytocholasin B is on the WI38 cells, the sporozoites, or both.

3. Characterization of developing EE stages by a) immunofluorescence and b) animal infectivity.

a) Immunofluorescence antibody (IFA) tests.

It was originally proposed to study the IFA reactivity of *P. berghei* EE stages at 24 hr. and 48 hr. post-infection, with anti-sporozoite and anti-red blood cell (RBC) sera. With the culture of *P. yoelii* in WI38 cells it has also been possible to study cross-reactivity between these two species (Table 5). *P. berghei* EE stages were easily identified by IFA, and this became the first positive demonstration that the parasite-like bodies produced in this system are indeed *P. berghei*. Anti-red blood stage serum, known to react with sporozoites, demonstrated strong fluorescence at 24 and 48 hr. The parasitophorous vacuole membrane strongly fluoresced, and in many cells appeared to be continuous with deposits of immunofluorescence on the host WI38 cell nuclear membrane including a mass of reactive material next to the nucleus on the opposite side with respect to the EE parasite. EE parasites typically develop close to the WI38 nucleus, which suggests that the EE parasite interacts with the nuclear membrane possibly to satisfy its biosynthetic requirements. Similar nuclear membrane fluorescence was also seen with anti-sporozoite serum (known to react only with sporozoites, not RBC stages) indicating its specific sporozoite antigenicity. The parasitophorous vacuole membrane fluoresced with anti-sporozoite antibody and the significance of this is discussed below. The peripheral parasite membrane also fluoresced with anti-sporozoite antibody, but internal fluorescence was almost absent. A similar pattern of immunofluorescence was shown by *P. yoelii* EE parasites reacted with *P. yoelii* anti-RBC and anti-sporozoite sera. Finally, *P. berghei* EE parasites strongly reacted with *P. yoelii* RBC serum, and *P. yoelii* EE parasites strongly reacted with *P. berghei* RBC serum, but no cross-reactivity was seen using anti-sporozoite sera. These results do show that EE parasites grown *in vitro* can be detected by IFA by methods previously applied to EE parasites grown *in vivo* (4), and that the EE stage *in vitro* has common antigens with both erythrocytic and sporozoite antigens, as previously demonstrated *in vivo* (5).

The success in applying IFA techniques to cultured EE parasites allowed two further sets of experiments, firstly the determination of the reactivity of hybridoma produced monoclonal antibodies, and secondly, experiments designed to explain the interaction of sporozoites with WI38 cells. These results have led to a hypothesis of the mechanism of entry of *P. berghei* sporozoites to WI38 cells. Table 6 summarizes the reactivity of two hybridoma antibodies. Both hybridomas B6 and F9 were produced by fusion of spleen cells of mice immunized with irradiated *P. berghei* sporozoites and mouse myeloma cells, and subsequently cloned (Danforth and Campbell in preparation). Hybridomas B6 and F9 both reacted in IFA with the surface of the sporozoites, but only B6 and not F9 produced a circumsporozoite precipitation (CSP) reaction, and neutralized sporozoites *in vivo* in mice. The B6 antibody reacted with EE stages after 24 and 48 hr. of culture; the reaction was strongest with an antigen on the parasite periphery thought to be the parasitophorous vacuole membrane, and weak with the internal cytoplasm. In contrast, F9 antibody reacted with the EE stages only after 24 hr. of culture and not after 48 hr. of culture. Thus, a protective antigen located on the surface of the sporozoite is also present on the periphery of the developing EE parasite.

Further experiments have been done to detect very young EE parasites, and from these the whole sequence of attachment and entry of sporozoites to WI38 cells has been studied. The infectivity of irradiated sporozoites to WI38 cells was compared to non-irradiated sporozoites. Irradiated sporozoites are the antigen used successfully to immunize mice and induce protective immunity (6) and it was of great interest to determine whether irradiated sporozoites would develop into EE stages. Earlier *in vivo* work (7) had suggested that irradiated sporozoites may develop into small EE parasites, but subsequent development into mature segmenters does not occur. This was confirmed *in vitro*. *A. stephensi* mosquitoes were irradiated at 8000 — 15000 rads in a ⁶⁰Cobalt source. Salivary gland sporozoites were added and cultures were fixed at 6, 8, 10, 12, 22 and 48 hr. later and examined by IFA and phase contrast microscopy, and after Giemsa staining. Both non-irradiated and irradiated sporozoites entered WI38 cells, were transformed and subsequently developed to trophozoites located next to the nucleus. However even at 22 hr., the trophozoites originating from irradiated sporozoites were smaller than those originating from non-irradiated sporozoites, rarely grew larger and never produced merozoites. By examining cultures between 6 and 12 hours after the addition of sporozoites, an apparent method of entry of sporozoites could be hypothesized. By IFA, using sporozoite specific antiserum, the sporozoites appeared to lie on the surface of the WI38 cell membrane, and developed a rounded "bulb" midway along its length. Sporozoite-specific antigen(s) were later detected on the WI38 membrane adjacent to the "bulb", and this area appeared to develop an invagination, into which the sporozoite entered, "bulb" first. Finally the WI38 membrane

Invagination with sporozoite-specific fluorescence sealed behind the rounding up sporozoite forming a parasitophorous vacuole. It is hypothesized that sporozoite antigen(s) are secreted from the sporozoite and cause the WI38 membrane to invaginate and that this process can be inhibited by cytochalasin B (Section A.2), the antigen(s) persist during the development of the EE parasite on the parasitophorous vacuole membrane as described using anti-sporozoite serum with EE parasites after 24 and 48 hr. of culture, and such antigen(s) may be related to the protective sporozoite antigen located by the B6 hybridoma on the parasitophorous vacuole membrane. Experiments are in progress to test this hypothesis by studying entry using B6 hybridoma antibodies.

b) Animal infectivity.

In vitro cultured EE parasites have been shown to be infective when injected into mice. Groups of cultures of WI38 cells were inoculated with salivary gland *P. berghei* sporozoites. After 24 and 48 hr. culture, the cells were trypsinized, pooled and injected intraperitoneally into a limited number of mice. In one experiment, an aliquot of the trypsinized cells from 24 hr. cultures was allowed to re-adhere to coverslips. These cultures subsequently developed mature schizonts indicating that EE parasites withstood trypsinization. The number of schizonts injected was calculated, and it was found that each mouse received approximately 600 schizonts. Each mouse developed a patent RBC infection 7 or 8 days after injection. Sporozoites held for 24 or 48 hr. in culture medium without WI38 cells, with and or without trypsinization were not infective to mice. Similarly, sporozoites added to a non-susceptible cell line (VERO cells), and injected after 24 and 48 hr. into mice were non-infective. Mice injected with cultured EE stages also developed gametocytes and experiments are in progress to determine if such gametocytes are infective to mosquitoes.

4. Antigenicity and protective immunity of EE stages.

The IFA data has shown that EE stages *in vitro* develop serologically similarly to those growing *in vivo*. It was proposed to collect merozoites for further antigenic and biochemical studies. Our results to date have shown that merozoites are liberated over an extended period of time and are extremely fragile. The successful establishment of techniques for the *in vitro* cycle of development of *P. berghei* described above strongly suggests the applicability of these techniques to human malaria. It is felt that at this time, further experiments in this area be subordinated to human malaria cultivation.

A. Scientific Aspects of Proposed Work

1. Specific New Objectives.

The primary goal of this new project is to achieve the full *in vitro* cycle of development of the exoerythrocytic stage of *P. vivax* and *P. falciparum* in a suitable cell culture system.

- a) to apply sporozoite purification techniques successful for *P. berghei* to *P. vivax* and *P. falciparum*.
- b) to test selected cell lines, particularly human embryonic lung cells, and different culture media, to support entry of *P. vivax* and *P. falciparum* sporozoites, their subsequent exoerythrocytic development, and release of merozoites.

Once *P. vivax* or *P. falciparum* have been established in a culture system, the scope of the work can be increased to include:

- c) the characterization of the developing EE stages of *P. vivax* or *P. falciparum* by immunofluorescence, as was done for *P. berghei*.

Further experiments are proposed using the *P. berghei* culture system already in existence:

- d) to study sporozoite-host cell interactions by IFA, scanning and transmission electron microscopy-*berghei*.
- e) to determine whether frozen (cryopreserved) *P. berghei* sporozoites will initiate *in vitro* EE development and whether fresh or cryopreserved sporozoites can be used for the development of an *in vitro* assay of immunity.

2. Experimental Design

The proposed research will use many of the techniques successfully applied to *P. berghei* as summarized in Section A. The work will be in the following areas:

- a) The method used to purify *P. berghei* sporozoites will be applied to sporozoites of *P. vivax* and *P. falciparum*. This will permit all cultures to be quantitatively infected with sporozoites and will allow quantitative comparison between different culture conditions.
- b) Human embryonic lung cells, uniquely susceptible to *P. berghei* sporozoites, will be tested for susceptibility to sporozoites of *P. vivax* and *P. falciparum*. If necessary, other cell lines will be tested.
- c) Different culture media will be tested on the basis that *P. berghei* grew significantly better in a given cell line in some media than in others. The primary goal would be the complete development cycle of the EE stage of *P. vivax* and *P. falciparum*.

- d) Immunofluorescent antibody (IFA) techniques proved useful in the *P. berghei* system to study the initial sporozoite interaction with cultured cells and the subsequent development of the EE stages. These methods will be applied to *P. vivax* and *P. falciparum* to determine if sporozoites of these species attach and enter cultured cells, and whether they transform into developing EE stages. *In vitro* EE development would allow the first comprehensive serological analysis of the EE stage of human malarial.
- e) The high infections achieved in the *P. berghei* system has made possible for the first time the feasibility of studying sporozoite-host cell interaction and EE development by electron microscopy. Initially scanning and transmission electron microscopy will be used to study the *P. bc. ghei* system.
- f) Examination of the infectivity of frozen *P. berghei* sporozoites to cultured cells to determine whether sporozoites may realistically be stored and standardized for future experiments. A standardized batch of sporozoites will be used to determine whether protective antibody can be measured *in vitro* by inhibition of sporozoite infectivity of cultured cells.

3. Procedures and Methods

The complete cycle of *Plasmodium berghei* will be maintained *in vivo* in mice and *Anopheles stephensi* mosquitoes, at the Biomedical Research Institute. *P. vivax* and *P. falciparum* will be maintained in *Aotus trivirgatus* monkeys and *A. freeborni* and *A. balabacensis* mosquitoes in the laboratory of Dr. W. E. Collins, Center for Disease Control, Public Health Service, Atlanta, Georgia 30333.

a. Purification techniques of sporozoites infective for cell monolayers.

The dissection of salivary glands from *A. stephensi* mosquitoes has been improved sufficiently in the *P. berghei* system to allow for more cultures to be inoculated free of microbial contaminants, and this technique will be applied to *P. vivax* and *P. falciparum*. Purification of *P. berghei* sporozoites from mosquito thoraces free of mosquito tissue and microbial contaminants has been successful in that they retain infectivity *in vitro* for cultured cells, although dose response curves have indicated that infectivity is lower than salivary gland sporozoites. However, purification has the advantage of allowing uncontaminated cultures, thus conserving sporozoites, important where the supply of human malaria is more limited. Therefore, the purification procedure will be applied to *P. vivax* and *P. falciparum*. Earlier work (2) showed that *P. knowlesi* and *P. cynomolgi* could be purified by ion exchange chromatography, indicating that modification of the technique used for *P. berghei* to other malarial sporozoites should be relatively easy.

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b. Examination of cell lines for susceptibility to sporozoite infection.

The WI38 line of human embryonic lung cells has proven more susceptible to infection with *P. berghei* sporozoites than any other line, including rodent cells. Another line of human embryonic lung cells, MRC-5 has recently been found to be as susceptible to *P. berghei* as WI38, and has the advantage of better growth for more subcultures. Production of *P. berghei* merozoites occurred in both WI38 and MRC-5 cells. It is proposed, therefore, that initially WI38 and MRC-5 cells be tested for susceptibility to *P. vivax* and *P. falciparum* sporozoites. Should WI38 or MRC-5 prove not to be susceptible to *P. vivax* or *P. falciparum*, other lines will be tested. Preliminary data (Section A2) has shown that the entry of sporozoites involves an active invagination by the host WI38 cell, which may resemble phagocytosis. Therefore, cell lines known to be phagocytic will be examined. The availability of cell lines from the American Type Culture Collection, also in Rockville, Maryland, will greatly assist this investigation. Cell monolayers will be monitored by Giemsa staining and IFA to detect developing EE stages, and to determine their maturation and release of merozoites.

c. Examination for culture media for EE growth.

Complete maturation and release of merozoites was achieved in WI38 cells grown in NCTC-135 medium. EE stages did not undergo segmentation in WI38 cells grown in RPMI-1640. Thus, composition of the culture medium is critical in the *in vitro* growth of EE stages. Initial experiments will be in WI38 cells grown in NCTC-135 medium. However, other culture media will be tested if necessary, to increase infections or induce maturation. With the complete cycle of *P. berghei* available, it should be possible to determine the basic metabolic requirements for EE parasites. It is already known that folic acid, necessary for the *in vitro* cultivation of avian EE stages, is not required for *P. berghei*. Moreover, NCTC-135 contains nucleic acid derivatives absent in RPMI-1640. Thus, essential co-factors for the culture of EE stages can be investigated using the *in vitro* system. All culture media used for *P. berghei* were supplemented with fetal bovine serum. It is proposed that if no growth of *P. vivax* and *P. falciparum* is achieved with fetal bovine serum, sera from other species will be evaluated.

d. Characterization of developing EE stages by immunofluorescence.

As described in Section A. 3a, the immunofluorescent antibody (IFA) technique was used to demonstrate the method of entry of *P. berghei* sporozoites into WI38 cells, and their EE development. It is proposed that this technique be applied to *P. vivax* and *P. falciparum*. As with *P. berghei*, *P. vivax* or *P. falciparum* sporozoites will be added to cultured cells, fixed with cold methanol after different times, and examined by IFA and phase microscopy.

It would be of interest to determine whether *P. vivax* and *P. falciparum* enter a susceptible cell by the same mechanism as *P. berghei*. Moreover, should WI38 or MRC-5 cells prove not to be susceptible to EE development of *P. vivax* or *P. falciparum*, it would be possible, by IFA, to examine whether the sporozoites do or do not attach, or if they attach fail to enter the cell, or if they enter but fail to develop to EE stages. Little is known of the serological reactivity of developing EE stages of malarial species other than those of *P. berghei*. With an *in vitro* culture system it would be possible for the first time to determine the immunological relationship between sporozoites, EE stages and RBC stages of human malarial species as has been done for *P. berghei* (Section A. 3a). The proposed sporozoite vaccine is based upon irradiated sporozoites. It has been determined (Section A. 3a.) that irradiated sporozoites enter WI38 cells by the same mechanism as non-irradiated sporozoites, but do not develop to mature EE schizonts. It is proposed that the infectivity of irradiated *P. vivax* and *P. falciparum* sporozoites also be examined for their entry and development in a susceptible cell line:

e. Examination of sporozoite — host cell in interaction and penetration by electron microscopy.

The development of the *P. berghei* culture system has allowed an *in vitro* investigation of the mechanism of attachment and entry of sporozoites to a susceptible cell. To date, all data is from IFA and phase contrast microscopy (Section A. 3a), and has shown that irradiated and non-irradiated sporozoites enter by the same mechanism. Since irradiated sporozoites induce protective immunity, elucidation of their mechanism of entry and fate once inside the cell is of importance. Only by electron microscopy will it be possible to extend and confirm the data obtained from light microscopy. It is proposed that the interaction of irradiated and non-irradiated sporozoites of *P. berghei* be studied by scanning and transmission electron microscopy. This will be done in collaboration with Dr. Susan Langreth, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20014, (letter enclosed). Sporozoites will be added to cell monolayers and fixed during the cycle of EE development. With the large numbers of developing EE stages we have achieved in this system, phase microscopy can be used to localize sporozoites and developing EE stages for further examination by electron microscopy. In addition factors influencing infectivity of sporozoites, such as cytochalasin B (Section A. 2), will be examined by electron microscopy. Thus, for the first time a comprehensive investigation by electron microscopy of the cycle of development of the EE stage of mammalian malarial species will be possible.

f. Infectivity of frozen, stored sporozoites and development of an *in vitro* assay of protection

The infectivity of sporozoites of *P. berghei* varies from batch to batch of mosquitoes. There is also a considerable variation in the numbers of sporozoites found in the salivary glands, even though oocyst production may remain relatively constant. The *P. berghei* data has suggested that salivary gland sporozoites are the most infectious to cultured cells (Section A. 1). Thus, for the proposed *P. vivax* and *P. falciparum* experiments, it would be of considerable advantage to be able to freeze and store sporozoites when they are available in quantity, and use a standardized batch for a series of experiments. It is proposed to freeze *P. berghei* sporozoites under conditions most favorable for retention of *in vivo* infectivity (8), and then test their *in vitro* infectivity to WI38 or MRC-5 cells. Once a system for the development of EE stages of *P. vivax* and *P. falciparum* has been established, freezing methods for these sporozoites will be investigated. The *in vitro* culture system would become a valuable assay of measuring the effects of freezing upon sporozoites on human malarias, removing the need to use an *in vivo* assay in monkeys. It would also have the additional benefit of being able to accumulate viable sporozoites at the Institute for a prolonged series of experiments.

In the development of an vaccine against malaria using sporozoites, an effective measure of protective immunity must be established. Currently, all methods require the use of an *in vivo* infection. However, establishment of an *in vitro* EE culture system permits the development of an *in vitro* assay of protection. Antibody raised to potential protective antigens could be assayed *in vitro* by the inhibition of entry of sporozoites into cultured cells. It is proposed that, initially using the *P. berghei* - WI38 system, sera from immunized mice or rabbits be used to inhibit sporozoite infectivity. With the availability of hybridoma derived monoclonal antibodies (see Section A. 3a), an *in vitro* test for protection would be of considerable assistance in identification and characterization of antigens eliciting protection.

g. Vaccine potential of EE antigens.

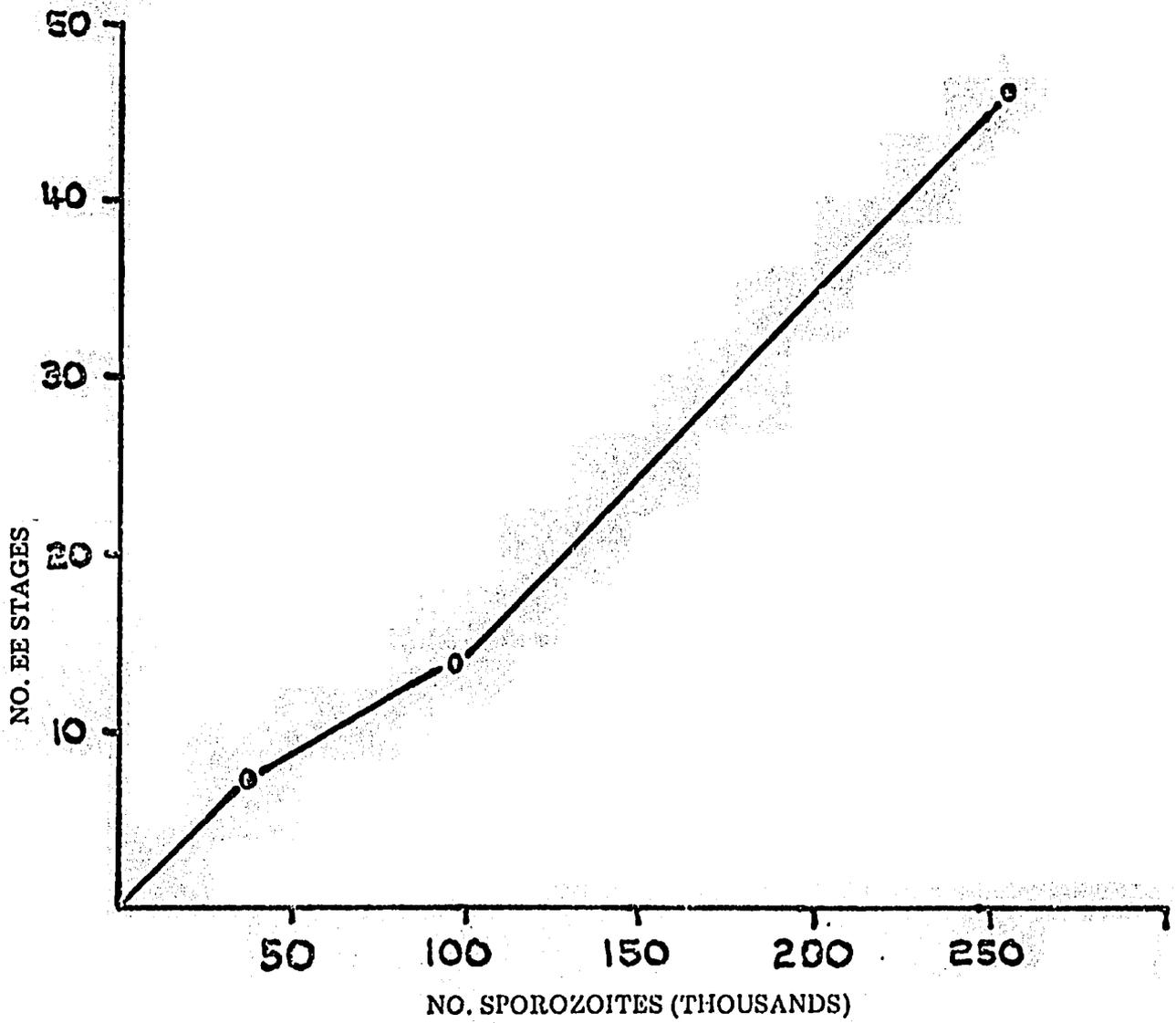
This is to be attempted only in the long term when *P. vivax* and *P. falciparum* have been established in culture. Serological data has suggested that segmenting *P. berghei* EE parasites more closely resemble RBC stages than sporozoites (Section A. 3a). This confirms other work (9) which has shown that rat liver sections, infected with EE stages of *P. berghei* *in vivo*, when subcultured into rats immunized against sporozoites, develop a patent RBC infection. That is sporozoite immunity does not extend to the EE stage. To date it has not been possible to immunize with mammalian EE stages. The *in vitro* culture system of *P. berghei* does offer the possibility of immunization: However, with one cycle of development, the numbers of available merozoites are too small to consider immunization at this

stage. It is proposed, that improvements to the culture system allowing greater synchrony of infection, with release of merozoites over a short time period, be investigated. Then it would be possible to consider active immunization. Once available in *in vitro* culture, the antigenicity of merozoites of *P. vivax* and *P. falciparum* would be of considerable importance. In this respect, the *in vitro* assay as proposed above (Section B. 2f) would be of considerable advantage. The inhibitory activity of sera from mice or rabbits immunized with EE merozoites could be assayed by the *in vitro* inhibition of entry of sporozoites into cultured cells. This would substantially reduce the number of merozoites required, and avoid the use of expensive monkeys in these initial experiments. If successful, immunization of *Aotus trivirgatus* monkeys could be attempted at a later date.

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Figure 1
Infectivity Pure P. berghei Sporozoites



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Figure 2.

P. berghei: CELL TYPES AND CULTURE MEDIA TESTED

CELL TYPE	MEDIUM				
	MLM	NCTC-135	MEM- α	RPMI-1640	WAYMOUTHS
RAT embryonic BRAIN	+	+	+	+	+
LIVER	+	+			
LUNG		+			
MOUSE embryonic WHOLE		+			
LIVER	+				
HEART	+				
MOUSE adult LIVER	-	-	-	-	
HAMSTER embryonic BRAIN	-	-	-	-	
LUNG	-	-	-		
TURKEY embryonic BRAIN	+	+			
HUMAN embryonic LIVER	-	-	-		
LUNG	+				
W138 LUNG		+	+	+	+
+	+ Low infectivity		- No infectivity		

905

Fig. 3. Growth Curves of *P. berghei* and *P. yoelii* in Rat Embryonic Brain Cells.

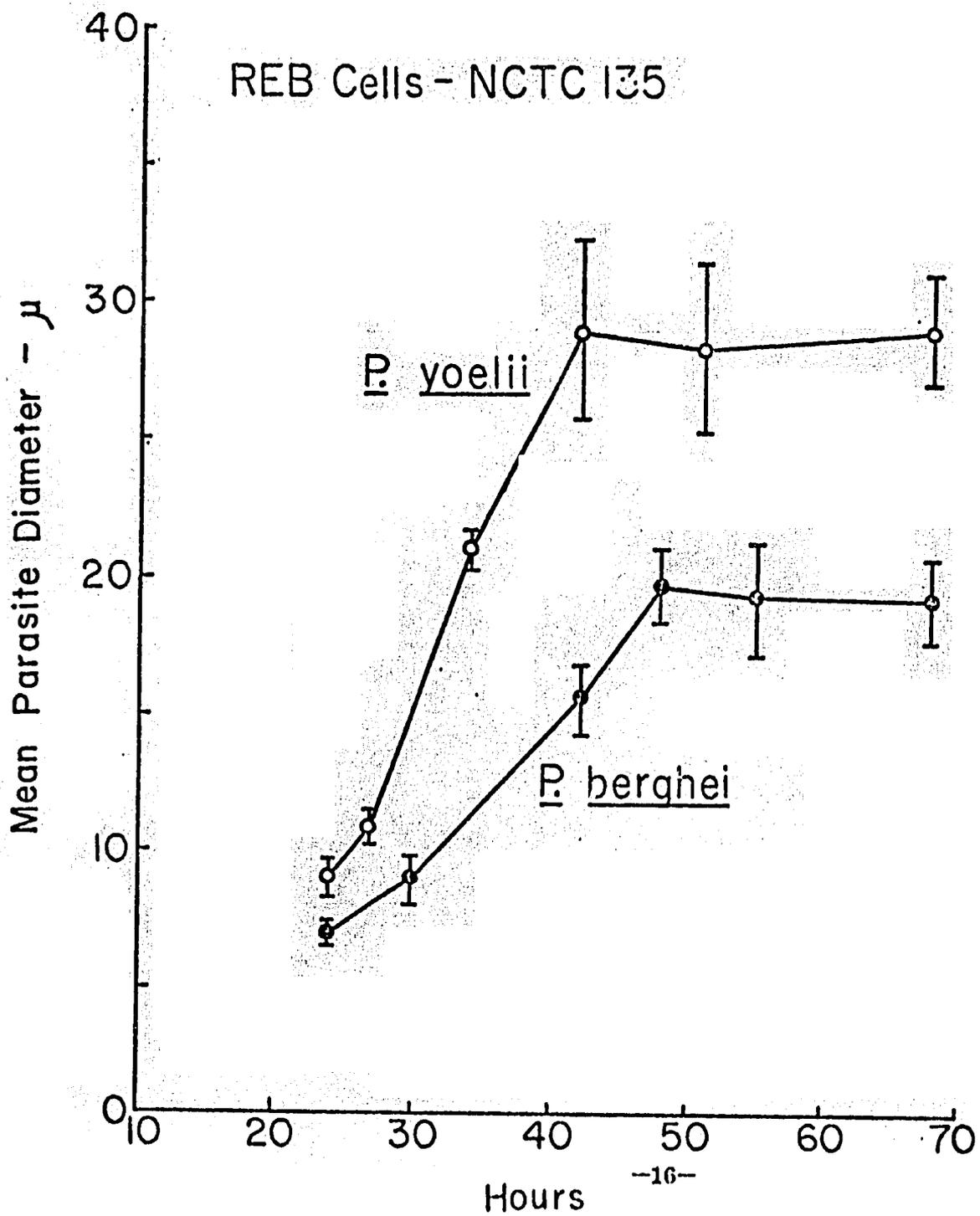


Fig. 4. *P. berghei* merozoites in WI38 cells.



Table 1.

In vitro development of P. berghei and P. yoelii EE stages

Species	Cell type	Medium	No. EE stages	
			Range	Current average
Berghei	REB	MLM	1-10	
		NCTC-135	1-36	8
		MEM- α	4-400	8
		WAYMOUTH'S	5-211	
	HEL	RPMI-1640	1-49	
		NCTC-135	1-2000	100-500
		MEM- α	3-94	50
WAYMOUTH'S		1-33		
Yoelii	REB		1-40	10
			1-13	6
	HEL		1-6	
			3-22	10
			1-15	8

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Table 2.

In vitro development of P. berghei and P. yoelii EE stages

Species	Cell type	Medium	Time max. size development Hr.	Max. schizont size μ
Berghei	REB	MLM	48	19.1 \pm 4.3
		NCTC-135	48	19.2 \pm 5.7
		MEM- ϕ	48	20.8 \pm 3.2
		WAYMOUTH'S	48	17.2 \pm 3.8
	HEL	RPMI-1640	48	15.0 \pm 3.0
		NCTC-135	48	30.5 \pm 3.8
		MEM- ϕ	48	29.4 \pm 5.5
		WAYMOUTH'S	48	19.2 \pm 4.6
Yoelii	REB	NCTC-135	42	29.0 \pm 5.5
		MEM- ϕ	42	27.5 \pm 4.2
	HEL	RPMI-1640	45	25.0 \pm 3.1
		NCTC-135	45	40.5 \pm 5.3
		MEM- ϕ	45	25.8 \pm 4.9

Table 3.

In vitro development of P. berghei and P. yoelii EE stages

Role of centrifugation

Species	Cell type	Medium	Centrifugation requirement
Berghei	REB	MLM	required
		NCTC-135	enhances infection
		MEM- α	enhances infection
	HEL	WAYMOUTH'S	required
		RPMI-1640	not required, may inhibit
		NCTC-135	not required
		MEM- α	not required
Yoelii	REB	WAYMOUTH'S	not required
		NCTC-135	not required
	HEL	MEM- α	enhances infection
		RPMI-1640	not required
		NCTC-135	not required
	HEL	MEM- α	not required
		MEM- α	not required

Table 4.
 Cytocholasin B inhibition of P. berghei
 sporozoite infectivity to IHEL cells

Cells incubated 24 hr. with 10 mg cytocholasin B in 0.1% DMSO in NCTC-135 medium

Salivary gland sporozoites isolated in cytocholasin B medium and added to cultures

Cells incubated 24 hr. further in cytocholasin B medium, then switched to NCTC-135 medium without cytocholasin B

	Slides positive	No. EE stages	No./slide
Cytocholasin B+	10/11	880	88
Cytocholasin B-	0/14	0	0

Controls = Zymosan latex

Table 5.

Immunofluorescent reactivity of P. berghei and P. yoelii EE stages

Antigen		Antiserum to			
Species	Stage	<u>P. berghei</u>		<u>P. yoelii</u>	
		SPZ	RBC	SPZ	RBC
<u>P. berghei</u>	SPZ	+	+	+	+
	EE	+	+	+	+
	RBC	-	+	-	+
<u>P. yoelii</u>	SPZ	-	+	-	+
	EE	-	+	-	+
	RBC	-	+	-	+

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Table 6.

Reactivity of hybridoma antibody to *P. berghei* sporozoites
with *P. berghei* EE stages

Antiserum	Reactivity in				
	CSP Reaction	<u>In vivo</u> Protection	IFA SPZ	EE 24 hr.	EE 48 hr.
Anti-SPZ	+	NT	+	+	±
Anti-RBC	-	NT	+	+	+
Hybridoma B6	+	+	+	+	+
F9	-	-	+	+	-

Publications from initial Research Proposal

Hollingdale, M. R., McCullough, M., Leef, J. L and Beaudoin, R. L. In vitro cultivation of exoerythrocytic stages of *Plasmodium berghei* and *P. yoelii* from sporozoites. Proceedings Tenth Intl. Mtg. Trop. Med. Malaria, Manila, Philippines, In Press. 1981.

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JUSTIFICATION FOR BUDGET ITEMS — PERSONNEL

Senior Tissue Culture Technician

This person must be proficient in many aspects of tissue culture methodology. In addition, he or she must have a well rounded background in malariology, parasitology, and biochemistry. Specific requirements are: at least five years of experience in the tissue culture of various stages of malarial organisms; in addition, he or she must have a history of successful research efforts as judged by published manuscripts and/or previous job descriptions demonstrating semi independent research capabilities.

Insectary Supervisor

This position must be filled by a person well acquainted with the life cycle of various Anophelese mosquitoes. The responsibility includes knowledge of insectary protocols for handling mosquitoes infected with strains of malaria transmissible to man. The insectary supervisor must have 3-5 years of experience in the insectary and have a past record of training personnel to rear, infect and maintain mosquitoes.

Insectary Technician

This person must develop proficiency in the insectary with respect to rearing mosquitoes. The job requires long working periods in the insectary where mosquito larvae are fed and monitored as they develop to pupae and must then be caged prior to their emergence as adults. Animal handling is a prerequisite since mice are used for maintaining the complete life cycle.

Laboratory Aide

This position requires an ability to learn and perform fundamental laboratory duties such as simple media preparation, record keeping, proper glassware washing and other routine laboratory duties.

Animal Handler

The animal handler must have had experience in a rodent facility and developed the ability to handle animals in an orderly fashion.

JUSTIFICATION FOR BUDGET ITEMS — NON PERSONNEL

Supplies

1. The expenses anticipated for the culture supplies are for media, antibiotics, buffers and other normal reagents necessary for carrying on work of this nature for a period of one year. These costs were estimated from past experience.
2. The amount needed for plasticware and other disposable supplies was estimated as above.
3. Animals - The number of mice, rats and rabbits along with the cost of their food and bedding was estimated as projected cost of the number of animals needed.

Equipment

1. Incubator, CO₂ (single chamber); This is a necessary piece of equipment required for incubating our cultures since our present capacity must be enlarged to accommodate more cultured material. The price given was for that of Napco Automatic CO₂ Incubator, Model 7341. We obtained several quotations and found this to be the least expensive incubator which would fit our needs.
2. Insectary Completion — Our existing high security insectary and associated laboratory requires two modifications to be considered high security in the strict sense. The first is a backup system for temperature control and secondly, moving all controls and machinery to a remote location from the work areas. This latter allows service by maintenance personnel.
3. Maintenance of Equipment — The maintenance is for normal laboratory operation of centrifuges, microscopes, electronic gear in our cryobiology facility and the compressors and associated refrigeration systems in our three insectories. It is an estimate based on past experience.

Travel

Part of our study involves the use of mosquitoes infected with human malaria. Such mosquitoes cannot be sent via air freight so one of our personnel as a courier will need to make trips to CDC in Atlanta to hand carry the insects. This cost also includes attendance at one national meeting per year for each investigator.

Publication

The amount stated was based on our past experience.

Budget: Year 1

	<u>%</u>	<u>Salary</u>
Principal Investigator	100	30.0
Co-Investigator	50	16.0
Senior Tissue Culture Technician	100	22.0
Insectary Supervisor	100	15.4
Insectary Technician	100	10.0
Laboratory Aide (Part-time)	100	7.0
Animal Handler (Part-time)	100	6.0
		<hr/>
		106.4
Overhead at 119.53%		127.2
Equipment: CO ₂ Incubator		4.0
Insectary Completion		6.0
Maintenance		2.0
Supplies		15.0
Travel		5.0
Publications		2.0
		<hr/>
GRAND TOTAL		267.6

*The proposed budget will supercede the budget approved for Year 2 of existing contract.

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3 Year Budget

	<u>Year 1</u>	<u>Year 2</u>	<u>Year 3</u>
Personnel	106.4	117.0	128.7
Overhead	127.2	139.9	153.9
Equipment	12.0	2.0	2.0
Supplies	15.0	17.5	20.0
Travel	5.0	5.0	6.0
Public	2.0	2.5	2.5
	<hr/>	<hr/>	<hr/>
	267.6	283.9	313.1

GRAND TOTAL= 864.6

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UNIFORMED SERVICES UNIVERSITY
OF THE
HEALTH SCIENCES
SCHOOL OF MEDICINE
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20014



MICROBIOLOGY

4 February 1981

TEACHING HOSPITALS
WALTER REED ARMY MEDICAL CENTER
NATIONAL NAVAL MEDICAL CENTER
MALCOLM GROW AIR FORCE MEDICAL CENTER
WILFORD HALL AIR FORCE MEDICAL CENTER

Dr. Michael R. Hollingdale
Biomedical Research Institute
12111 Parklawn Drive
Rockville, MD 20852

Dear Michael:

As a member of the AID Malaria Immunity and Vaccine Research Collaborative Network, I would be pleased to collaborate with you on electron microscopic aspects of your work on invasion of sporozoites and exoerythrocytic development in culture. I understand that the species involved will be Plasmodium falciparum, vivax and berghei.

Sincerely,

Susan G. Langreth

SUSAN G. LANGRETH, Ph.D.
Associate Professor

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UNITED STATES INTERNATIONAL DEVELOPMENT COOPERATION AGENCY
AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

March 16, 1981

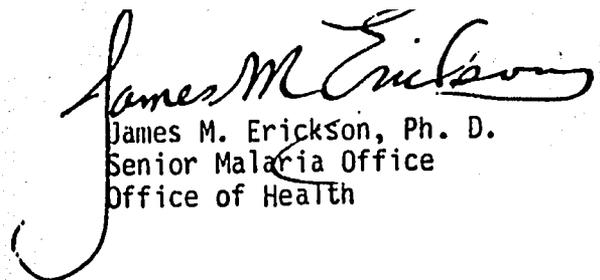
Dr. Michael Hollingdale
Biomedical Research Institute
1211 Parklawn Drive
Rockville, Maryland 20852

Dear Dr. Hollingdale:

On March 2, 1981, the Research Advisory Committee received your request to expand and redirect your current malaria research project on the exo-erythrocytic stage of this parasite. The subcommittee concluded that more work was necessary on the P. berghei system before you should start on P. falciparum and P. vivax. I have enclosed the committee comments that were read into the record on March 4, 1981.

Please review the subcommittee comments. When you feel you can address the contained comments with supporting data, we will be most happy to resubmit your request for funding consideration.

Sincerely yours,


James M. Erickson, Ph. D.
Senior Malaria Office
Office of Health

enclosure: a/s

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31 March 1981

James M. Erickson, Ph.D.
Senior Malaria Office
Office of Health
United States International
Development Cooperation Agency
Agency For International Development
Washington, D. C. 20523

Dear Dr. Erickson:

The current contract held by the Biomedical Research Institute entitled "*In vitro* cell culture of the exoerythrocytic stage of malarial parasites" was awarded in May 1980. It is a modification of the original proposal (same title) submitted to AID in March 1980. The RAC recommended that the goals and areas of research of the original proposal be reduced in scope, and the approved contract has as its ultimate objective the achievement of the full *in vitro* cycle of development of the exoerythrocytic stage of *Plasmodium berghei* in a suitable cell system. As we reported at the AID meeting in January 1981, this ultimate goal has been achieved; *P. berghei* sporozoites inoculated into cultures of human embryonic lung cells go through the complete cycle of exoerythrocytic development with release of merozoites.

The RAC also specifically modified the areas of research of the original proposal, and mentioned six areas where they felt meaningful results could be obtained. The majority of the research goals have been accomplished and are discussed below.

- a. Testing of suitable cell lines in various culture media to permit full maturation of the exoerythrocytic stage with release of infective merozoites. *Completed.* Of all culture media tested, only Medium NCTC-135 supplemented with 10% fetal calf serum successfully supported the complete cycle. Other media increased susceptibility of cultured cells to sporozoites without maturation, and differences in media composition may suggest the essential cofactors for exoerythrocytic development.
- b. Examination of cell lines for increased susceptibility to sporozoites. *Completed.* The human embryonic lung line WI38 was found to be the most sensitive of a total of 10 lines tested, and was the only line in which the development of the complete exoerythrocytic cycle occurred.
- c. Purification of sporozoites retaining infectivity to cultured cells. *Completed.* A technique using density gradient centrifugation followed by elution from an ion exchange column produces sporozoites infectious to cultured human embryonic lung cells. It is reproducible and allows quantitative experiments.

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- d. Characterization of the developing exoerythrocytic stage by immunofluorescence. *Completed.* As the Exoerythrocytic stage develops, anti-sporozoite activity is increasingly restricted to the parasite periphery and the parasite cytoplasm giving rise to merozoites which become exclusively cross reactive with the RBC stage. The results show that mature merozoites serologically more closely resemble the RBC stage than the sporozoite stage.
- e. Determination of the infectivity of exoerythrocytic stage to mice. *Completed.* Cultured exoerythrocytic stages were subinoculated into mice at intervals through the development cycle. Such mice developed a red blood cell infection accompanied with gametocytes. *Anopheles stephensi* mosquitoes fed on these mice developed a normal sporozoite infection in their salivary glands, which upon inoculation into cell cultures initiate the cycle of exoerythrocytic development.
- f. Preparation and testing of candidate vaccines from exoerythrocytic stages. *Proposed.* This area of research was suggested in the original contract proposal and was retained in the current approved contract. However, it was our understanding that *in vitro* cultivation of the ET stage of *P. berghei* was our primary objective, and determination of vaccine potential was one of six areas to be pursued as secondary goals. If the RAC are suggesting that we now accumulate sufficient quantities of EE antigens to conduct a thorough evaluation of their immunizing potential, the primary focus of the contract has been changed from cultivation to vaccine testing. Our whole system has been on a small scale suitable for comparing large numbers of media - cell combinations. To produce enough merozoites to use as an immunizing antigen will require a substantial increase both in equipment and supplies and also technical assistance, and this would have to be reflected in an increased budget. We are quite prepared to do this, and indeed in our new proposal, vaccine testing is listed as an area of research. With the proposed primary focus to cultivate the human malaria, we calculated that the increased budget and expanded facility would also allow a proper evaluation of vaccine potential of *P. berghei*, while also allowing us to begin studies on cultivation of *P. vivax* and *P. falciparum*.

The serological data, section d, suggests that exoerythrocytic merozoites closely resemble red blood cell stage merozoites. Other investigators have shown that red blood cell stage merozoites will induce protective immunity in rodents and primates. This close similarity is of importance in the human system as merozoites from blood culture are contaminated with red blood cell components, and an alternate source of merozoites from tissue culture cells would be of considerable advantage. We feel it is an appropriate time to include human malaria, particularly *P. vivax*, because it is the most important relapsing malaria species and for which no culture system is available.

Sincerely,

Michael R. Hollingdale, Ph.D.

James L. Leef, Ph.D.
Head Malaria Department

Contract No. DSPE-C-0079, *In vitro* Cultivation of the Exoerythrocytic Stage of Malarial Parasites
by M. R. Hollingdale

Progress Report No. 1 for May 15, 1980 — May 14, 1981

The principal object of this contract was to establish the full *in vitro* development cycle of the exoerythrocyte (EE) stage of *Plasmodium berghei* in a suitable cell culture system. It can be reported that this principal objective has been achieved.

Sporozoites of *P. berghei* enter a human embryonic lung cell line, WI38, undergo EE development ending in segmentation and release of merozoites.

In addition, during experiments to complete the cycle, five of the six proposed areas of research have been fulfilled, and progress is summarized below.

1. Sporozoite purification

Purified sporozoites used to initiate EE cultures must be free of microbial contaminants, as free as possible of mosquito tissue, and be infectious to cultured cells. The procedure developed meets these requirements. The method utilizes isolated *Anopheles stephensi* salivary glands disrupted to release sporozoites, which are then semi-purified on a discontinuous density gradient and finally purified by passage through a DEAE-cellulose ion exchange column. Approximately 1 in 100 sporozoites produce 1 EE parasite, while sporozoites isolated from whole thoraces are less infective, and those from abdomens non-infectious.

2. Evaluation of selected cell lines.

Cell cultures from embryonic or adult mice, rats, hamsters, turkeys, monkeys and humans have been tested to support EE development. All, except hamster, showed some infectivity, but infectivity was highest in monkey and human tissues, and the complete cycle was found to develop in these tissues.

3. Evaluation of selected culture media.

Contrary to expectations, the type of culture medium was of less importance in establishing EE cultures than the cell type. Using cultured human lung (WI38 cells) the complete cycle occurred with NCTC-135, MEM-alpha, Waymouth's, and BME media supplemented with 10% fetal bovine serum. Unlike the avian EE cycle, folic acid was not required.

Additionally, the EE stage of *P. yoelii* has been established in conditions identical

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to that of *P. berghei*, but release of merozoites has not been demonstrated.

4. Characterization of developing EE stages by immunofluorescence antibody (IFA) tests.

In collaboration with Dr. R. Beaudoin and Ms. J. Ramsey of the Naval Medical Research Center, Bethesda, MD, IFA tests have been used to study the entry and serological development of *P. berghei* EE parasites in cultured WI38 cells. Briefly, sporozoites appear to develop a rounded "bulb" midway along its length, and secrete sporozoite-specific antigen(s) onto the membrane of the WI38 cell. The WI38 membrane appears to invaginate at this point enveloping the sporozoite in a parasitophorous vacuole bounded by a membrane thus derived from the WI38 cell. Trophozoites at 24 hr strongly fluoresce with anti-sporozoite and anti-red blood cell (RBC) stage sera, but by 48 hr reactivity is strongest with anti-RBC sera. In addition, two hybridoma clones secreting monoclonal antibodies to two distinct surface components of the *P. berghei* sporozoite were available from Dr. H. Danforth of this institute; B6 elicited a circumsporozoite reaction and neutralization *in vivo* in mice, but F9 did not. In IFA, B6 antibody reacted with both 24 and 48 hr cultures, whereas F9 only reacted at 24 hr.

Gamma-irradiated sporozoites also were found to infect WI38 cells by the same mechanism as non-irradiated sporozoites and developed into trophozoites. However, nuclear division did not occur and persisting parasites at 48 hr were generally abnormal in appearance.

Finally, in collaboration with Dr. Ruth Nussenzweig at New York University Medical School, purified hybridoma monoclonal antibody, and its Fab moiety, produced against a sporozoite surface antigen (Pb44) and able to elicit the circumsporozoite reaction and *in vivo* neutralization, and thus analogous to the B6 hybridoma, were tested for *in vitro* neutralization of *P. berghei* sporozoites. Both the complete antibody and Fab were as active in blocking infection; at 40 µg/ml blocking was absolute, and inhibition occurred even as low as 2 µg/ml.

5. Infectivity of cultured EE stages to mice.

Cells containing EE stages grown for 48 hr in culture were removed by trypsin and approximately 600 EE schizonts were injected intraperitoneally into mice. Each developed a patent RBC infection 7 or 8 days later. Controls never developed an RBC infection. The mice developed normal gametocytes, and *A. stephensi* mosquitoes fed on these mice developed heavy salivary gland infections of sporozoites, which were used successfully to again establish *in vitro* EE cultures.

6. Antigenicity and protective immunity of EE stages.

The IFA tests have demonstrated that cultured EE stages have an antigenic development similar to that reported *in vivo*. However, to date no experiments have been performed to elicit antisera to EE schizonts or merozoites. It is anticipated that such work will be done during year two of the contract.

In addition to these six areas of research, additional experiments have been done to determine the potential of using *in vitro* cultured EE parasites as a test for tissue schizonticides. Preliminary data has shown that primaquine is effective at physiological levels. However, unexpectedly, chloroquine was also directly effective at similar concentrations. Work is in progress to evaluate further this system.

BIOMEDICAL RESEARCH INSTITUTE

Telephone: 881-3300
Area Code: 301

12111 Parklawn Drive
Rockville, Maryland 20852

28 September 1981

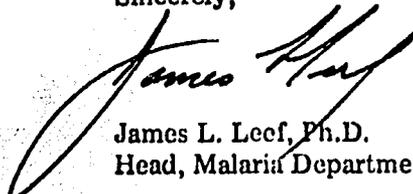
To whom it may concern:

In a recent letter to Dr. Erickson (28 August 1981), Dr. Hollingdale confidentially disclosed some developments regarding the work going on here at BRI. I'll paraphrase the content because it represents an up-date on progress report No. 1.

The mechanism of entry of *Plasmodium berghei* sporozoites has continued to be studied in cultured WI38 cells since WI38 cells support the complete exoerythrocytic (EE) development of *P. berghei*, as just published in Science 213:1021. *P. berghei* sporozoites carry on their surface a protective antigen (Pb44), and monoclonal antibodies to Pb44 neutralize sporozoite infectivity when incubated together *in vitro* and injected into mice, or when the antibodies are passively transferred into mice. Recently, in a joint project with Drs. Ruth and Victor Nussenzweig, at New York University, we have shown that monoclonal antibody, and its Fab fragment to Pb44, also neutralizes sporozoite infectivity to WI38 cells.

This data has generated a hypothesis for the entry mechanism of sporozoites and the role played by the Pb44. The hypothesis may be general in nature since preliminary data have shown that similar events may be occurring with *P. knowlesi*. Thus it should be possible to use inhibition of attachment as an assay for the protective antibody to *P. knowlesi*, as is now used in the murine system. This would be a considerable improvement over using primates or human volunteers.

Sincerely,



James L. Leef, Ph.D.
Head, Malaria Department

JLL/rmh

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In-vitro Cell Culture of the Exoerythrocytic Stage of Malaria Parasites - Biomedical Research Institute

I. SUBCOMMITTEE REPORT

Dr. Weller, Chairman of the subcommittee that also included Drs. Black, Carter and Schweigert reported on the project.

The project was first reviewed by RAC on March 4, 1980 when it was decided that a more sharply focused proposal was needed and a site visit to the research facility was recommended.

The site visit by Dr. Weller, of RAC, consultants Contacos and Boudoin, and AID staff Smith (DS/HEA) and Rechcigl (DS/PO/RES), was carried out in April. The team concluded that the revised proposal was responsive to RAC's previous recommendation. Dr. Weller thought the project had potential but is a high risk endeavor. It is a new area of research that is concerned with a different stage of the parasite than the rest of the malaria network.

II. SUBCOMMITTEE COMMENTS

Dr. Black questioned why the revised proposal which was reduced in scope from the original showed only a small drop in the budget. He was also interested in what activities would be assigned to some of the research team. Dr. Black asked for a clarification of the \$77,000 which had already been provided to the researchers. Would it be a part of the overall budget or supplemental to it? He found the research aspects of the proposal to be satisfactory.

Dr. Carter agreed that the research is risky and involves a new stage of the malaria parasite than other AID research.

Dr. Schweigert pointed out that for a very small budget reduction, AID was getting a great deal less research than in the original proposal. Also, the overhead charges appeared to be extremely high. He thought these two points merited careful review.

III. OTHER COMMENTS

Dr. Heady wondered if the Biomedical Institute is a profit-making organization. Dr. Weller provided some written information about the Institute and replied that it was non-profit.

Mr. Smith of AID told of the earlier interest in WHO in concentrating on the merozoite stage of malaria. It was decided then that no approach should be abandoned. The proposed research was in a new area but was justified.

Dr. Erickson of AID explained that one of the technicians that Dr. Black had asked about was from the laboratory of Dr. Nussensweig and would be doing essentially the same activities as he had in Nussensweig's lab. Dr. Erickson also clarified that the \$77,000 was part of the total budget and not a supplement.

Dr. Contacos explained that it was difficult to lower the budget very much with a reduction in scope because the budget primarily paid the salaries of the principal investigators.

IV. ACTION

Motion: That the revised proposal be funded as requested for a period of two years with possibility of extension to a third year if, on review after 18 months, it appears that satisfactory progress was being made.

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be reported to the RAC in June.

Dr. Carter asked if the three new proposals before the RAC are approved, will funds be available to implement them?

Dr. Erickson stated that funds are available for all of the projects except the one project on vector control.

No action by the RAC was necessary at this time.

In-vitro Cell Culture of the Exo-erythrocytic Stage of Malaria Parasites
- Biomedical Research Institute

Dr. Weller, subcommittee chairman, reported for himself and Drs. Black, Carter and Schweigert.

I. SUBCOMMITTEE REPORT

RAC is asked to review a proposal from the Biomedical Research Institute entitled "In-vitro Cell Culture of the Exoerythrocytic Stage of Malaria Sporozoites." The proposal requests a total of \$482,000 over a three year period.

The project is new and would add a unique and previously unexplored approach to the AID malaria vaccine network. The background is next summarized briefly. Current AID funded efforts to develop a malaria vaccine focus on obtaining immunogenic material from two stages of the parasitic life cycle, i.e. the use of sporozoites obtained from infected mosquitoes, and the use of erythrocytic stages - malaria organisms harvested from parasitized red blood cells. A third approach, the use of immunogens obtained from the exoerythrocytic cycle has for technical reasons not been explored in mammalian models. In man, this is the phase of the cycle that begins with the infection of a liver cell on penetration by a sporozoite, extends through the massive intra-cellular replication of the parasite within the hepatocyte, and terminates with the release of infectious merozoites that then enter red blood cells and initiate the red blood cell - i.e. erythrocytic cycle.

The exoerythrocytic stages of mammalian parasites were, prior to 1979, uncultureable. In contrast, the same stages of two species of avian

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malaria parasites have been cultured. Continuous cultivation of the exoerythrocytic stages (EE) was achieved by Dr. Ruff's group at the U.S. Naval Malaria Research Insititue, Bethesda, in 1966. Under Dr. Beaudoin's direction, the Navy research group has continued to pursue the possibility that exoerythrocytic stages of mammalian species can be similarly cultured. In a collaborative study, at the Biomedical Research Institute, financed by the Navy, the first cultivation of the EE stages of the rodent parasite, Plasmodium berghei was achieved last year and the results published by Strome, de Santis and Beaudoin (In-vitro; volume 15, pp. 531-536, 1979). Currently, according to Dr. Beaudoin, the cutback in funding for malaria research by the Navy will not permit continued support of the work at the Biomedical Research Institute. This is the background for the request for funding by AID.

The paper by Strome et al. has been reviewed. The results are convincing and indicate that EE stages of P. berghei can be grown to the late schizont stage in cultures of embryonic rat brain and rat liver and in embryonic turkey brain. However, the percentage of such cultures showing infection was low, being 9.4% for turkey brain cells, 14% for rat brain cultures, and 3% for rat liver cultures. Development progressed for 48 to 56 hours, and then aborted; no mature segmenters or free merozoites were seen. A basic problem involved contamination of the sporozoite inoculum; some 43% of all culture preparations had to be aborted because of contamination, usually with yeasts.

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The present proposal is submitted by Dr. Michael Hollindale who worked for one year (1978-79) in Dr. Trager's laboratory on growth of EE stages of P. lophurae in embryonic turkey brain cell cultures. He joined the Biomedical Research Institute in July, 1979, and has since focused on growth of EE stages of P. berghei. The undocumented statement is made that use of a human embryonic lung cell line (WI38), with nutrient medium of RPMI 1640 plus 10% fetal calf serum yields a culture infectivity rate of 60% and a 10 fold increase in numbers of parasites per culture.

In brief the proposed research will seek to:

- 1) Develop methods for purification of sporozoite inocula free of mosquito contaminants, that will permit better infections. Currently, a maximum of four parasitized cells is seen per field.
- 2) Screen cell lines to retest culture strains with enhanced susceptibility to the EE stages.
- 3) To study sporozoite - host cell interactions by ultrastructural microscopic studies.
- 4) To characterize the developing EE stages by immunologic, morphologic, and animal infectivity studies.
- 5) To examine the possibility that the EE stages of P. vivax and P. falciparum can be cultured.
- 6) To determine the immunogenicity potential of cultured EE stages of P. berghei.

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The research potential outlines a tremendous amount of work with considerable variation as to procedural detail in different sections. In general all of the objectives are desirable. However, the total amount of work outlined is not reasonable for this small group of investigators. For example, it is stated that if merozoites that are infective can be obtained "retested mouse strains will be inoculated with EE merozoites attenuated by irradiation, freezing, formalin or other agents, and with or without adjuvants. Such mice will be challenged with sporozoites, EE merozoites, and erythrocytic merozoites and protective immunity measured." This section alone would be a full-time endeavor for a team of investigators. In fact, there is only one full-time professional (Dr. Hollingdale, the P.I.) and only one full-time technician on the project.

C. V.'s of two other recent doctoral degree holders, Drs. Leef and Danforth, are included, but only one other professional individual - a malariologist at 60% effort is budgeted. Two part-time technicians complete this small team. (No mention is made of Dr. Strome, the senior author on the single paper published to date; has he left the Research Institute?).

In general this proposal embodies a potentially valuable new approach to vaccine production. The objectives appear overly ambitious, although of merit. RAC suggests that the proposal be narrowed in scope and more sharply focused on the rodent malaria system. The primary objective should be on the tissue culture system per se; efforts should be made to complete the EE cycle in-vitro and to increase yields. A move at this time into studies of P. vivax and P. falciparum using infected mosquitoes brought by

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courier from Atlanta at great expense is not justified. At the same time this initiative, if funded with more limited objectives should be integrated into the ongoing network. The group should not prepare their own P. berghei antisporezoite sera; such can be obtained from Dr. Nussenzweig at N.Y.U. Similarly, the principal investigator should visit Dr. Nussenzweig's lab to learn methods of purifying sporozoites; this work should not proceed independently. Conversely, it is recalled that Dr. Nussenzweig wished to establish a colony of Anopheles balabacensis in New York, to cover the contingency that the colony at the NIH might be lost; there is no reason why the Biomedical Research Institute cannot serve as an alternate source.

Reference is made to other unspecified projects on malaria now funded at the Biomedical Research Institute. A list of such projects, funding sources, and this staffing should be furnished before AID acts on this application. AID and RAC should have a complete picture of ongoing malarial research at this non-academic operation for the protection of all concerned.

In summary, it is recommended that this application be funded at a reduced figure with concentration on exploiting an important discovery, namely, that the EE stages of a rodent malaria will develop partially in a mammalian culture system. The emphasis should be on improving and achieving production of EE merozoites in-vitro.

II. SUBCOMMITTEE COMMENTS

Dr. Black expressed concern about making antiserum for human use out of "crude extracts." He noted that a very positive aspect of the project was the number of younger research workers who would emerge if the project were funded.

Dr. Carter concurred with the suggestion that the proposal should be rewritten and suggested that funding be set at the same level as present Navy support.

Dr. Schweigert felt the project should be reduced in funding level. He requested that more details about the Biomedical Research Institute be provided.

III. GENERAL COMMENTS

Dr. Erickson, project manager, DS/HEA, replied first to Dr. Schweigert, stating that funding for the Biomedical Research Institute came largely from the Navy and that the Institute had a staff of about 86. He expressed confidence in the staff and noted that the Institute has "first class" research facilities including insectaries secure enough to handle highly infectious species. He noted Navy funding for the project previously set at \$700,000 had been reduced to less than \$200,000.

Dr. Erickson indicated that the data on the successful culture work on the human embryonic lung cell line was unpublished, but submitted data of Dr. Hollingdale. These results were recently corroborated by Dr. Robert Sinder at the Imperial College of London during an AID site visit to London, England in 1979. The successful demonstration that P. berghei

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could be grown in human lung cells would allow the researchers at Biomedical Research Institute to quickly jump to P. falciparum and P. vivax, since these parasites normally grow in human cells.

Dr. Erickson further pointed out that Dr. Strom has recently retired from the government and is no longer an active scientist. With reference to Dr. Weller's suggestion that the Biomedical Research Institute researchers obtain antiparasite sera from Dr. Nussensweig, Dr. Erickson indicated that the U.S. Navy was already providing the necessary anti-sera to the project. With reference to the need of Biomedical Research Institute researchers to learn Dr. Nussensweig's purifying techniques, it was pointed out that Dr. Danforth, now of Biomedical Research Institute, developed the column separation techniques while he was a member of Dr. Nussensweig's research team.

Lastly, Dr. Erickson indicated that the Biomedical Research Institute has a colony of A. stephensi and could not, therefore, provide A. balabacensis to Dr. Nussensweig. They have facilities to establish such a colony but that is way beyond the scope and budget of the research program proposed.

Dr. Joseph, DS/DAA, stressed the need to sharpened the focus on all projects, including the malarial research program, given the budget cuts proposed this past weekend by the President.

Dr. Weller commented that results achieved to date by the Biomedical Research Institute staff do reflect the \$700,000 invested by the Navy. Weller suggested that AID provide funds in the range of \$150,000 - \$250,000 after the project is rewritten.

Dr. Wishik urged that the role of the Navy in funding the project be better defined before AID makes a commitment. Wishik suggested that a site visit (to Rockville, Md.) would also be useful.

Dr. Erickson suggested that the site visit be coordinated with the work of a malaria network committee, recently established so that the program would not be delayed. In checking on current program funding by the Navy during lunch he found that only \$50,000 will be provided by Navy.

Dr. Smuckler stated that a delay for the purpose of "sharpening" the project proposal ought to be tolerable.

IV. ACTION

Motion: (1) RAC approves, in principle, support of a sharply focused project to obtain growth of mammalian exo-erythrocytic stages in tissue culture. The project has great potential and AID should participate at this stage; (2) however, except for a brief preliminary trial to see if P. vivax or P. falciparum might grow readily, the project should concentrate on completing the EE cycle of P. berghei in-vitro. A rewritten application should be submitted for consideration with this objective as the point of emphasis; (3) a project site visit should be made before the project is resubmitted to RAC; and (4) the project is now funded by the US Navy, but this support is precarious. In considering AID funding, a maximum of \$300,000 per year from all sources might be reasonable.

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At the request of Dr. Erickson, the Committee returned to this project proposal later in the day. Dr. Erickson had additional information concerning the funding of the researchers at the Biomedical Institute. Funding by the Department of the Navy would be exhausted in the next few months. The research team may no longer be available to work on A.I.D. research once it is dispersed. He asked if it would be appropriate for the RAC to recommend interim funding until the research proposal could be revised and brought back to the Committee. After a brief discussion the Committee accepted Dr. Falcon's recommendation that the malaria subcommittee be delegated the authority to act on behalf of the RAC in an emergency, and within the limits of the previous motion. Dr. Pimentel would participate as a member of the malaria subcommittee.

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Contract Proposal

Submitted to

The Agency for International Development
(Modification submitted October 1, 1981)

TITLE: In Vitro Cultivation of Human Malaria

PROPOSED CONTRACT PERIOD: January 1, 1982 thru December 31, 1984

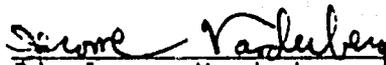
PRINCIPAL INVESTIGATOR: Jerome Vanderberg, Ph. D., Professor
Division of Parasitology, Department of Microbiology
Social Security No. 125-26-1205

PERFORMANCE SITE: 341 East 25th Street
New York, New York 10010

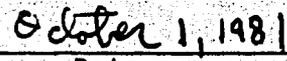
CONTRACTOR INSTITUTION: New York University Medical School
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APPLICATION APPROVALS:



Dr. Jerome Vanderberg
Professor
Principal Investigator



Date

Dr. Milton Salton
Professor and Chairman
Department of Microbiology

Date

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ARTICLE I: STATEMENT OF WORK

OBJECTIVES

The purpose of this project is to develop improved culture techniques for the production and maturation of gametocytes of Plasmodium falciparum in vitro. The mature gametocytes will be fed to mosquitoes for the production of sporozoites to be used in the development of a sporozoite vaccine against malaria. It is proposed to standardize and optimize these production procedures so that the technology can be readily transferable to other laboratories (such as that of Dr. R. S. Nussenzweig), actually engaged in the development of a vaccine. It is also proposed to develop an in vitro correlate test to measure the status of protective immunity in hosts being immunized against sporozoites. The basis of this test would be the ability of serum from immunized hosts to inhibit invasion of cultured hepatocytes by sporozoites.

BRIEF BACKGROUND AND RATIONALE

In recent years, considerable progress has been made in the development of a sporozoite vaccine against malaria. It should be pointed out that thus far, the only humans successfully immunized against malaria were immunized with sporozoites. More recent progress in immunization, largely reported by Nussenzweig, et al., has led the A. I. D. to make the development of a sporozoite vaccine a continuing item of priority in its program.

My laboratory was involved in the initial studies on sporozoite immunization of rodents during collaborative studies from 1967 through 1972. We were also involved in the work which led to the first immunization of human volunteers against sporozoites (Clyde, Most, McCarthy and Vanderberg, 1973). Subsequently, our group moved away to a series of studies on the basic physiology

and in vitro culture of sporogonic stages and sporozoites of rodent and human malaria. After several years of divergence between our group and the immunization studies of Dr. Nussenzweig's group, recent progress and events in both of our laboratories have led us back towards a more direct involvement in immunization studies.

a) Important progress has been made by Dr. Nussenzweig and her associates on immunization and on understanding the immune response in rodent and simian malaria. The time is now right for a direct assault on falciparum malaria. However, this step continues to be held back by the general unavailability of P. falciparum sporozoites in sufficient numbers.

b) We have recently solved this production problem. During the period Jan. 15, 1978 - Jan. 14, 1981 I was the Principal Investigator for an A. I. D. contract, "In Vitro Cultivation of Human Malaria" (AID-ta-C-1486). The chief aim of this contract was to develop methods for in vitro cultivation of gametocytes of human malaria, and subsequent infection of mosquitoes with these gametocytes. We made gradual advances in resolving the problem throughout our contract period, and by the end of the contract we were able to show that the addition of hypoxanthine to our culture system permitted the production of mature, infective P. falciparum gametocytes in vitro. When these gametocytes were fed to mosquitoes, we were able to produce large numbers of P. falciparum sporozoites. It was fortunate that this problem could be solved at this time, because now more than ever before (as we will show in the subsequent sections of this proposal) large numbers of P. falciparum sporozoites will be needed on a regular and continuing basis for immunization studies and for genetic engineering.

In addition, we have made some interesting recent findings on sporozoite invasion within the intact animal (Shin, Vanderberg, and Terzakis, 1981). We found (contrary to common belief) that P. berghei sporozoites invade hepatocytes directly, rather than indirectly via Kupffer cells. This has given us further

insight into mechanisms of sporozoite invasiveness. These insights can be applied in follow-up in vitro studies on the role of anti-sporozoite antibodies in blocking invasion of the liver.

SPECIFIC AIMS

1. To develop improved methods for the in vitro cultivation and maturation of P. falciparum gametocytes, and to standardize these procedures so that the improved technology can be readily transferred to laboratories involved in sporozoite immunization studies. These studies will be done by:
 - a. Analyzing and assessing our current procedures
 - b. Testing different strains and clones of the parasite
 - c. Determining the importance of strain "senescence" in continued production of gametocytes
 - d. Testing substitutes for human serum in gametocyte culture
 - e. Testing improved media for more efficient gametocyte maturation
 - f. Determining the effects of antibiotics on gametocyte production and maturation
 - g. Testing automated culture procedures for more efficient gametocyte production
 - h. Studying the kinetics of gametocyte maturation
 - i. Developing procedures for gametocyte concentration
 - j. Comparing A. freeborni and A. stephensi as vectors for culture - produced gametocytes
2. To develop an in vitro correlate test that measures the state of functional immunity to sporozoites in hosts being immunized against sporozoites.

WORK PLAN

I. In Vitro Cultivation of Gametocytes

A. Background and Rationale

1. Justification: The need for P. falciparum Sporozoites

As progress continues on the development of a P. falciparum sporozoite vaccine, there is an ever increasing need for sporozoites to conduct these studies with. Some of the requirements are as follows:

a) Sporozoites for Genetic Engineering Studies

It is becoming increasingly clear that the practical "sporozoite vaccine" will eventually consist of pure, defined protective parasite antigens produced on a large scale by recombinant bacteria. Currently, three types of approaches are in use for genetic engineering studies, and could be applied to the P. falciparum sporozoite: (1) DNA can be isolated from the parasite, and sequences of various lengths can be inserted into bacteria. The object of this so-called "shotgun" approach is to select for cloned colonies of bacteria that synthesize the desired parasite protein from genetic information provided by parasite DNA. (2) Messenger RNA can be isolated from the parasite. From this it may be possible to prepare complementary sequences of DNA for insertion into bacteria. (3) Small quantities of the desired antigen can be isolated directly from the parasite in pure form. After determination of the amino acid sequence of this protein, it is possible to synthesize specific nucleotide sequences that code for the desired protein or for antigenic portions of it, and to insert these sequences in bacteria.

The details of these various approaches, and a comparative analysis of the advantages and disadvantages of each are beyond the scope of this proposal. The key point to be made is that each of these approaches requires

large numbers of sporozoites supplied on a regular basis. This could best be achieved by collaborative efforts between a sporozoite production facility and a laboratory specializing in genetic engineering studies.

b) Sporozoites for Hybridoma Studies

A hybridoma that produces monoclonal antibodies against a single surface component of the P. falciparum sporozoite has recently been developed by Dr. R. S. Nussenzweig and her associates (personal communication). It will now be necessary to continue producing new such hybridomas. The monoclonal antibodies produced will be needed for continued characterization of sporozoite surface antigens (to study surface components other than the one previously characterized), for determination of which of these antigens are functionally protective (by sporozoite neutralization studies), for comparing different geographical isolates of P. falciparum (to test cross immunity across different geographical strains), and for testing the antigens produced by the recombinant bacteria described above. (By employing these monoclonal antibodies in a radio-immuno-assay it would be possible to screen the clones of recombinant bacteria to identify those producing the desired antigen.) For all of these studies, P. falciparum sporozoites must be available for production of these required hybridomas.

c) Sporozoites for Neutralization Studies

When a new hybridoma is produced, it is necessary to determine whether the monoclonal antibody secreted by it is functionally active against sporozoites. This is most readily done with a sporozoite neutralization assay. Viable, infective sporozoites are incubated with the test monoclonal antibodies, and are then inoculated into susceptible hosts to determine whether the sporozoites have been neutralized. A supply of sporozoites is required for this (as well as sporozoites for the positive control inoculation).

d) Sporozoites to Determine Immune Status of Host

As experimental hosts (human or simian) continue to be immunized in the future, it will be necessary to determine the actual state of their immunity at any given time after immunization. The development of such a test is an important component of this proposal (see section beginning on p. 15). Obviously, a continuing supply of sporozoites will be needed to carry out these correlative tests.

This correlate test will be of considerable importance because of three serious potential shortcomings of sporozoite vaccination: (1) In all studies done so far with rodents, simians, or humans, the duration of protective immunity has been shown to last only a few months. Is it possible to develop an immunization procedure that gives long lasting immunity? (2) Thus far, effective protective immunity has been achieved only with the intact sporozoite. Attempts to immunize by homogenization or fractionation of the sporozoite have been unsuccessful. Can an immunization schedule be developed that gives good protective immunity when only pure antigens are used? (3) Individuals in hyperendemic areas may be poor subjects for sporozoite immunization because they often have a high degree of non-specific immunosuppression stemming from infection with other parasites (including malaria). Can a schedule be developed for immunizing such individuals?

In view of these potential problems, it is unlikely that effective immunization of humans against sporozoites will prove to be a simple matter, even if large quantities of in vitro produced parasite antigen becomes available. Quite clearly, a considerable number of studies with isolated purified antigens will have to be done to answer these questions. Dosages, routes of immunization, scheduling of dosages, possible use of adjuvants, etc. will all have to be looked into. Rather than challenge all of the

experimental hosts being immunized under these protocols, it would be more strategic to periodically collect serum from them, and to assess the changing status of immunity during the trials. All of this will require much work, and a continuing supply of sporozoites.

e) Sporozoites for Challenge

The ultimate test of immunity is refractoriness to challenge. During initial pilot immunizations, and after eventual large scale trials, it will be necessary to challenge with controlled doses of sporozoites to test whether or not the vaccine actually works. Obviously, a continuing supply of sporozoites will be needed.

2. Background of Culture System for Parasitic Production

One of the most interesting aspects of the Trager and Jensen (1976) system for cultivation of P. falciparum was that this system was capable of producing gametocytes as well as asexual parasites. However, studies in our laboratory (Vanderberg et al., 1977) and elsewhere soon showed that these gametocytes did not reach maturity in culture, as judged by their failure to infect susceptible mosquitoes, and the failure of the microgametocytes to exflagellate. We pointed out in our original contract proposal (March, 1977) that "it seems highly unlikely that P. falciparum gametocytes which develop within a culture system are inherently genetically sterile. It seems more reasonable that these gametocytes, which appear to develop normally for most of their development, fail to reach final maturity because of unfavorable environmental conditions. The problem thus appears to be largely one of culture. For successful culture of asexual parasites, erythrocytes must be kept alive for the 48 hour cycle of the parasite. On the other hand, successful gametocyte culture may require survival of the host erythrocyte for close to 2 weeks. Thus, culture conditions sufficient

for asexual parasites may be inadequate for gametocytes."

The detailed background and rationale relating to culture of gametocytes and the improvement of culture conditions for them are presented in the reprints attached as Appendix I of this proposal: "Culture of the Invertebrate Stages of Plasmodia and the Culture of Mosquito Tissues", by I. Schneider and J. Vanderberg (especially pp. 237-244, "Gametocytogenesis In Vitro", and Gametogenesis In Vitro); and Appendix II: The Transmission by Mosquitoes of Plasmodia in the Laboratory", by J. Vanderberg and R. Gwadz (especially pp. 158-172, "Gametocytes in the Vertebrate Host").

During the past 1 - 2 years, a number of laboratories working on this problem have reported slight successes with in vitro culture of these gametocytes (Carter and Beach, 1977; Chin et al. at CDC; Verhave et al. in Holland; Smalley et al. in England; Hink at Ohio State; and ourselves). Most laboratories began to report the attainment of exflagellation on a regular basis, and in some cases rare infection of mosquitoes. Workers at CDC reported what up to now were the most promising results. By allowing the gametocytes to mature in a "flow vessel" with continuous perfusion of medium, they were able to infect more mosquitoes than had been previously possible (personal communication). This completely bore out my argument that better gametocyte maturation was primarily a culture problem. Though the results of CDC were extremely important, their infection rates of mosquitoes were relatively low, and in many cases were unrepeatably.

Our new finding that hypoxanthine is the limiting factor in the culture of these gametocytes now permits any laboratory to grow large quantities of mature gametocytes both for gamete and sporozoite production. (See details in my End of Contract Report, covering funded portion of Contract Period January 15, 1978 - January 14, 1981).

B. Proposed Culture Procedures for Gametocyte Maturation

Though the problem of gametocyte culture has been solved by our findings that hypoxanthine is required for their maturation, some further studies are required in order to optimize this discovery.

1. Analysis of Current Gametocyte Production Procedure.

Though we have been able to obtain regular maturation of infective gametocytes in our laboratory, another laboratory (N.I.H.) has not been able to duplicate our results by adding hypoxanthine to their cultures, and applying their own procedures. It seems clear that hypoxanthine is a necessary but not sufficient element in gametocyte maturation. That is, we appear to be doing other essential things which have led to our regular success. It is therefore important to experimentally analyze all of the procedures in our protocol in order to determine which of these are absolutely essential, and should therefore be part of the protocol of other laboratories. The aim of this is to have our technology readily transferable to other laboratories that might want to go into gametocyte production.

Components of our protocol to be tested are: (1) serum increase - (We normally initiate new gametocyte cultures at 15% human serum and maintain them for a week at this level before reduction of the concentration. We will reassess this.) (2) hematocrit adjustment - (We normally initiate new gametocyte cultures at 12% hematocrit and reduce them to 6% after a week. This procedure was initiated in order to get larger numbers of gametocytes. We should also assess its effects on gametocyte maturation.) (3) addition of fresh erythrocytes - (We normally initiate new gametocyte cultures with red blood cells within a day or two of their collection. This is an expensive procedure, since it requires frequent purchases of blood. We will reassess this approach.) (4) temperature control during media changing - (During the daily changing of medium, we maintain our culture dishes on a warming tray

while we withdraw the old medium and add fresh prewarmed medium. Some other laboratories do not do this procedure, and we will assess its importance.) (5) gassing - (We standardly use the "Scheibel gas mixture of 3% oxygen, 2% carbon dioxide, 95% nitrogen. We find that other laboratories use other mixtures. We therefore will make a comparative assessment.) (6) stock for initiation of new gametocyte cultures - (We standardly initiate our cultures with "spent" cultures that have been growing for 12 - 16 days, as we find that these give larger numbers of gametocytes. Other laboratories tend to initiate new cultures with stock from younger, rapidly growing cultures. We will therefore do a comparative assessment of the procedure.)

2. Testing Different Strains and Clones of P. falciparum

Experience with asexual parasite culture has shown that different strains and geographical isolates vary considerably in their culture responses. Some are easy to establish in culture; others are difficult. Some are good growers; others grow slowly. Presumably, similar variations exist with P. falciparum gametocytes. We propose to test a range of different geographical isolates to determine how many of these are good gametocyte producers. The aim is to choose several especially good gametocyte producers representing different geographical regions. These selected isolates could then be used as standards in the various laboratories engaged in this research. As previously pointed out (p. 5) different geographical isolates will be required for testing cross immunity of sporozoites from different regions of the world.

If certain strains can be identified as "good" gametocyte producers, and it can be demonstrated that these strains can be cultured over a long period of time without becoming "senescent", it would be useful to establish clones of these parasites. The purpose would be to standardize the strains to insure comparability of results in different laboratories. The standard dilution procedure for cloning would be used.

Several laboratories within the A. I. D. Malaria Network (Trager, Green) as well as outside the Network (Wallicker, Carter) have already begun to establish clones of parasites. It thus might be more expeditious to obtain an already cloned line from one of these workers, and to use this line for our gametocyte production studies.

3. "Senescence" of Parasite Strains

There is some controversy over how long parasite strains may be cultured in vitro before they lose the ability to produce gametocytes that can go on to mature. Verhave (unpublished) has suggested that only recently isolated strains are useful in this regard; others have suggested that this is not so. We have frozen a number of stabilates of several strains at various intervals after their original isolation. We propose to follow these stabilates in order to test them. It will be important to determine whether a "good" strain, once identified, can continue to be used, or whether it will be necessary to keep going back to early passages or even to keep establishing new isolates from nature.

4. Substitution of Peptones for Human Serum in Gametocyte Culture

The use of human serum in cultures involves several difficulties. It may be difficult to obtain in large quantities on a regular basis; it may be expensive to purchase, and its efficacy may vary from donor to donor. In a region endemic for malaria, further problems may result from a high incidence of antimalarial antibodies among blood donors, and a relatively high incidence of Hepatitis B virus, which could pose hazards for experimenters. We were successful in resolving these problems for culture of asexual parasites by the development of a procedure for replacing human serum by addition of peptones and calf serum to the media. Our results (Ifediba and Vanderberg, 1980) made it possible to culture P. falciparum on a regular basis without human serum.

The culture of gametocytes can be even more expensive, because higher concentrations of human serum may be required for their full maturation. We therefore propose studies in which we will attempt to adapt our procedure for

use with gametocytes. Already established gametocyte-producing strains (Z, FCN-2, and Honduras) will be gradually adapted by step-wise, progressive reduction of the human serum component, together with a concomitant step-wise increase in peptone concentration (all in the presence of calf serum). Hypoxanthine will be added, and attempts will be made to wean the cultures away from human serum. Our previously adapted peptone strains cannot be used for this, inasmuch as they are not gametocyte producers.

5. Media Improvement for Gametocytes

The most significant deleterious changes in media used for culture of P. falciparum asexual stages involve depletion of glucose by the parasite, and depression of the pH (because of lactate production by the parasite). Recent studies have shown that these problems can be reduced by simple adjustment of the medium, thereby resulting in better parasite growth and a less compelling need for frequent changing of the media (Osisanya, Gould, and Warhurst, 1980; Butcher, 1979). The glucose component of RPMI-1640 is doubled, and the buffer TES is used in place of HEPES to obtain better buffering capacity. We will attempt to duplicate these studies with gametocyte-producing cultures. The aim is to see whether we can get better and more dependable growth of gametocytes.

It is entirely possible that this approach may actually depress gametocyte production, since gametocytes are known to be produced in cultures that have begun to deteriorate under nutritional stress. Therefore, we will test this modified medium not only on young gametocyte cultures undergoing induction, but also on older ones that have already produced gametocytes.

6. Antibiotics

We currently maintain our cultures without the addition of antibiotics. However, many beginning laboratories tend to have periodic contamination problems when antibiotics are not used, and this often leads to considerable amounts of lost time and resources. The use of antibiotics in culturing gametocytes could be helpful in combating this occasional problem, in the same way that they have been useful in the culture of asexual parasites. We therefore plan

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to assess the effects of penicillin, streptomycin, gentomycin and 5-fluorocytosine (as a fungicide) in our gametocyte cultures. Normal maturation of gametocytes in the presence of these agents could lead to their routine use in culture media.

8. Automated Culture Procedures

Maintenance of culture systems is a labor intensive procedure. Cultures must be fed and manipulated 365 days a year, including weekends and holidays. This strain can be somewhat alleviated by use of an automated feeding device. Two such systems appear especially useful, i.e., the flow vial (Trager and Jensen, 1980) and the orbital incubator (Butcher, 1981).

Flow vials have limitations, in that they and their accessories are expensive to purchase, extremely expensive to maintain, and have a relatively low capacity. The flow vial developed by Trager has a culture volume capacity of 12 ml, and requires 50 - 60 ml of fresh medium per day, in addition to a continuous flow of a custom gas mixture. Our tissue culture dishes are extremely cheap, hold 20 ml of culture volume apiece and use about 1/5 of the daily amount of fresh medium required for flow vials. As no special equipment is required, the static culture dishes can be used in almost unlimited numbers in an incubator. Flow vials have been used by the CDC group of researchers, who have succeeded in a partial solution of the gametocyte maturation problem. Perhaps, with the addition of hypoxanthine, the flow vial can be made to be more effective for gametocyte culture, and the flow rate can be significantly reduced. We propose to test this.

A simpler automated device has been described by Butcher (1981). Cultures are grown in flasks and are kept in suspension by means of continuous orbital shaking. Less medium is required than needed for the flow vial, because medium is delivered discontinuously (once or twice daily), rather than by continuous perfusion as in the flow vial. The apparent advantages of this system over the flow vial are that it is cheaper to construct, cheaper to maintain, and

more parasites can be grown per unit volume of medium (because the cells are in suspension rather than in a thin layer at the bottom of the flow vial). We propose to test this system for our gametocyte production.

8. Kinetics of Gametocyte Maturation.

We have shown that gametocytes cultured by our procedure are most infective when fed to mosquitoes on days 12-14 of their culture period. Obviously, this could be affected by the various modifications that we have proposed above. Therefore, we would continue to do timing studies to determine the best days for recovery of gametocytes for feeding to mosquitoes. In addition, we have relatively limited data on how long infective gametocytes continue in culture beyond this 14 day period. We propose to study this.

9. Gametocyte Concentration

It would be useful to separate developing gametocytes from asexual parasites in order to reduce "competition" for nutrients, and the excessive build-up of toxic waste products. The separated gametocytes could then be returned to culture. Concentration of gametocytes would also be helpful for further studies with them (feeding to mosquitoes; gamete production and harvesting; further in vitro development to ookinetes, etc.). Preliminary studies have now shown that centrifugation on a continuous percoll gradient can be used to greatly concentrate P. falciparum gametocytes. We plan to return these gametocytes to culture and see if they retain their infectivity to mosquitoes.

10. Use of Anopheles freeborni

As I pointed out in my review article (page 156 and 215-216 of Appendix II), "Many species of anophelines are efficient vectors only for geographically coindigenous strains of P. falciparum." We will therefore test one other species, Anopheles freeborni, to determine if we can improve sporozoite production. A. stephensi is clearly not the most efficient vector

for falciparum malaria, and we may be able to increase our production of sporozoites by turning to another mosquito.

II. Development of an In Vitro Correlate Test to Measure Anti-sporozoite Immunity

1. Previous Studies in Our Laboratory

An important element of the A. I. D. network program in malaria immunization is the development of a vaccine effective against sporozoites, being pursued by Dr. Ruth S. Nussenzweig and her associates. We have considerable previous experience in this area, as our group collaborated on the problem with Dr. Nussenzweig's group during the years 1967 - 1972 when we jointly initiated this approach with rodent and simian malaria (See publications 23-26, 31, 33-34, 36-41 in my attached Bibliography, pp. 35-36. Indeed, our ability even to initiate these studies had been conferred by our earlier studies in which we worked out and established the laboratory model system for sporozoite transmission of rodent malaria (See publications 12-17, pp. 34 - 35). The procedures that we first developed (Vanderberg and Yoeli, 1966) are still the standard procedures used today by virtually all workers in the field (See review in Vanderberg and Gwadz, 1980).

Furthermore, we initiated the procedures for immunization by whole-body irradiation of infected mosquitoes (Vanderberg et al., 1970). The development of this approach allowed us to attempt the same procedure with humans, and led to the first successful vaccination of humans against malaria (Clyde, Most, McCarthy, and Vanderberg, 1973).

In addition, my group has long been involved in studies on basic biology of the sporozoite. The relevance of this is that a number of important findings developed in our laboratory have subsequently been applied for use in practical immunological ways. Two examples of this are as follows:

(1) We have done a series of basic studies on the development of sporozoites from the time that they are in the oocyst, until the time that they reach full maturity in the salivary glands. During this differentiation period, the sporozoites undergo a maturation in their morphology, antigenicity, infectivity, motility, and their response to antibodies (Vanderberg et al., 1967; Vanderberg and Rhodin, 1967; Vanderberg et al., 1972; Vanderberg, 1973; Sterling et al., 1973; Vanderberg, 1974; Vanderberg, 1975; Vanderberg, 1977). The understanding of this concept of sporozoite maturation has been essential in establishing appropriate immunization schedules, and in learning about the importance of different sporozoite antigens in inducing immunity to sporozoites.

(2) We did some studies on the in vitro maintenance of isolated sporozoites (Vanderberg, 1974) and on the metabolism and biochemistry of these in vitro sporozoites (Mack and Vanderberg, 1978). As part of these studies we developed a medium that could keep isolated sporozoites alive for long periods of time, and we found a number of specific inhibitors that acted against sporozoites. These procedures and findings have recently been used by Dr. Nussenzweig and her group to study the in vitro synthesis of Pb44, the functional antigen of the sporozoite.

Many other examples exist, including our studies on column purification of sporozoites, surface characteristics of sporozoites, etc. There is now a new immunological need, namely, the development of an in vitro correlate test for ant sporozoite immunity. Many of the basic biological procedures that we have established in recent years lend themselves to application in such a test. The time is now ripe to attempt what we have successfully

done before, namely, the application of basic biological procedures to work out a practical immunological problem. We thus propose to apply some of the basic in vitro studies that we have been doing to develop such an in vitro correlate test to measure antisporozoite immunity.

2. The Problem

The chief problem is to be able to determine the immune status of a simian or human subject without resorting to a direct challenge. Immunization of simians or humans can be an expensive and time consuming affair. If the immunized subject is challenged too early, before protective immunity has been attained, the entire experiment can be lost. A similar problem relates to determining the duration of protective immunity, once it has been attained. It would be easier and more practical to follow the course of immunity of a subject by taking serum samples, and by assaying these samples in an in vitro test system. This is especially true for sporozoite immunity to P. falciparum. None of the simian models used (Aotus monkeys, gibbons, and chimpanzees) are very susceptible to sporozoites in the first place. These simians are all difficult to acquire and are extremely expensive to buy and maintain. It is unlikely that large numbers will ever be available for experimental use with P. falciparum sporozoites. Thus, as much information as possible must be obtained from the few that will be immunized in the future. This requires a dependable in vitro test to determine immune status.

3. Present Procedures for Assessing Immunity

The most direct procedure for assessing the immune status of an immunized host is direct challenge with sporozoites (subject to the problems

above). Another variation on this procedure is the sporozoite neutralization assay (Nussenzweig, Vanderberg and Most, 1969). Sporozoites are incubated with serum from the immunized host, and then injected into a non-immunized host to test whether the serum has inactivated the sporozoites. This can readily be done with rodent malaria. However, it has limited value with simian or human hosts, because new experimental hosts (including controls) are required. If it is necessary to test many samples of serum (either from vaccinated animals or from hybridoma sources) this procedure obviously cannot be used.

The first in vitro assay described for mammalian malaria ant sporozoite antibodies was the circumsporozoite precipitation (CSP) reaction (Vanderberg, Nussenzweig and Most, 1969). At the time of its description, we had the impression that it gave a good correlation for protective immunity. This impression still remains, although there are cases when this correlation does not hold. For instance, mice immunized with sporozoites broken up by homogenization or repeated freeze-thawing will develop CSP antibodies, but no protective immunity. Likewise, splenectomized mice may develop protective immunity, but no CSP antibodies after immunization with sporozoites. Another in vitro test, the fluorescent antibody test (FAT) is more sensitive but the results obtained with it cannot always be correlated with protective immunity. What is needed is a sensitive test that can assay the ability of the sporozoite to invade a host liver cell in vitro, and also to assay the ability of immune serum to block this invasion. This hypothetical test will be known as the sporozoite invasion test (SIT).

4. The Sporozoite Invasion Test

This test aims at a procedure for obtaining regular invasion of normal host liver cells by sporozoites in vitro. Strome, DeSantis, and Beaudoin (197

were able to obtain partial development of exoerythrocytic (EE) forms in vitro by inoculating P. berghei sporozoites into cultures of rat embryo cells. More recently, this same group of investigators (Hollingdale et al., 1981) has been able to achieve complete EE form development of P. berghei in vitro in human embryonic lung cells. These important results establish the possibility of repeatable culture systems for EE forms. However, one must be cautious about interpreting results on the mode of sporozoite invasion into these highly abnormal cells. Up until now it was not known whether sporozoites in vivo invade hepatocytes directly, or endothelial cells which line the sinusoids of the liver. We have recently demonstrated that hepatocytes are invaded directly (Shin, Vanderberg and Terzakis, 1981). It now becomes possible to concentrate on hepatocytes as the appropriate host target cells. Since hepatocytes are normally invaded in vivo it should be possible to establish a sporozoite-hepatocyte system that does this in vitro.

I first proposed the concept behind this test at a meeting at A.I.D. between Drs. Erickson, Nussenzweig, and myself. It was agreed that there was a real need for such a test, and I accordingly later submitted it as part of my detailed new proposal of May 1, 1981. Based on this proposal, Dr. Nussenzweig subsequently began some preliminary collaborative studies with Dr. Hollingdale on some of the experiments described. The success of these preliminary experiments proposed in my application (reported to A.I.D. by Dr. Nussenzweig) is encouraging, and supports the feasibility of my overall proposal. However, a number of problems remain to be resolved. These include quantitation procedures, and the development of a system for primate malaria.

B. Proposed Studies

1. Sporozoite Sources

Plasmodium berghei sporozoites will be collected by procedures that have been described in detail (pp. 203 - 206 in Vanderberg and Gwadz, 1980). In brief, gametocytes are raised in young hamsters. Three to four days after inoculation of hamsters with infected blood, Anopheles stephensi mosquitoes are fed on the hamsters and maintained for 16 - 18 days to permit sporozoite maturation and migration to the salivary glands. Sporozoites are harvested by dissection of salivary glands (for studies requiring small numbers of sporozoites), or by grinding up isolated mosquito thoraces and separating out the sporozoites by centrifugation on a density gradient (Schulman, Oppenheim and Vanderberg, 1980). Further purification of these sporozoites can be obtained (especially when it is desirable to remove bacteria and fungi) by passage through a DEAE-cellulose column (Mack, Vanderberg and Nawrot, 1978) or by passage through a lectin-affinity column (Vanderberg, 1979). Both of these column procedures allow sporozoites to pass through the column, while contaminants are retained on the column.

Newer procedures that we have been working on involve the use of Percoll gradients to separate out sporozoites. Sporozoites so prepared are cleaner than can be obtained with any other procedure that we have attempted. A major goal of ours will be to carry this procedure further in order to eliminate all or most of the cotaminating micro-organisms. The rationale for this is the need to do the SIT with a suspension of sporozoites introduced into the cell culture. Preliminary studies done thus far (Nussenzweig and Hollingdale; personal communication) used whole infected salivary glands to infect cultures. In control cultures, this results in a great variation in EE forms from culture to culture, and in an uneven distribution of EE forms within each culture. Thus, quantitation of the percentage of sporozoites that

convert into EE forms is impossible. Quantitation of the system will be necessary for such studies as titration of serum activity during an immunization procedure, comparison of different immunization procedures, etc.

Accordingly, we propose to do further studies on sporozoite purification by gradient centrifugation procedures. Studies that we have done so far suggest that these sporozoites are contaminated with a relatively small number of different species of bacteria. It therefore may be possible to use specific antibiotics to control the multiplication of these bacteria in culture. We propose to do standard bacteriological assays with our preparations in order to specifically identify the contaminating micro-organisms and to assess which antibiotics they are sensitive to. Our goal is to find a mixture of antibiotics which on the one hand will control contaminating micro-organisms, and on the other hand will have negligible effects on the sporozoites and their host cells.

Plasmodium falciparum sporozoites will be obtained by permitting mosquitoes to feed on gametocytes that have been grown in culture (see pp. 9 - 15) of this proposal for details.

Plasmodium vivax sporozoites will be obtained from infected A. stephensi mosquitoes in a collaborative effort with Drs. Richard Baker and Vincent McCarthy of the University of Maryland Medical School. Dr. Baker has made arrangements with Port of Baltimore health officials for receiving blood specimens from merchant seamen who arrive infected with vivax malaria. Drs. Baker and McCarthy have already visited our laboratory to discuss this collaborative effort. At the same time, we are helping them to establish the in vitro P. falciparum gametocyte system within their laboratory. (We have had past collaborative experiences with Dr. McCarthy during our immunization studies with P. falciparum sporozoites.)

Plasmodium knowlesi and P. cynomolgi sporozoites will be obtained from Dr. Ruth S. Nussenzweig as part of a collaborative study. She and her

associates have been immunizing rhesus monkeys with these sporozoites, and also have established hybridomas that produce monoclonal antibodies against sporozoite surface components. If we are successful in developing a practical and correlative in vitro sporozoite invasion test against rodent malaria, we will initiate similar correlative tests with Dr. Nussenzweig, using her sporozoites, and serum from her immunized animals.

2. Antibodies to be Tested

Serum from mice and rats immunized against P. berghei sporozoites will be collected according to standard procedures (Cochrane et al., 1980). In brief, animals will be immunized by multiple intravenous inoculation of gamma irradiated sporozoites obtained from salivary glands of A. stephensi mosquitoes. We have done this procedure on numerous occasions, and am quite familiar with it. In addition, mice will be immunized with freeze-thawed sporozoites (to permit them to develop CSP antibodies without concomitant protective immunity); other mice will be immunized after they have been splenectomized (to permit them to develop protective immunity without CSP antibodies). Antisporozoite monoclonal antibodies that have been produced by hybridomas will also be tested. These will become available from Dr. Ruth Nussenzweig, as part of a collaborative effort to test these monoclonal antibodies in our in vitro system. An especially interesting series of monoclonal antibodies to test would be those developed at the Naval Medical Research Institute. Several different cloned lines of hybridomas have been established. One of these lines produces monoclonal antibodies that protect against sporozoites; other lines produce antibodies that give a positive fluorescent antibody test, but do not protect against sporozoite challenge (Beaudoin et al., personal communication). If we are successful in developing a sporozoite invasion test against serum collected from immunized mice, we will discuss the possibility of collaborative testing of the NMRI hybridomas with the workers involved.

Serum from simians immunized with sporozoites of simian or human malaras will be obtained from Dr. Ruth Nussenzweig. Likewise, monoclonal antibodies that react with these human or simian malaria sporozoites will be obtained for collaborative testing in our proposed in vitro system.

3. Host Liver Cells

As previously pointed out, we will restrict our observations to hepatocytes, since these appear to be the cells normally invaded by sporozoites in vivo. Young rats will be used because of their relatively high susceptibility to P. berghei sporozoites (Vanderberg et al., 1968). Primary cultures of isolated hepatocytes will be initiated by enzymatic dissociation of livers. The technique to be used for dissociation and for initial culturing of these cells is our own modification of the procedures of Bonney et al. (1974); Seglen (1973); and Wanson et al. (1977). We have had extensive experience with this procedure for obtaining normal rat hepatocytes, and hepatocytes infected with exoerythrocytic forms of P. berghei, and for maintaining these cells in culture for extended periods of time (Foley, Kennard and Vanderberg, 1978 a, b).

In brief, donor rats were anesthetized with sodium pentobarbital and a cannula was introduced into the venous circulation via the abdominal inferior vena cava. The thoracic inferior vena cava was then ligated, and the portal vein opened to serve as the outflow for the perfusion media. The liver was perfused in situ using a peristaltic pump at a flow rate of 6 ml/min with two solutions. First, a calcium-free salt solution was perfused through the liver for 10 min to remove the blood and plasma components and to initiate cleavage between liver cells. The liver was then perfused for 10-15 min with the same salt solution to which had been added 0.05 g/100 ml of the enzyme collagenase. Both solutions were aerated

at 37°C with 5% CO₂, 95% O₂ for the 15 min before use. When the liver was well digested, it was removed from the donor, and placed in tissue culture medium. The capsule was cut in several places and the liver was then gently suspended in the medium and the resultant suspension of liver cells and debris filtered through a 253 µm mesh nylon filter and washed twice with tissue culture medium by slow centrifugation. The cell suspension was then counted in a hemacytometer and diluted. The viability of cells was determined by the trypan blue exclusion method.

In previous studies that we did, these cells were not found to be susceptible to invasion by P. berghei sporozoites in vitro (Vanderberg, unpublished). However, it is now well understood that the surface of cultured hepatocytes changes dramatically within hours after isolation from rats, and new ways have been described that enable the cultured hepatocytes to retain their surface receptor characteristics. One of the most effective ways is to culture the cells on a connective tissue matrix (Biomatrix) prepared from rat liver tissue (Rojkind et al., 1980). Dr. Lola M. Reid, a member of this group that developed the procedures at Albert Einstein Medical School in New York, has agreed to collaborate with us on this problem.

For invasive studies with simian and human sporozoites, we plan to test two different types of human hepatocytes: transformed and normal:

A number of different human hepatocarcinoma lines have been isolated for culture and characterized. One of these (SK-HEP-1) has retained many characteristics of the normal hepatocyte and has been obtained by us from the Sloan-Kettering Institute in New York. It is growing well in our laboratory (procedures in Fogh and Trempe, 1975; Fogh et al., 1977 a, b), and will be tested with P. falciparum. Other transformed human

hepatocyte lines are available, and will be obtained and screened during the project.

There are no reports in the literature yet on the extended culture of normal human hepatocytes. However, Dr. Reid has been successfully culturing such cells on human liver "biomatrix", and has agreed to collaborate with us on the project. Both the cells and the "biomatrix" material are obtained from autopsy specimens. For practical and medical-legal reasons it would be impossible for us to obtain such material directly myself. However, Dr. Reid, in association with the Liver Institute at Albert Einstein Medical School has access to human donor material on a continuing basis. (Another source of human liver cells that I have explored is the portion of normal liver removed during surgical resection of a hepatocarcinoma. Such material may be obtained at many hospitals, provided normal consent protocol is followed. However, a high incidence of hepatitis B virus is found in these cases, and this poses risks to the experimenters).

4. Culture Procedures

A wide range of different media will be tested in these studies, including: mouse liver medium, and diploid growth medium (as used by Strome et al., 1979), new medium used by Hollingdale et al. (personal communication), standard media used for culturing human hepatoma cells (Fogh et al. publications), standard media used for culturing rat and human hepatocytes on "biomatrix" (Reid, personal communication), and various media that we have used for culturing rat liver cells infected with exoerythrocytic forms (Foley, Kennard and Vanderberg, 1978 a, b).

5. Experiments

When a system has been established for in vitro penetration of sporozoites into hepatocytes, attempts will be made to quantitate this

with measured numbers of sporozoites and measured numbers of hepatocytes under standard culture conditions. The following alternatives will be compared to assess which gives the best morphological indication of normal invasiveness: (a) direct observation of sporozoite penetration, by means of phase - contrast microscopy, (b) fixed and Giemsa-stained preparations done to determine sporozoite penetration, (c) fixed and Giemsa-stained preparations at 48-72 hours, to determine exoerythrocytic form development, (d) fluorescent antibody determinations (Krotzski et al., 1981) at 48-72 hours, to determine exoerythrocytic form development.

If many preparations have to be tested, we will attempt to develop a radio-assay based on incorporation of ^3H hypoxanthine. Erythrocytic forms of the parasite have been shown to have a high requirement for hypoxanthine, while other purines are only slightly incorporated. This has resulted in the development of a sensitive assay for blood-stage growth of the parasite (Desjardins et al., 1979). We have been using this assay on a regular basis within our laboratory. We would attempt to determine whether the same preferential hypoxanthine requirement exists for developing exoerythrocytic forms. If so, we would attempt to feed our cultures with a medium containing tracer quantities of labelled hypoxanthine and very large quantities of other purines, such as adenine. Presumably, the uninfected liver cells would incorporate little of the labelled hypoxanthine, while the infected cells would incorporate much more. After incorporation, the cells would be stripped from the culture dish by trypsinization, and the incorporation measured. (It should be pointed out that this is a speculative and untested assay system for exoerythrocytic forms. It could turn out to be highly sensitive, and susceptible to semi-automation, or it could prove to be non-workable. Even if it does not work, however, it is still possible to fall back on

the morphological assays).

By use of any of these assays, serum from immunized animals, and monoclonal antibodies from hybridomas will be tested for their ability to prevent sporozoite invasion of hepatocytes. It has already been established that ant sporozoite immune serum has no effect on further development of the sporozoites, once they have invaded the liver (Vanderberg, 1973).

Another assay system to be evaluated is that of sporozoite motility. I have previously developed in vitro procedures for "turning on" sporozoite motility (Vanderberg, 1974). I pointed out at the time, "In addition to inducing CSP reactions, ant sporozoite immune serum completely immobilizes sporozoites and often causes the sporozoite to detach from the slide surface. This phenomenon so far appears to be species-specific in that ant sporozoite immune serum will immobilize sporozoites of only the homologous species. Whether the CSP reaction and sporozoite immunization are induced by separate antibodies, or instead represent 2 different effects of a single antibody remains to be determined. It is conceivable, for instance, that the CSP antigen plays a role in sporozoite motility, and that precipitation of the antigen as it is secreted might make it impossible for the sporozoite to continue locomoting for any length of time."

Indeed, sporozoite motility may be necessary for sporozoite invasion of the liver, and the chief action of ant sporozoite antibodies may be to block this motility and thereby prevent invasion. Thus, sporozoite immobilization might be usable as a simple in vitro assay to determine the immune status of a host.

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ARTICLE II: KEY PERSONNEL

1. Dr. Jerome Vanderberg, Principal Investigator
(see curriculum vitae and bibliography, following)
2. Dr. Seymour Schulman received a Ph. D. in the biochemistry of insect development, at the Biology Department of N. Y. U. in 1977. He spent 2 years with me as a post-doctoral fellow, and last year was appointed a Research Assistant Professor within our Department. He will continue his association with me on this project, and will focus primarily on parasite cloning, concentration procedures for gametocytes, sporozoite preparation and purification for our tissue culture studies, and on sporozoite-hepatocyte interactions in vitro.
3. Mr. Richard Nawrot has closely worked with me on mosquito maintenance and mosquito-parasite studies for the past 17 years. He is thoroughly trained and competent in all aspects of technical work with mosquitoes, gametocyte production, and infection of mosquitoes by feeding on hosts or through membranes.
4. Mr. Titus Ifediba, who is a native of Nigeria, completed his Ph. D. work with me February 1981. His studies have been on replacement of human serum by peptone substitutes, and on in vitro maturation of P. falciparum gametocytes. He hopes to stay in my laboratory for an additional year for further training and to complete studies on gametocyte maturation and on culture of EE forms. (We would hope to replace him with another post-doctoral fellow when he leaves.)

Notes:

1. The 4 staff members listed above have worked together for many years in my laboratory. We work smoothly and efficiently together as shown by our output and by our repeated demonstrated ability to meet the goals that we propose.

2. At this point, there is no guarantee that all of the individuals listed in the budget request will be available in the event of funding. Because of a lack of funds, two individuals who had been working with me have already been terminated. If new research funds do not become available shortly, it is likely that others will also be terminated. In that event, the personnel list mentioned above would no longer hold, and I would have to recruit new individuals. A number of such potential candidates are available.

Date of birth: [REDACTED]

Dependents: Wife and two children

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Education:

B. S. (Biology) City College of New York, 1955
M. S. (Entomology) Penn State University, 1957
Ph. D. (Medical Entomology) Cornell University, 1961

Professional Employment:

1974-present: Professor, Department of Microbiology, NYU Medical School
1967-1974: Associate Professor, Department of Preventive Medicine, NYU
Medical School
1963-1967: Assistant Professor, Department of Preventive Medicine, NYU
Medical School
1961-1963: Post-doctoral Fellow, Department of Biology, Johns Hopkins
University.

Teaching Experience:

Department of Preventive Medicine, NYU Medical School; Course in Parasitic Diseases;
Graduate Courses in Parasitology, Methods in Parasitology, Seminars.
Department of Entomology, Cornell University: Assisted in course in Parasitology,
Medical Entomology, and Insect Morphology (As Graduate Student) 1957-61.
Department of Zoology, Penn State University: Assisted in courses in General
Zoology and General Entomology (As Graduate Student) 1955-57.

Grants & Contracts (Current):

Research Grant "Studies on the Sporozoite of Plasmodium" NIH - NIAID
Research Contract "Cell Physiology of the Malaria Parasite" U. S. Army Medical
Research and Development Command
Graduate Training Grant "Graduate Training in Infectious Diseases" NIH-NIAID
Research Contract "In Vitro Culture of the Malaria Parasite" U. S. Agency
for International Development

N.Y.U. Administrative Functions:

Director of Graduate Program, Division of Parasitology
Member, NYU Basic Medical Sciences Board
Member, NYU Medical Center Faculty Council (1970-73)
Member of various Medical Center committees and sub-committees

Membership in Professional Organizations:

Entomological Society of America	American Mosquito Control Association
American Institute of Biological Sciences	American Society of Tropical Medicine and Hygiene
Gamma Alpha Scientific Fraternity	American Society of Parasitologists
Sigma Xi	American Society of Protozoologists

Local Arrangements Committee, Treasurer - NY Meeting of American Society of Tropical Medicine and Hygiene
Local Arrangements Committee, Chairman, NY Meeting of Entomological Society of America
Secretary-Treasurer, New York Society of Tropical Medicine

Invited Symposia:

International Colloquium on Plasmodium berghei, Antwerp, 1964
International Panel Workshops on Malaria, Washington, 1966, 1969, 1972
Symposium on Vaccination Against Malaria, National Academy of Science, Washington, D. C., 1974
Presidents Symposium - American Society of Parasitologists, Kansas City, 1974
International Workshops on Biology and Culture of Malaria, World Health Organization, 1976, 1977, 1978, 1979.

Other Activities:

Member, Advisory Panel on Parasitic Disease Therapy for U. S. Pharmacopeia Revision.
Contributor, Manual of Clinical Microbiology (American Society for Microbiology).

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FACILITIES

Our Department has extensive facilities both for maintenance of tissue cultures and for maintenance of the malaria cycle. A modern animal facility and insectaries are available. Large numbers of Anopheles stephensi mosquitoes are reared on a regular basis. A wide array of biological, optical and biochemical equipment are close at hand. The library, faculty and technical resources of the N.Y.U. Medical Center are also close at hand, and available for consultation.

The large numbers of falciparum-infected mosquitoes that we plan to have on hand creates a potential safety hazard. Therefore, we have instituted rigorous handling and safety precautions, and in cooperation with Dr. R.S. Nussenzweig we have constructed new safety facilities for working with these mosquitoes. We intend to maintain a high level of control and caution in our work.

Relationship of Proposal to Other Research Projects

The budget and research plan for this proposal have been carefully drawn to coordinate with other research projects being conducted in the laboratory of the Principal Investigator. Most of the individuals listed also have responsibilities on other projects. There is no overlap on these projects. Though some of the projects are complementary in nature, each has specific and non-duplicating aims and approaches. The percentage effort and percentage salary for each person represents our best estimate of that person's actual involvement and time spent with the particular project. The status of each of these projects is as follows:

1. N.I.H. Research Grant AI09560

"Studies on the Sporozoite of Plasmodium"

Principal Investigator - Jerome Vanderberg

Termination Date: 7/31/82

Total approved funds for terminal year grant (8/1/81 - 7/31/82) = \$72,671

This project has dealt with the basic physiology of the P. berghei sporozoite, and its interactions with the liver. The specific aims have been:

1. To determine which cell in the liver is invaded by the Plasmodium berghei sporozoite, and to trace the route of this invasion.
2. To establish whether a histidine-rich protein is present in the sporozoite, to characterize it, and to establish whether it plays a role in penetration of the liver by the sporozoite.
3. To study the process of rhoptry secretion, and to establish whether this secretory material plays a role in penetration of the liver by the sporozoite.

4. To study receptor sites on the sporozoite, which might aid in recognition and penetration of a liver cell.

The basic rationale of this project has been to study fundamental biological mechanisms. Information learned from these studies has been subsequently applied to more "goal oriented" research, such as projects supported by A.I.D. or the U. S. Army Medical Research and Development Command. This complementary approach has worked well in the past. I plan to submit a new competing application to enable me to continue this N.I.H.-supported research after the termination of the current grant on 7/31/82.

2. U. S. Army Medical Research and Development Command Contract

DADA 17 73 C 3027

"Cell Physiology of the Malaria Parasite"

Principal Investigator - Jerome Vanderberg

Termination Date: 8/31/81

Total approved funds for terminal year of contract (9/1/80 - 8/31/81)
= \$60,860

The most current contract proposal had listed the following aims:

1. To develop an in vitro system for exoerythrocytic forms of P. falciparum and to investigate drugs which might interfere with exoerythrocytic forms and their metabolism.
2. To identify and characterize human erythrocyte receptors for P. falciparum merozoites, and to investigate drugs which might interfere with the process of recognition.

However, as noted in the letter from General Rapmund reproduced on the following page, this contract is being terminated. Accordingly, a portion of the Army proposal listed under aim 1 above has been modified and is being submitted as a portion of this current A.I.D. proposal (see pp. 15-27 of this proposal).

Among the areas of progress within this Army-supported work, we did some studies on P. falciparum merozoite receptor proteins, and on the changing



DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
FORT DETRICK, FREDERICK, MD 21701

REPLY TO
ATTENTION OF

SGRD-OP

10 FEB 1981

Jerome Vanderberg, Ph.D.
New York University Medical Center
New York, NY 10016

Dear Doctor Vanderberg:

Due to budget decrements, severe inflation, and new missions in chemical warfare defense and occupational health research on new Army systems, the US Army Medical Research and Development Command is unable to support many valuable and productive contracts on infectious diseases. I regret to inform you that your contract is among those which cannot be renewed upon its termination date.

Therefore, your present contract, No. DADA17-73-C-3027, entitled "Cell Physiology of the Malaria Parasite," will be terminated on 31 Aug 81. A final report should be submitted at that time.

I fully recognize this action diminishes our research base on militarily relevant infectious diseases which in past wars have caused many more casualties than hostile action. I want to thank you for your important contributions to our infectious disease research program and for your continuing interest in it. I shall strive for a correction in the funding priority of infectious disease research in coming years and hope that such correction might enable my Command again to support your research.

Sincerely,

GARRISON RAPMUND
Brigadier General, MC
Commanding

patterns of protein synthesis during the life cycle of the parasite. These merozoite studies were supported almost entirely by the Army Contract, and by a N.I.H. pre-doctoral fellowship held by the person actually doing much of the work, Chris Lambros. We used information gained from these studies as the basis for a portion of a previous A.I.D. Contract proposal (isolation of merozoite receptor protein for possible use as an antigen). However, we were requested to drop this portion of our proposal, and accordingly have done so in this current modification of the proposal. It should be emphasized that the actual pilot studies were done with the approval and financial support of the Army Medical Research and Development Command. In no way did they interfere with the actual A.I.D. contract-supported work that we did concurrently, and in no way can this Army-supported work be considered to be a departure from my approved A.I.D. objectives.

3. W.H.O. Research Grant I.D. No. 800400 (Funded by W.H.O. Working Group on Chemotherapy of Malaria)

"Inhibition of the Interactions Between Erythrocytes and Plasmodium falciparum Merozoites"

Principal Investigator: Seymour Schulman

Approved Dates 7/1/81 - 6/30/84

Total approved funds for first year: \$34,892

This project was based on results obtained from a previous Army-supported Contract, and in a sense will now take over that aspect of the terminated Army project (see Aim 2 of Army Contract above).

4. A.I.D. Contract No. ta-C-1486

"In Vitro Cultivation of Sporogonic Stages of Human Malaria"

Principal Investigator - Jerome Vanderberg

Effective Dates: This 3 year contract was originally scheduled to be terminated on 1/14/81. The contract period was extended to 7/1/81, without additional funds, to permit completion of some of the objectives, as could be accomplished with available funds.

Total approved funds for terminal year of Contract = \$94,820

Details of work accomplished were presented in my Annual Progress Reports, and End of Contract Report. In summary, four major goals were accomplished:

a) a technique for establishing synchrony of asexual cultures was perfected. This "sorbitol" procedure has now become the standard synchronization procedure in the field of malaria culture work. The goal was proposed, as follows, in my original approved A.I.D. technical proposal (page 8).

"Attempts will also be made to improve synchrony of developmental stages within these cultures. This may be an especially important problem to solve in gametocyte culture, in view of the fact that mature gametocytes may be produced synchronously, and may be short-lived in their maturity when produced (See Appendix I, page 15). Thus, if all of the gametocytes within a culture could be produced in synchrony, a high percentage of them might be shown to be mature at the same time after an appropriate period of development. Attempts will be made to impose such synchrony by fluctuations of levels of oxygen, CO₂, hormones, temperature, cyclic AMP, and various regulators of cell division and the cell cycle."

b) A technique for replacing human serum with peptones and calf serum was developed. This procedure and subsequent modifications of it have been confirmed and used by other members of the A.I.D. Network (Jensen and Siddiqui). The goal was proposed, as follows in my original A.I.D. approved technical proposal (pp 7-8).

"Another problem of mass production relates to the expense and difficulty of supplying large quantities of human serum. Possible transfer of hepatitis virus may also pose a problem. Substitutes will be sought. The possibility of sera from other animals such as bovines, sheep, horses, etc. will be investigated. Human plasma extenders, as well, will be tried. Such substitutes may possibly be used if specific growth factors are added. What is needed is a better understanding of the specific factors in human plasma which are required for parasite growth. Studies with the various fractions of human plasma (which are commercially available from Miles Laboratories) may give insight into which of these are required by the parasite. The use of lyophilized plasma will be tested as well, to determine whether plasma can be stored and shipped under tropical conditions. The rationale for this is that if the Trager-Jensen system is introduced into African laboratories, there might be problems associated with plasma from local, immune populations."

c) A technique for enhancing induction of P. falciparum gametocytes in culture was described, and

d) A procedure that permits complete maturation of P. falciparum gametocytes in culture was described.

These latter two accomplishments were the main goals of the prior contract. Two important elements in the consideration of any new contract proposal are whether the proposed goals actually fit in with the new goals and priorities of the A. I. D. Network Program, and whether there is a credibility that the principal investigator can actually accomplish these new proposed goals. Whether the proposed goals fit in with the overall A.I.D. program is best left to the judgment of the reviewers and advisors involved. However, our credibility to accomplish proposed goals within a given contract period, and within a given budget speaks for itself.

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(Modification submitted October 1, 1981)

U.S.A.I.D. CONTRACT PROPOSAL

JEROME VANDERBERG
PRINCIPAL INVESTIGATOR

PROPOSED BUDGET FOR FIRST YEAR

(January 1, 1982 - December 31, 1982)

A. <u>Personnel</u>	Effort	<u>Amount</u>
Dr. Jerome Vanderberg, Principal Investigator	55	\$ 22,229
Dr. Seymour Schulman, Assoc. Research Scientist	35	7,578
Mr. Richard Nawrot, Assistant Research Scientist	50	10,218
Dr. Titus Ifediba, Post-Doctoral Fellow	100	15,500
Dishwasher (shared)	20	1,899
Secretary (to be hired)	50	<u>6,571</u>
TOTAL SALARIES		63,995
B. Fringe Benefits (at 21.5% salaries effective 9/1/81; at 22.5% salaries effective 9/1/82)		13,972
C. <u>Equipment</u>		
1. Harvard perfusion pumps (2), plus tubing and glassware apparatus for Trager's Flow vial system		5,500
2. Orbital shaker and platform for tissue culture flasks.		1,660
3. Low-high temperature incubator for items 1 and 2.		1,565
4. Carbon dioxide incubator		3,800
5. Liquid Nitrogen storage tank-freezer		945
6. Sartorius analytical balance		3,150
7. Wang Word Processor (shared cost)		4,500

8. Microscope slide storage system	565
9. Microgasometer	1,155
10. Chemical safety cabinet	650
11. Sorvall RC-5B Centrifuge with rate controller and SS-34 rotor	10,595
12. Air Conditioner	<u>650</u>

EQUIPMENT TOTAL

34,740

D. Supplies (Media, gases, chemical, plasticware, etc.)
(based on actual analysis of prior monthly
purchase records) 8,500

E. Blood serum and cells 6,000
(based on 2 units of serum and 0.5 units of cells
per week at \$50 per unit)

F. Equipment maintenance, service contracts 1,000

G. Animals (Mice, rats, hamsters) 500

H. Animal care and shared insectary maintenance 1,000

I. Travel (meetings, and conduct of joint research) 1,000

J. Publications (reprints and page costs) 500

TOTAL FIRST YEAR DIRECT COSTS \$ 131,207

OVERHEAD AT 49.5% OF DIRECT COSTS \$ 47,751
(Exclusive of Equipment)

TOTAL FIRST YEAR DIRECT AND INDIRECT COSTS \$178,958

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PROPOSED BUDGET FOR SECOND YEAR
(January 1, 1983 - December 31, 1982)

A.	<u>Personnel</u> (As indicated in first year budget plus 7% increase as required by N.Y.U.)	\$68,475
B.	Fringe benefits at 22.5% salaries	15,407
C.	Supplies (media, gases, chemicals, plasticware, etc.)	8,500
D.	Blood serum and cells	6,000
E.	Equipment maintenance. service contracts	1,000
F.	Animals (mice, rats, hamsters)	500
G.	Animal care and shared insectary maintenance	1,000
H.	Travel (meetings and conduct of joint research)	1,000
I.	Publications (reprints and page costs)	<u>500</u>
	TOTAL SECOND YEAR DIRECT COSTS	\$ 102,382
	OVERHEAD AT 49.5% OF DIRECT COSTS (Exclusive of equipment)	\$ 50,679
	TOTAL SECOND YEAR DIRECT AND INDIRECT COSTS	\$ 153,061

PROPOSED BUDGET FOR THIRD YEAR
(January 1, 1984 - December 31, 1984)

A.	<u>Personnel</u>	\$73,268
	(As indicated in second year budget plus 7% increase as required by N.Y.U.)	
B.	Fringe benefits at 22.5% salaries	16,485
C.	Supplies (media, gases, chemical, plasticware, etc.)	8,500
D.	Blood serum and cells	6,000
E.	Equipment maintenance, service contracts	1,000
F.	Animals (mice, rats, hamsters)	500
G.	Animal care and shared insectary maintenance)	1,000
H.	Travel (meetings and conduct of joint research)	1,000
I.	Publications (reprints and page costs)	500
	TOTAL THIRD YEAR DIRECT COSTS	\$ 108,253
	OVERHEAD AT 49.5% OF DIRECT COSTS (Exclusive of Equipment)	\$ 53,585
	TOTAL THIRD YEAR DIRECT AND INDIRECT COSTS	161,838

Justification for Equipment

1. Flow vial system will be used to determine feasibility of mass production of mature P. falciparum gametocytes, for eventual large scale production of sporozoites (see pp. 13 - 14 of proposal).
2. Orbital shaker is to be used for orbital incubator set-up for mass production of gametocytes, as in item 1 above (see pp. 13 - 14 of proposal). The same perfusion pumps as in item 1 will be adapted for use with the orbital incubator.
3. As an economy measure, a single incubator will be used to house items 1 and 2 above. The studies will necessarily have to be done at different times, since only one system can fit in the incubator at a given time. A low-high temperature incubator (with compressor as well as heating unit) is required for maintaining constant temperatures while heat producing equipment (the orbital shaker) is maintained within.
4. A carbon dioxide incubator is required for maintaining many cell lines (human embryonic lung cells, for instance) (see pp. 23-25 of proposal).
5. The liquid nitrogen freezer will be required to maintain frozen samples of parasite strains, preserved at different times after their initial isolation (see pp. 10 - 11 of application).
6. An analytical balance is used on a daily basis in our studies. At present, there is only one in our entire Division (in Dr. Nussenzweig's laboratory), and the pressures for the use of this balance often requires long waits, or postponements of experiments.
7. Our Division is planning to purchase a word processor. In view of the considerable amount of time that we spend on contract-related applications, reports, publications, the use of the word processor would permit a substantial saving in typing time, and in literature retrievals for preparing

bibliographies. The indicated cost would be my share of an overall purchase.

8. The slide storage system is for organized filing and storage of microscope slides of blood smears. Many of our studies require maintenance of large numbers of slides.
9. The microgasometer is used for accurate measurement and delivery of a gas mixture to gametocyte cultures (see p. 10).
10. A chemical safety cabinet is for storage of flammable solvents and reagents in our laboratory. We have been informed that without one, we are currently in violation of New York City Fire Dept. regulations.
11. The Sorvall centrifuge is primarily for high speed centrifugation of Percoll gradients for purification of sporozoites (see p. 20). Our preliminary studies showing the value of such a gradient were done with a centrifuge in another laboratory. This will no longer be available for the extended centrifugation times that we will need.
12. The air conditioner is for a small laboratory (room 509) adjacent to our regular laboratory. We are presently quite crowded in our laboratory, and are attempting to organize this adjacent facility for our own work.

Salary increments included in budget years beyond the first are based on estimated percentages including merit and inflationary factors. Percentages applied vary depending on job descriptions, time and grade, etc. for each employee. Actual salaries to be paid in future years must be in agreement with the official wage and salary program consistently applied at N.Y.U. Medical Center.

Resources for the Future

1755 Massachusetts Avenue, N.W.
Washington, D.C. 20036

October 5, 1981

Ms. Pamela L. Baldwin
AID Office of Energy
Room 306 SA-18
Washington, D.C. 20523

Dear Pamela:

Enclosed is a list of the ARDEN documents and projected dates of delivery of the final reports to RFF. We have made tentative plans to print most of the final reports as part of the RFF/CEPR Discussion Paper Series or submit them to the RFF Publications Committee for consideration as RFF Research Papers. (These are indicated by asterisks on the list.) We reserve the right to revise these plans--analysis on some projects is not yet complete and financial restrictions may necessitate a reduction in the number of papers we can have printed.

Papers marked with a checkmark (✓) on the enclosed document list are available for examination by the AID Research Advisory Committee members at your meeting in November. A package including each of these documents is being sent to you under separate cover (a list of the titles is enclosed here). We have included all ARDEN materials except those which are not completed or those on which we have significant unresolved technical questions. Also included in this package are background papers on the rural electrification study (including the original design document, minutes of RE conference meetings, working drafts from the Operations Research Group, questionnaires, preliminary data reports and tables, etc.). The papers concerning rural electrification are preliminary working drafts and are labelled as such. They are not yet available for reproduction or citation without permission from RFF.

As noted, the list of ARDEN documents includes research studies and methodological studies. Research studies include in-house reviews and analyses of existing data and investigations carried out in specific developing areas by consultants or developing area institutions. Methodological studies are papers addressing questions of project design and analysis in ARDEN subproject investigations.

Also enclosed are copies of the following documents for your files which you have not previously received:

1. Ramesh Bhatia, "Fuel Alcohol From Agro-Products in India: A Study of Crop Substitutions, Food Prices and Employment," July 1981;
2. Ashok V. Desai, "Interfuel Substitution in the India Economy," July 1981;
3. RFF, "Comments on Operations Research Group (ORG) Chapters and Household and Village Tables," September 15, 1981;

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Pamela L. Baldwin
October 5, 1981
Page Two

4. Operations Research Group (JRG), "Rural Electrification and Socio-Economic Development: Consequences and Determinants--Chapterisation Plan," September 8, 1981;
5. ORG, "Impact of RE on Rural Industrialisation," September 1981;
6. RFF, "Comments on ORG's 'Impact of RE on Rural Industrialisation,'" September 30, 1981;
7. Administrative Staff College of India (ASCI), "A Study of Conservation Possibilities in Manufacturing Industries in India: Progress Report - July 20, 1981"

Fifty copies of the Desai Discussion Paper, "Interfuel Substitution in the Indian Economy" will be mailed to you this week for distribution to AID missions.

Sincerely yours,



Marilyn M. Voigt
Project Assistant

Enclosures



Resources for the Future

1755 Massachusetts Avenue, N.W.

Washington, D.C. 20036

October 5, 1981

ARDEN Documents

For Review by U.S. AID Research Advisory Committee

Biomass Studies:

Julia C. Allen, "Charcoal Industry Location and Transport Study"

_____. "Modeling the Environmental Effects of Forest Management in a Watershed: A Multiobjective Approach to Forest Management for Developing Countries"

Ramesh Bhatia, "Fuel Alcohol From Agro-Products in India: A Study of Crop Substitution, Food Prices and Employment"

Donald I. Hertzmark, William Ramsay, Stephen Polasky, "A Simple Analytic Model for Agricultural Price Impacts Due to Alcohol Fuels Program"

Instituto de Investigaciones en Ciencias Economicas, "Desbalance Comercial Externo y Crisis Alimentaria: Resultados Esperados de un Programa Agresivo de Produccion de Alcohol Carburante en Costa Rica" (Translation: "Foreign Trade Imbalance and the Food Crisis: Anticipated Results of an Aggressive Program of Alcohol Fuel Production in Costa Rica" available end of October 1981.)

Energy Demand and Conservation Studies:

Administrative Staff College of India, "A Study of Conservation Possibilities in Manufacturing Industries in India: Progress Report - July 20, 1981"

Ashok V. Desai, "Interfuel Substitution in the Indian Economy"

Joy Dunkerley, Gunnar Knapp with Sandra Glatt, "Factors Affecting the Composition of Energy Supplies in Developing Countries"

Instituto Nacional de Energia, "Industrial Energy Consumption in a Developing Country: The Ecuadorian Case"

John J. Jankowski, Jr., "Industrial Energy Demand and Conservation in Developing Countries"

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Rural Electrification Studies:

Part I. Research and Project Design

- A. RFF, "An ARDEN Study Design Document--Rural Electrification and Socioeconomic Development"
- B. RFF, "International Comparative Studies of Rural Electrification--Coordinated Research Projects for Studies in India"
- C. RFF, "Summary of India Rural Electrification Washington Coordination Conference, July 1-3, 1980"
- D. Douglas F. Barnes, "Specification of Rural Electrification Analysis"
- E. RFF, "ARDEN Rural Electrification Meeting, Tuesday, March 31, 1981, Minutes of the Meeting"

Part II. Questionnaires

- F. Instituto SER de Investigacion,
 - "Evaluation of the Impact of Rural Electrification in Colombia"
 - "Coding Manual"
 - "Stratification"
 - "Household Questionnaire"
 - "Community Questionnaire"
- G. Operations Research Group,
 - "Socio-Economic Impact of Rural Electrification--Field Instructions"
 - "Household Questionnaire--Part I"
 - "Household Energy Use--Part II"
 - "Village leader Schedule"
 - "Village Level Schedule From Panchayat Office/SEB Records/Local Leaders"

Part III. Preliminary Tables

- H. Operations Research Group,
 - "Village Tables"
 - "Household Tables--Summary Observations on Relationship Between Household Level Indicators and Rural Electrification"
 - "Sample of Preliminary Raw Data from Village Survey (12 villages)"

Part IV. RFF Comments on Research Projects

- I. RFF, "Comments on ORG Rural Industries Paper"
- RFF, "Comments on ORG Chapters and Household and Village Tables"
- RFF, "Comments on ORG Rural Electrification 'Response' and 'Literature' Drafts"

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Part V. Preliminary Draft Research Reports

- J. Operations Research Group, "Rural Electrification and Socio-Economic Development: Consequences and Determinants--Chapterisation Plan"
- K. _____, "Studies Relating to Rural Electrification in India--Summary of Past Evidence"
- L. _____, "Preconditions for Successful Response in the Residential Sector"
- M. _____, "Temporal Response to Rural Electrification Agricultural, Residential and Industrial Sectors"
- N. _____, "Impact of RE on Rural Industrialisation"
- O. Administrative Staff College of India, "Summary of ASCI Cost-Benefit Study"

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ARDEN DOCUMENTS

Biomass Studies

<u>Author</u>	<u>Title</u>	<u>Projected Delivery Date to RFF</u>
Research Studies:		
Julia C. Allen	Forest Soils in Developing Countries	April 1982
Julia C. Allen and Douglas F. Barnes	Deforestation, Wood Energy and Development	October 1981
* Julia C. Allen, Douglas F. Barnes and Margaret Skutsch	Social Forestry in Tanzania	January 1982
* Douglas F. Barnes	Social Forestry	January 1982
* Ramesh Bhatia (consultant at Harvard)	Fuel Alcohol From Agro-Products in India: A Study of Crop Substitution, Food Prices and Employment	Received
* Instituto de Investigaciones en Ciencias Economicas (Juan Manuel Villasuso)	Foreign Trade Imbalance and the Food Crisis: Anticipated Results of an Aggressive Program of Alcohol Fuel Production in Costa Rica	Received (translation available October 1981)
* Shafiqul Islam and William Ramsay	Fuel Alcohol: Some Complexities in the Brazilian and American Programs	November 1981
* William Ramsay with R. Bhatia, P. Ehrlich, A. Poole, J. Villasuso	Macroeconomic Factors for Fuel Alcohols in the Third World	December 1981
* University of Dar es Salaam (Margaret Skutsch, A. B. Temu)	Socioeconomic Aspects of Existing Woodfuel Energy Programs: Village Afforestation in Tanzania-- Policy and Case Studies	April 1982 (delayed for medical reasons)

ARDEN DOCUMENTS

Biomass Studies (cont'd)

<u>Author</u>	<u>Title</u>	<u>Projected Delivery Date to RFF</u>
Methodological Studies:		
Julia C. Allen	Charcoal Industry Location and Transport Study	Completed
Julia C. Allen	Modeling the Environmental Effects of Forest Management in a Watershed: A Multiobjective Approach to Forest Management for Developing Countries	Completed
Donald I. Hertzmark, William Ramsay, Stephen Polasky	A Simple Analytic Model for Agricultural Price Impacts Due to Alcohol Fuels Program	Completed

ARDEN DOCUMENTS

Energy Demand and Conservation Studies

<u>Author</u>	<u>Title</u>	<u>Projected Delivery Date to RFF</u>
Research Studies:		
* Administrative Staff College of India (R. K. Pachauri)	Study of Conservation Possibilities in the Manufacturing Industries in India	October 1981
* Centro de Estudios Sobre Desarrollo Economico (Jose Eddy Torres)	Nonhousehold Rural Energy Demand	November 1981
* Ashok V. Desai	Interfuel Substitution in the Indian Economy	Completed
* Joy Dunkerley, Gunnar Knapp with Sandra Glatt	Factors Affecting the Composition of Energy Supplies in Developing Countries	Received
* Instituto Nacional de Energia (Eduardo Moran)	Industrial Energy Consumption in a Developing Country: The Ecuadorian Case	Received
* John J. Jankowski, Jr.	Industrial Energy Demand and Conservation in Developing Countries	Completed
** Lawrence Berkeley Laboratory (Lee Schipper)	Energy Conservation in Kenya	October 1981
* Scott Noll	The Potential for Transportation Energy Conservation in Developing Countries	November 1981
* Alan Poole	The Brazilian Transport Sector: Historical Background and Assessment of Conservation Possibilities	November 1981

* Scheduled for RFF/CEPR Discussion Paper Series

** Will be submitted to RFF Publications Committee for consideration as a published RFF Research Paper.

ARDEN DOCUMENTS

Rural Electrification Studies

<u>Author</u>	<u>Title</u>	<u>Projected Delivery Date to RFF</u>
Research Studies:		
* Administrative Staff College of India (R. K. Pachauri)	International Comparative Studies of Rural Electrification	January 1982
* Janice Brodman (consultant at Stanford)	Socioeconomic Impacts of Rural Electrification in Indonesia	March 1982
* Operations Research Group (B. B. Samanta)	Socioeconomic Impact of Rural Electrification	March 1982
* Instituto SER de Investigacion (Eduardo Velez)	Rural Electrification in Colombia	February 1982
** Douglas Barnes and others	Rural Electrification and Energy in Developing Countries	April 1982
Methodological Study:		
Douglas F. Barnes	Specification of Rural Electrification Analysis	Completed

* Scheduled for RFF/CEPR Discussion Paper Series

** Will be submitted to RFF Publications Committee for consideration as a published RFF Research Paper.

MEMORANDUM

May 11, 1981

TO: DS/EY, Mr. Alan Jacobs

FROM: DS/PO, Thomas O'Keefe

SUBJECT: Extension of Energy Policy and Planning Project
(936-5703)

We have sent forward for authorization the extension-refunding package on this project.

There are three matters that we consider significant aspects of the implementation of the extension.

1. The RAC will review the results of the RFF work.
2. FY 81 funding of RFF should specify that this is final funding for the current cooperative agreement.
3. The Scopes of Work for the several energy planning activities to be funded in FY 81 should include the financial contributions of the USAIDs.

UNITED STATES INTERNATIONAL DEVELOPMENT COOPERATION AGENCY
AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

JUL 10 1981

Mr. John E. Herbert
Secretary-Treasurer
Resources for the Future, Inc.
1755 Massachusetts Avenue
Washington, D. C. 20036

Subject: AID/DSAN-CA-0179
Amendment No. 1

Dear Mr. Herbert:

Pursuant to the authority contained in Sections 297 and 635 of the Foreign Assistance Act, as amended, and the Federal Grant and Cooperative Agreement Act of 1977, the Agency for International Development hereby amends the subject Cooperative Agreement effective the date of this letter to extend the expiration date of the Agreement from August 31, 1981 to April 30, 1982.

I. Cover Page

- A. From block entitled "Expiration Date" delete "August 31, 1981" and substitute "April 30, 1982."
- B. From block entitled "Amount Obligated" delete "\$800,000 and substitute "\$1,075,000."

II. Program Description

Add "Program Description September 1, 1981-April 30, 1982"

III. Article L Budget

Delete "L Budget" and substitute -

"L. Budget

The funds herein shall be used to finance the following items:

Item	Firm Budget		Total Budget & Amount Obligated
	FR: 9/1/79 TO: 3/31/81	FR: 9/1/81 TO: 4/30/82	FR: 9/1/79 TO: 4/30/82
1. Salaries	\$259,324	\$ 86,949	\$ 346,273
2. Fringe Benefits	59,645	20,086	79,731
3. Overhead	143,536	64,437	207,973
4. Travel	44,400	5,405	50,210
5. Other Direct Costs	23,095	24,623	47,958
6. Subgrants	270,000	73,500	343,500
	<u>\$800,000</u>	<u>\$275,000</u>	<u>\$1,075,000</u>

IV. Delete Article N, "Overhead Rates" in its entirety and substitute the following in lieu thereof:

N. Overhead Rates

Pursuant to the provision of the Standard Provisions entitled "Negotiated Overhead Rates - Non-Educational", a rate or rates shall be established for each of the Recipient's accounting periods during the term of this Cooperative Agreement. All rates set forth below are provisional rates and are subject to downward adjustment only, for the period(s) stipulated.

<u>Rate</u>	<u>Base</u>	<u>Period</u>
45.0%	Direct Salaries and Fringe Benefits	Fr: 9-1-79 To: 9-30-80
"	"	Fr: 10-1-80 To: Until amended

Rates for subsequent periods shall be established in accordance with the terms of the "Negotiated Overhead Rates - Non-Educational Institutions" clause of the Standard Provisions.

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Except as provided herein all terms and conditions of the subject Cooperative Agreement remain unchanged and in full force and effect.

Please sign the original and ten copies of this amendment. Return the original and nine copies to the undersigned.

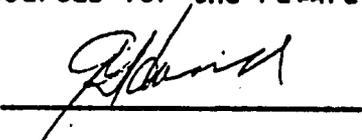
Sincerely yours,



Morton Darwin
Agreement Officer
Agriculture/Nutrition Branch
Central Operations Division
Office of Contract Management

ACCEPTED:

Resources for the Future

BY: 

TITLE: Secretary-Treasurer

DATE: 7/16/81

Fiscal Data

Amendment Amount: \$275,000
Total Obligated Amount: \$1,075,000
Appropriation No: 72-1111021.6
Allotment No: 146-36-099-00-20-11
PIO/T No: 3611450
Project No: 936-5703

"PROGRAM DESCRIPTION"
September 1, 1981-April 30, 1982

Purpose of Cooperative Agreement

The purpose of this agreement is to utilize the recognized expertise and demonstrated research and analysis competence of Resources for the Future (RFF) to develop and manage an energy studies project. This extension will enable RFF to continue increasing its capability to analyse developing country energy issues and the relationship between energy use and development. The recipient, by developing and managing a multidisciplinary study of energy use and development, will contribute significantly to the design of well-conceived energy assistance programs of donor organizations; to the preparation of national and sectoral energy plans in developing countries, and to the exchange of information, analyses and research designs among academic and government institutions in developed and developing countries.

B. Specific Objectives

1. To provide data and analyses of specific energy concerns thus contributing to a greater understanding of energy and development issues in general.

2. To improve the quality and variety of background materials on LDC energy issues available to energy decision-makers and project managers.

3. To increase understanding of interaction between rural and urban energy supply and demand scenarios.

4. To encourage interaction and information exchange among LDC energy analysts and their counterparts in donor countries.

C. AID-RFF Cooperation

The Office of Energy (DS/EY) in AID and the recipient will continue to cooperate and collaborate closely in the implementation of this project. The project officer in the appropriate technical office (DS/EY) will work closely with the project director and other principal investigators in RFF and serve as the channel for AID guidance in this project. The AID project officer will be consulted on issues such as: selection of senior professional staff to participate in the project; selection of cooperating institutions in developing countries and elsewhere; selection of LDC energy issues to be studied. She will participate as AID's representative at RFF-sponsored conferences on the implementation of this project and be the principal liaison between AID and RFF.

D. Implementation

RFF will complete the implementation and management of general studies relating to energy use and economic development in LDCs, with principal focus on AID-assisted countries. In addition, RFF will devote considerable attention and resources to the dissemination of research results. In accordance with the program of research initiated in FY 79-80, the three principal areas of research will continue to be:

1) The costs and benefits of rural electrification programs, as determined through surveys and case studies of rural electrification programs in selected countries;

2) Issues of energy demand, including conservation and fuel substitution, in industry and transportation; and

3) Energy supplies from biomass, with special emphasis on alcohol fuels, community forestry, large-scale commercial fuelwood plantations and charcoal production.

Most of the work to be done under the extended agreement will involve the completion and dissemination of research subprojects already begun, although a limited number of new projects may be undertaken to fill gaps in existing knowledge and round out research results for comprehensive topical publications. All subcontracts will be with institutions in developing countries that are included in AID's development assistance program, unless special permission is received from DS/EY to subcontract with institutions outside these countries. Subcontracts with U.S. institutions will be permitted for the purpose of disseminating research results through publications, seminars, workshops, visual presentations, etc.

E. Advisory Committee

The recipient will continue to convene an Advisory Committee created during the first funding period of the Cooperative Agreement. In the event that membership changes are necessary or advisable, the grant recipient will maintain the following general categories of representation on the Advisory Committee (the exact number to be determined by the recipient's project director and the DS/EY project officer):

- (a) AID (DS/EY and the Regional Bureaus)
- (b) Sociologist/social scientist
- (c) Development Economist
- (d) Physical Scientist
- (e) LDC Representative
- (f) Representative of foundations working on energy.

(g) Representative of RFF Management Governing Board.

The Advisory Committee shall meet at least once during the period of the proposed extension to review research results and proposed dissemination activities. The institution will follow its established outside peer review procedures before publishing studies.

F. Project Subgrants

The recipient may make project subgrants to institutions and/or individuals in developing countries. These subgrants can fund individual studies or components of larger studies as approved by AID and the Advisory Committee. Prior to making any project subgrant over \$5,000 the grantee shall submit the following information to the Project Officer, Office of Energy, for approval: (Approval will be based on criteria set forth in Paragraph G below.)

- (a) Subproject title.
- (b) Subproject description.
- (c) Duration of sub-project for which funding is proposed.
- (d) Name and address of the proposed sub-grantee and the location(s) where activities will take place.
- (e) Estimated cost of proposed subproject.
- (f) Qualifications of the subgrantee relevant to proposed subproject.
- (g) Resources which grantee proposes to contribute and inputs others will make to subproject, including dollar value.
- (h) Plan for carrying out proposed subproject.
- (i) Results expected from proposed subproject.
- (j) Data to be reported by subgrantee.

G. Criteria for AID Approval of Project Proposals

The RFF project officer will submit detailed proposals and implementation plans to the AID project officer for approval. The AID project officer, after consultation with AID technical and regional offices and USAID field missions, as appropriate, will provide final approval of those proposals in writing to RFF. Criteria for AID's approval of proposed projects are:

- (a) Consistency with AID's energy strategy.
- (b) Appropriate LDC government and AID regional and mission clearance.

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Reporting Requirements

RFF will submit a comprehensive report on all activities and progress to date as of October 31, 1981. The report will serve, along with the substantive reports arising out of the program of research and studies, as a principal focus of the evaluation scheduled for that time."

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Resources for the Future

Emery N. Castle, President

June 2, 1981

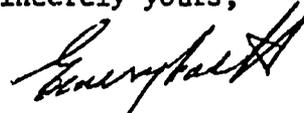
Mr. Morton Darwin
Office of Contracts Management
CM/COD
Agency for International
Development
Washington, D.C. 20525

Dear Mr. Darwin:

In accordance with your request, I am submitting the attached proposal "Extension of the ARDEN (AID-RFF Development and Energy) Cooperative Agreement with AID for an Energy Policy and Planning Studies Program."

The proposal seeks to extend the Agreement itself through April 30, 1982. In addition, it requests \$275,645 in additional FY 81 funding.

Sincerely yours,



Emery Castle
President

EC/mas

Enclosure

W. Ramsay
Re-Revised Draft
June 1, 1981

DRAFT

Proposal

EXTENSION OF THE ARDEN (AID-RFF DEVELOPMENT AND ENERGY)
COOPERATIVE AGREEMENT WITH AID
FOR AN ENERGY POLICY AND PLANNING STUDIES PROGRAM

Introduction

Resources for the Future proposes here to extend an existing ARDEN cooperative agreement with AID to carry out a program of policy and planning studies on energy issues in developing countries. The original cooperative agreement, entered into September 1, 1979, is due to expire August 31, 1981. In addition, the original funding for the cooperative agreement, according to the proposal submitted by Resources for the Future and accepted by the AID Office of Contract Management, was to cover only the 18 months of operation from September 1, 1979 through February 28, 1981. However, at our present rate of expenditure, existing funds should last through May 31, 1981.

This proposal therefore seeks to extend the cooperative agreement through April 30, 1982, and to secure incremental supplementary FY 81 funding.

This proposal is made by RFF in the belief that the existing agreement for cooperation has served its original main purpose of advancing the goals of AID in developing a better energy assistance.

program while helping RFF consolidate and extend its existing capabilities for analyzing developing country energy issues. In addition, it is clear that the existing program has succeeded in gaining the active participation of research institutions in LDC's and that an extension of the program would continue to help develop expertise within the developing areas themselves to improve national energy planning and policymaking. Finally, the interaction of RFF with a number of LDC institutions and governments and with AID headquarters and field missions has helped and should continue to help develop new avenues for the exchange of energy data, analytic methods and other relevant information among world energy analysts.

Goals of the ARDEN Program

The research done under this project has been aimed at providing new data and analyses of specific energy concerns, thus contributing to a greater understanding of energy and development issues in general. It has also sought to improve the background information available to LDC energy policy planners and to the staff of AID, other government agencies, and international donor institutions. It has also, as noted above, been designed to develop the capabilities of LDC energy analysts and has contributed to data and idea exchanges among analysts and planners in various developing countries. Among the specific issues treated in the ARDEN work are the role of renewable resources in satisfying LDC energy supply needs, the nature of energy demand and conservation possibilities,

and the interaction of energy sector plans with broader economic development strategies.

Review of ARDEN Activities Carried Out with FY 79-80 Funds

The operations to date, funded by FY 79-80 appropriations, have supported an RFF study team comprising two (originally three) senior researchers, one economist and one sociologist at the doctoral level, and research and clerical staff. Numerous subgrants have also been made to LDC researchers. The work has centered on three project areas: (1) rural electrification evaluation, (2) energy conservation, inter-fuel substitution, and other energy demand areas, and (3) selected problems in biomass energy policy research.

The level of effort on the project has built up gradually. A key part of the ARDEN effort lies in grants and contracts to institutions and consultants in developing areas. Negotiation of these efforts at long distance inevitably takes a good deal of time, and it has been necessary to coordinate the build-up of in-house efforts with our outside contracts in LDC's. Current indicated levels of effort have reached about 1200 man-hours monthly.

Progress has been fastest in the field of rural electrification, where pre-existing RFF research had identified promising areas of study and likely collaborators. Substantial progress has also been made in the field of energy demand, where advantage has been taken of the keen interest already existing in many developing areas in studying conservation

possibilities in industries and other sectors. In the field of biomass, efforts have been made to identify key research gaps in this field, and RFF staff have proceeded carefully in trying to select useful problems that can be studied on as rigorous a scientific basis as possible.

Dissemination of ARDEN Results

The ARDEN studies and their results will be disseminated by the formal and informal publication of papers and books, informal contact with other researchers and with policymakers around the world, and--perhaps at a later stage, resources permitting--the holding of formal seminars or workshops.

The analytic and methodological studies produced by the RFF staff or the ARDEN project will normally first be circulated by informal means. However, a good many of them will also be treated to a more formal process of dissemination through the new RFF working paper series. This series will use informal reproduction processes, but a standardized format and list of subscribers. The case studies produced by developing area institutions and other consultants for the project will also normally come out as working papers. However, some of them will probably be published also as RFF research papers. RFF research papers are printed by offset from typescript, but are otherwise treated by libraries and other research institutions as books.

The final ARDEN studies will normally be submitted for publication as RFF research reports or as RFF books. RFF books, which are published for Resources for the Future by the Johns Hopkins Press, have a well-established reputation in the field of resource economics as standard references in the field of resource policy research.

In practice, RFF will in effect carry out an informal communication of RFF research results all over the world through its wide network of contacts throughout the world, and its participation in such programs as the International Communication Agency tours, the Visitors Program Service, and other government agencies. Should resources permit, at a later stage of the program, it might be desirable to hold formal seminars and workshops to acquaint LDC policymakers and researchers with the ARDEN research results and to explore research topics of interest to all developing nations.

Progress on Institution-Building Aspects

Although most of the studies subcontracted to LDC institutions under this program are still in early or intermediate stages, the results already appear promising with respect to institution building and development of a collaborative network of researchers overseas. In particular, the ARDEN project has helped the Administrative College of India in Hyderabad expand its considerable potential in the field of energy analysis. Furthermore, other valuable contributions to energy planning in India should result--through, for instance, the retention as a consultant on this project of Dr. Lalit Sen, on leave from his post as Chief Evaluation Research Officer of the Rural Electrification Corporation (India).

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The ARDEN program has also helped solidify information contacts between the Instituto SER ("SER" is not an acronym) and CEDE (Centro de Estudios sobre Desarrollo Economico) groups in Colombia where our studies on rural electrification and on conservation promise to benefit from related efforts we have brought together. Furthermore, in Ecuador, a rather modest ARDEN subcontract has made it possible for a very able analytic group at the National Energy Institute to realize more of its potential for theoretical analysis by making possible a survey of industrial energy use and conservation possibilities. Similarly, in Costa Rica, an ARDEN subgrant has given support to researchers at the University of Costa Rica in helping them to make solid analytical contributions to the ongoing debate there on the effects on the Costa Rican agricultural sector of the present extensive national program of alcohol fuels.

Review of Work on ARDEN Subprojects

The ARDEN subprojects are briefly described here. Expenditures by subproject are reviewed in the "Costs" section below.

Rural Electrification Subproject

Nature of Subproject and Expected Output. Past research on the impact of rural electrification on development has led to contradictory conclusions. Critics claim that rural electrification, given current weak electricity demand in developing countries, is often too expensive, does not benefit all social classes equitably, and has no significant impact on agricultural or industrial development. By contrast, advocates of rural electrification argue that investments in electricity infrastructure produce long-term benefits by eventually creating more jobs, diminishing

rural-urban migration, increasing agricultural production, and generally improving the quality of life in rural areas.

The RFF ARDEN team is examining the effects of rural electrification in India in 96 village-level studies, supplemented by selected household surveys in 24 villages. Among the main questions addressed in the surveys is the long-term consequences of rural electrification in increasing agricultural production and stimulating growth of rural industries. A further very important goal of the study is to examine the social effects of rural electrification on the community--for example, on income distribution and tendencies toward rural out-migration. The research will also evaluate complementary inputs to rural electrification, including roads and access to change agents such as extension services. Finally, the question of the real opportunity costs of rural electrification, apart from subsidy measures, is being investigated in the context of a limited comparison of rural electrification with alternative energy sources.

The reports on individual studies that are part of the subproject will be given distribution as working papers and will be synthesized in the form of an RFF research report or book. This would give guidance to energy planners in developing areas and to AID and other donors by delineating as clearly as possible the type of economic and social results that can be expected from rural electrification projects.

Status. RFF has been exceedingly fortunate in gaining access to existing earlier surveys of villages in India, and has contracted with

two outstanding research organizations in India, the Operations Research Group at Baroda and the Administrative Staff College in Hyderabad, to resurvey villages previously surveyed in 1966 to try to trace the effects of subsequent rural electrification.

A comparable study has been undertaken under an ARDEN subgrant with the Instituto SER in Colombia.

Reports on the Indian case studies, "Benefit-Cost Problems in Rural Electrification", by R. K. Pachauri and others, and "Socio-Economic Impacts of Rural Electrification in India", by B. B. Samanta and others, should be available, respectively, in May 1981 and in July 1981. The report on the Colombia study, "Rural Electrification Impacts in Colombia", by Eduardo Velez and others, should be delivered by November 1981.

These case studies of course will also be utilized as part of the overall in-house analysis of this problem (see "Plans for June-October 1981" below).

Energy Demand Subproject

Nature of Subproject and Expected Output. The need for data on energy demand in developing countries is critical if energy policymakers are to be able to plan for future energy supply needs. The RFF team has picked out several areas where it believes that maximum payoffs can be obtained for policymaking in the near future. For example, as part of developing a general understanding of how energy demand may or may not change as development proceeds, studies of interfuel substitution have already been carried out. In addition, studies of energy demand in specific sectors

of the economy have been undertaken. The initial emphasis has been on the industrial sector, with some study now also being directed to the transport sector.

The outputs from this study will be geared to understanding industrial (and transport) energy use in developing areas in such a way that opportunities for energy conservation can be identified. It is believed that timely efforts at increasing energy efficiency in developing areas can be by far the most cost-effective way of meeting energy demand at the margin. The RFF reports will be directed toward giving guidance to AID and other donors as to what kind of technical assistance can be of most use in conserving energy in developing areas.

Status. RFF has taken advantage of several general demand studies to be carried out in developing areas by funding the parts of these studies of relevance to the RFF program. In at least one case this partial contribution by RFF has made possible the implementation of the entire study. Studies have been completed in Kenya and are well underway in Colombia and in Ecuador. The report on Kenya is now available in a very rough draft form (see "Documentation"); the report should be available in a final draft in May 1981. The report on Ecuador "Industrial Energy Use in Ecuador" by Eduardo Moran F., Pedro Vernet, and others, will be available also in May. "Energy Use Patterns in Colombia" by Jose Eddy Torres and others, the report on the Colombia study (which includes work financed by other agencies on energy demand in general), will be available in October 1981.

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An RFF analytic study (see "Documentation" heading below) of inter-fuel substitution used available data to document the changing relative usage patterns between fuels such as coal and oil on the one hand, and between commercial and traditional fuels on the other. A related case study contracted with Professor Ashok V. Desai of Trivandrum explored the Indian experience in this area. An in-house RFF analytic study has also been carried out on energy conservation in the industrial sector in developing countries. These studies are available now (see "Documentation" below).

A beginning has also been made in the study of energy conservation in transportation. A report from a consultant on energy transportation in Brazil is now available (see "Documentation" below).

The various case studies mentioned will also be used as inputs to in-house analyses (see "Plans for June-October 1981" below).

Biomass Subproject

Nature of Subproject and Expected Output. The biomass program subproject deals with three main study areas. First of all, the RFF team has been investigating the macroeconomic aspects of alcohol fuel programs, especially with regard to Brazil. In particular, the food versus fuel problem is being studied to trace the effects of diverting agricultural production to energy crops both on food prices and on energy prices themselves.

The alcohol studies will produce reports describing a methodology for analyzing the effect of an alcohol fuel program on national agricultural sectors in general and on key national macroeconomic variables. While a definitive quantitative analysis of these issues is beyond the scope of the ARDEN effort--and may indeed be beyond the power of any present economic models--the ARDEN staff believes it is essential that countries now planning to invest in the alcohol option should have the best information available. This information would then be of critical importance to AID in considering technical assistance in the field of alcohol fuels. The effect of fuel alcohol industries on balance of payments is now being studied; an analysis of employment effects is scheduled for the near future.

The other two study areas deal with afforestation. The possibility of combining conventional saw timber and pulp production with fuelwood or charcoal production for local or distant fuels markets is being considered for commercial plantations in developing areas. The other study area deals with the organizational aspects of community forestry programs, and is an attempt to identify the causes of their failures and successes as related to local social factors and to national forestry policies.

The forestry-related studies will lead to reports on the critical economic and non-economic factors in both commercial and community or individual afforestation schemes. These factors should be of use to AID in designing technical assistance projects for afforestation. As this study area progresses, it is planned to draw comparisons between

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commercial and community schemes so that donor agencies can better assess the advantages and drawbacks of these two alternative ways of dealing with biomass energy opportunities--and with the overall problem of deforestation in LDC's.

Status. Support has been given to researchers at the University of Costa Rica to model the effects of alcohol policy options on the agricultural sector in the northeast portion of that country. A report on this work "La Politica del Alcohol Carburante y sus Efectos en el Sector Agricola", by R. Celis U. and J. M. Villasuso, will be available in August 1981. In addition, some limited consultancies have been contracted for in this area in order to devise simple analytic methods for the general treatment of these complex problems.

Several in-house studies have been prepared on the alcohol question and on the forestry study areas (see "Documentation" below). Several LDC case studies in these areas have also been proposed under incremental FY 81 funding (see below).

Documentation of ARDEN Project

The following are the titles of completed documents on ARDEN problem areas. They are of four types, as explained below.

Study Design Documents

The study design document outlines the plan of action for the investigations in ARDEN subproject areas. It normally will incorporate by reference relevant analytic and methodological studies and will describe planned and ongoing case studies.

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Biomass Area:

1. Charcoal Transport Networks and Charcoal Kilns
2. The Environmental Impact of Forest Management in Selected Watersheds
3. Macroeconomic Effects of Alcohol Fuels Industry

Energy Demand and Conservation Area:

1. General Energy Demand and Conservation Studies
2. Industrial Energy Conservation Studies
3. Transportation Energy Conservation Studies

Rural Electrification Area:

1. Rural Electrification and Socioeconomic Development

Analytic Studies

Analytic studies are in-house reviews and analyses of existing data. They may be designed to explore the possibility of a more detailed investigation—that is, they may constitute a "mini-study." Alternately, they may provide contributions to a full-fledged ARDEN study.

Biomass Area:

1. Oil Imports, Foreign Exchange and the Brazilian Alcohol Program: Some Preliminary Observations by Shafiqul Islam
2. Sugar or Alcohol? A Billion Dollar Brazilian Question by Shafiqul Islam

Energy Demand and Conservation Area:

1. Factors Affecting the Composition of Energy Supplies in Developing Countries by Joy Dunkerley and Gunnar Knapp with Sandra Glatt
2. Industrial Energy Conservation in Developing Countries by John E. Jankowski, Jr.

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Methodological Studies

Methodological studies are developments of methodologies or other theoretical formulations that will be used in ARDEN subproject investigations.

Biomass Area:

1. Charcoal Industry Location and Transport Study by Julia C. Allen
2. Modeling the Environmental Effects of Forest Management in a Watershed: A Multiobjective Approach to Forest Management for Developing Countries by Julia C. Allen
3. A Simple Analytic Model for Agricultural Price Impacts Due to Alcohol Fuels Programs by Donald Hertzmark, William Ramsay and Stephen Polasky
4. Social Forestry in Developing Countries by Julia C. Allen and Douglas F. Barnes

Rural Electrification Area:

1. Specification of Rural Electrification Analysis by Douglas F. Barnes

Case Studies

Case studies are investigations carried out in specific developing areas by consultants or developing area institutions. They will normally contribute heavily to the final reports on ARDEN studies.

Energy Demand and Conservation Area:

1. The Brazilian Transport Sector: Historical Background and Assessment of Conservation Possibilities by Alan Poole
2. Energy Conservation in Kenya by Lee Schipper et al.
3. Inter-fuel Substitution in the Indian Economy by Ashok V. Desai

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Candidate Project Papers

Candidate project papers are brief--or sometimes longer--reviews of a potential study area with suggestions for a possible future ARDEN subproject. They are intended primarily as briefing material for the ARDEN Advisory Committee.

1. Direct Biomass Combustion Potentials for Industrial and Electric Generation Applications in LDCs by Scott Noll
2. LDC Coal Production, Importation, and Use--Issues and Strategies by Scott Noll

Summary of Existing LDC Subprojects
and Principal Consultants Funded Under ARDEN

The attached charts detail existing ARDEN subcontracts in rural electrification, energy demand and conservation, and alcohol fuels.

(Proposed forestry studies are discussed below.)

RURAL ELECTRIFICATION

February 1981

COUNTRY	INSTITUTION	CONTACT/PRINCIPAL INVESTIGATOR	AMOUNT	DATES	STATUS				REMARKS
					Exploratory	Proposal Received	Contract Signed	Government Clearance	
Columbia	Instituto SER de Invest. Igerión. (SER)	Eduardo Velez	\$40,000	Jan. 1, 1981- Sept. 1981	X			N.A.	Socio-economic impact survey of 90 "villages"; economic supply studies.
India	Administrative Staff College of India (ASCI)	R. K. Pachauri	\$16,500	Sept. 1, 1980- March 1981	X	X	X	X	Evaluation of benefit-cost ratio of rural electrification in financial and social terms.
	Operations Research Group (ORG)	D.B. Samanta	\$27,000	Sept. 1, 1980- May 1981	X	X	X	X	Socio-economic impact survey of 96 villages.
	Harvard Institute for International Development (HIID)	Lalit Sen (Consultant)	\$ 4,400	Jan. 21, 1980- July 1981	X	X	X	N.A.	Long-term consultant for India. Rural electrification project.
	HIID	Lalit Sen	\$15,000	Jan. 1, 1981- July 1981	X	X	X	N.A.	Will analyze household survey from 24 villages in the larger ARDH project.
Malaysia	HIID	Clem Jenkins	\$ 5,000	May 5, 1980- Nov. 1980	X	X	X	X	Grant in part of a larger study to evaluate the microeconomics of rural electrification in Malaysia.

N.A. = not applicable

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COUNTRY	INSTITUTION	CONTACT/PRINCIPAL INVESTIGATOR	AMOUNT	DATES	STATUS				REMARKS
					Exploratory	Proposal Received	Contract Signed	Government Clearance	
Brazil	(Consultant)	Alan Poole	\$ 9,000	June 1, 1980- Aug. 1981	X	X	X	N.A.	Conceptual possibilities in Brazilian transport sector.
Colombia	Centro de Estudios Sobre Democracia Economica (CEDE)	José Eddy Torres	\$19,714	Sept. 1, 1980- Aug. 1981	X	X	X	X	Rural industry and energy production.
Canada	Institute National de l'Énergie (INÉ)	Eduardo Norán	\$ 7,000	Sept. 1, 1980- Feb. 1981	X	X	X	X	Summary of energy use and related output of 800 industrial enterprises.
Kenya	Lawrence Berkeley Laboratory	Leo Schipper	\$73,700	July 1, 1980- June 30, 1981	X	X	X	X	Study of industrial energy demand and conservation in Kenya.

N.A. - not applicable

February 1981

ATTACH

COUNTRY	INSTITUTION	CONTACT/PRINCIPAL INVESTIGATOR	AMOUNT	DATES	STATUS			REMARKS	
					Expenditures	Proposal Received	Contract Signed		Governmental Clearance
Alcohol	(Consultant)	Donald Hertzmark	\$ 6,000	Jan. 1980- Aug. 1981	X	X	X	N.A.	Agriculture sector modeling.
Brazil	(Consultant)	Alan Foote	\$ 4,700	June, 1980- Aug. 1981	X	X	X	N.A.	Agricultural inputs.
	(Consultant)	Pierre-Jacques Ehrlich	\$ 1,600	Oct. 15, 1980- Dec. 15, 1980	X	X	X	N.A.	Balance of payments aspects.
Costa Rica	Instituto de Investigaciones en Ciencias Economicas (IICE), Universidad de Costa Rica	Juan Manuel Villanueva	\$21,500	Jan. 1, 1981- June 30, 1981	X	X	X	N.A.	Collection of data and analysis on the effects of an alcohol fuel program on balance of payments in Costa Rica.
India	(Consultant)	Ramesh Rautia	\$ 5,000	Jan. 29, 1981- Aug. 31, 1981					Fuel alcohol from agro-products in India.

N.A. - not applicable

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Plans for June-October 198

Supplementary support is requested in this proposal for the remainder of FY 81 and for October 1981. The original funding, from the FY 79-FY 80 budget, was projected in the RFF proposal to last eighteen months, from September 1, 1979 through February 28, 1980. Current projections indicate that these funds can actually cover twenty-one months, so that support for approximately 6100 man-hours more for the four months remaining in FY 81 and for the month of October 1981, is requested (see "Costs" below).

The activities planned for the remainder of FY 81 and for October 1981, to be supported by these supplementary funds, are summarized below for the various ARDEN study areas. The budgets for each subproject are given in more detail in the "Costs" section, showing plans for spending existing funds and for requested supplemental funds.

Rural Electrification

An analysis of the baseline data from the 1966 survey is proceeding, and will form a large part of in-house work for the remainder of the fiscal year. In addition, results from the studies in India and preliminary results from Colombia are expected to arrive during this period and will require extensive in-house analysis. A continuous level of effort involving ten percent of the time of a Senior Fellow and eighty percent of the time of a Fellow is anticipated through October 31, 1981.

In addition to in-house expenses, \$10,000 is requested to add the state of West Bengal to the existing Indian analysis. This addition should help to satisfy some of the concerns of members of the Advisory Committee as to data adequacy and is therefore strongly recommended.

A preliminary report on the rural electrification problem, "A Preliminary Analysis of the Effects of Rural Electrification in India" by Douglas Barnes, William Ramsay, and others, will be finished in September 1981.

Energy Demand

The industrial energy conservation effort will be continued during this period. The results of the Kenya and Ecuador studies will be combined, together with the existing in-house analytic study, to produce a document in September 1981: "Preliminary Analysis of Industrial Energy Use Patterns and Conservation Possibilities in Developing Areas", by Joy Dunkerley, John Jankowski and others.

As far as additional grants during FY 81 are concerned, a proposal from the Administrative Staff College of India, currently before the Indian government for approval, would add an important new dimension to our industrial conservation work. It would examine trade-offs between capital, labor and energy factors in the Indian context. This case study, with the provisional title "Conservation Possibilities in Manufacturing Industries in India", by R. K. Pachauri and others, could be completed in FY 81 if started promptly, producing a draft report by the end of the fiscal year. Therefore, \$12,000 for this study is included in the cost requests below.

In addition, through no technical fault of the subcontractor, the report on the Kenya investigation has been delayed and some overruns have occurred. Due to the groundbreaking nature of this study of the details of industrial and commercial energy structure in a developing country, we are requesting new FY funds of \$5,500 to cover this overrun.

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Additional work has begun in exploring the consequences and possibilities for energy conservation in the transport sector. Analyses of potential changes in the auto fleet, observed modal switching, (e.g., auto to bus), etc. are being made. This work, carried out through consultants, will also be followed up through October 1981. A case study will be finished on this problem by July 1981: "Freight and Urban Passenger Transport in Brazil: Development and Conservation Possibilities" by Alan Poole. In addition, an in-house analytic study will be completed by August 1981: "Energy Use and Conservation in Transportation in Developing Countries", by Scott Noll.

An estimated level of effort of about 40 percent of the time of a Senior Fellow and about 100 percent of the time of a Fellow (equivalent), plus necessary research assistant time, will be devoted to this area through October 1981.

In-house work on the connection between energy and development will also be undertaken. Efforts will be made to connect type of export and domestic-goods strategies with type and quantity of energy use. A report on this work, "Energy Implications of Development Strategies", by John E. Jankowski, Jr. would be available in August 1981.

Biomass

In-house work will continue in three areas: alcohol, community forestry and plantation forestry.

For the alcohol work, we anticipate shortly receiving some data on macroeconomic effects of alcohol programs from our subproject in

Resources for the Future

1755 Massachusetts Avenue, N.W.

Washington, D.C. 20036

October 5, 1981

Ms. Pamela L. Baldwin
AID Office of Energy
Room 306 SA-18
Washington, D.C. 20523

Dear Pamela:

Enclosed is a list of the ARDEN documents and projected dates of delivery of the final reports to RFF. We have made tentative plans to print most of the final reports as part of the RFF/CEPR Discussion Paper Series or submit them to the RFF Publications Committee for consideration as RFF Research Papers. (These are indicated by asterisks on the list.) We reserve the right to revise these plans--analysis on some projects is not yet complete and financial restrictions may necessitate a reduction in the number of papers we can have printed.

Papers marked with a checkmark (✓) on the enclosed document list are available for examination by the AID Research Advisory Committee members at your meeting in November. A package including each of these documents is being sent to you under separate cover (a list of the titles is enclosed here). We have included all ARDEN materials except those which are not completed or those on which we have significant unresolved technical questions. Also included in this package are background papers on the rural electrification study (including the original design document, minutes of RE conference meetings, working drafts from the Operations Research Group, questionnaires, preliminary data reports and tables, etc.). The papers concerning rural electrification are preliminary working drafts and are labelled as such. They are not yet available for reproduction or citation without permission from RFF.

As noted, the list of ARDEN documents includes research studies and methodological studies. Research studies include in-house reviews and analyses of existing data and investigations carried out in specific developing areas by consultants or developing area institutions. Methodological studies are papers addressing questions of project design and analysis in ARDEN subproject investigations.

Also enclosed are copies of the following documents for your files which you have not previously received:

1. Ramesh Bhatia, "Fuel Alcohol From Agro-Products in India: A Study of Crop Substitutions, Food Prices and Employment," July 1981;
2. Ashok V. Desai, "Interfuel Substitution in the India Economy," July 1981;
3. RFF, "Comments on Operations Research Group (ORG) Chapters and Household and Village Tables," September 15, 1981;

Pamela L. Baldwin
October 5, 1981
Page Two

4. Operations Research Group (ORG), "Rural Electrification and Socio-Economic Development: Consequences and Determinants--Chapterisation Plan," September 8, 1981;
5. ORG, "Impact of RE on Rural Industrialisation," September 1981;
6. RFF, "Comments on ORG's 'Impact of RE on Rural Industrialisation,'" September 30, 1981;
7. Administrative Staff College of India (ASCI), "A Study of Conservation Possibilities in Manufacturing Industries in India: Progress Report - July 20, 1981"

Fifty copies of the Desai Discussion Paper, "Interfuel Substitution in the Indian Economy" will be mailed to you this week for distribution to AID missions.

Sincerely yours,



Marilyn M. Voigt
Project Assistant

Enclosures

Costa Rica. This information will supplement earlier in-house work and reports from consultants in Brazil on balance of payments aspects. When the results of agricultural sector calculations are received later in the fiscal year, these will also be subjected to in-house analysis.

The community forestry work will deal with information supplied, we propose, by two new projects, one in Tanzania for \$26,000, now before AID for approval, and another one in Upper Volta for about \$20,000. (The Upper Volta project will replace a project proposed earlier for Korea.)

On the basis on these projects, we believe that an interim report on how and why community forestry projects do or do not work can be prepared during early FY 82. We therefore suggest an allotment of \$46,000 from new FY 81 funds for these projects.

In the field of plantation forestry, work will be mainly restricted to in-house efforts plus possibly some small consultant contracts. However, some beginning will be made on establishing a documented methodology for treating the critical environmental question of the effects of intensive harvesting of forestry plantations for wood fuels.

The level of effort in this area will be about 40 percent of the time of a Senior Fellow, about 80 percent of the time of a Fellow (equivalent) for the remainder of the year, plus extensive research assistant effort.

The FY 81 products of this work should be a draft final report, "Macroeconomic Consequences of Alcohol Fuels" by W. Ramsay, Scott Noll, and others, on the alcohol problem, a draft of key sections of a study on community forestry, and a preliminary study, "Preliminary Analysis of Environmental Impacts of Energy Forestry Management" by J. C. Allen, and others, on the environmental aspects of the plantation forestry problem

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Activities after October 31, 1981

While all research activities under this proposal will be completed by October 31, 1981, the need for disseminating results will entail some further activities throughout the rest of FY 82. As outlined above, RFF research results are normally propagated through our Discussion Paper series, through RFF Research Reports, and through books published for RFF by Johns Hopkins press. These documents are all subject to extensive verification and peer review processes, both internally at RFF and by the use of distinguished outside reviewers. Since this process inevitably is somewhat lengthy, we anticipate some level of activity throughout a good part of FY 82 in disseminating the research results, in order to realize as fully as possible the goals of the Cooperative Agreement.

Project Structure

The general nature of the studies to be carried out will, as in the existing cooperative agreement, be determined cooperatively by RFF and AID. RFF will have the responsibility for drafting the design of individual study areas. An existing Advisory Committee includes, in addition to AID and RFF representation, representatives of foundations interested in international energy issues, representatives of international organizations, and scholars from the academic world. This committee will be consulted at semi-annual meetings on project selection and design. Preliminary in-house studies will be prepared analyzing and reviewing key policy areas, as a guide to final study design. RFF will continue to establish collaborative relationships with institutions and individuals in developing areas to carry out studies or parts of studies in tandem with the RFF staff. RFF will submit proposals for such subgrants or subcontracts to the AID Office of Energy for its approval before final implementation.

RFF Capabilities

RFF has done substantial innovative research and analysis in both conventional and non-conventional energy. Its Center for Energy Policy Research (CEPR), directed by Milton Russell, examines all aspects of energy policy, as spelled out in more detail in the attached RFF annual report for FY 80. The Center can also call for support on the other divisions of RFF: Quality of the Environment and Renewable Resources.

RFF has been a pioneer in every aspect of energy research, ranging from initial efforts during the 1950's to the recent Energy in America's Future. It has also done extensive research on developing country energy use, work which has been supported by the World Bank, State Department and Ford and Rockefeller Foundations. In addition to AID. Some especially relevant publications are:

Books:

Cecelski, E., J. Dunkerley and W. Ramsay, Household Energy and the Poor in the Developing World, Research Paper R-15 (Washington, D.C., Resources for the Future, 1979).

Dunkerley, J., W. Ramsay, L. Gordon and E. Cecelski, Energy Strategies for Developing Nations, (Baltimore: Johns Hopkins University Press for Resources for the Future), April 1981.

Ramsay, W., Unpaid Costs of Electrical Energy: Health and Environmental Impacts from Coal and Nuclear Power (Baltimore, Johns Hopkins University Press for Resources for the Future, Inc., 1979).

Schurr, S., J. Darmstadter, H. Perry, W. Ramsay, and M. Russell, Energy in America's Future: The Choices Before Us (Baltimore, MD, Johns Hopkins University Press for Resources for the Future) August 1979.

Dunkerley, J., Trends in Energy Use in Industrial Societies: An Overview (Resources for the Future, Washington, D.C., 1980).

Darmstadter, J., J. Dunkerley and J. Alterman, How Industrial Societies Use Energy: A Comparative Analysis, published for Resources for the Future by the Johns Hopkins University Press, 1977.

Ramsay, W., Alcohol Fuels and Developing Countries, Research Paper X (Resources for the Future, Washington, D.C., submitted 1981).

Articles and Chapters:

Cecelski, E. and W. Ramsay, "Prospects for Fuel Alcohols from Biomass in Developing Countries", paper presented at the United Nations Conference on Long Term Energy Resources, Montreal, 26 November - 8 December 1979.

Ramsay, W., "The Alcohol Fuels Option in the Third World", Energy Journal, vol. 2, no. 1 (1981) pp 99-103.

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Dunkerley, J. and Andrew Steinfeld, "Adjustment to Higher Oil Prices in Oil Importing Developing Countries", Journal of Energy and Development, Spring 1980.

Cecelski, E. and J. Dunkerley, "Energy Use in the Rural and Urban Household Sectors of Developing Countries" in Proceedings of an International Workshop on Energy Survey Methodologies for Developing Countries, National Academy of Sciences, 1980.

Dunkerley, J., "International Energy Data and Information," in Energy Information: Description, Diagnosis, and Design edited by William W. Hogan.

Darmstadter, J., J. Dunkerley and J. Alterman, "International Variations in Energy Use," in Annual Review of Energy, vol. 3, 1978.

Dunkerley, J., "Estimation of Energy Demand and Conservation in the Developing Countries" Paper to be published in forthcoming Proceedings of IAEE Conference on International Energy Issues--the Next Ten Years.

Dunkerley, J., "Energy Conservation: potential and policies" published in Proceedings of Second Workshop of Western Hemisphere Energy, pp 125-132, sponsored by the Banco de Bogota and Universidad de los Andes in co-operation with the MITRE Corporation September 1979.

Dunkerley, J. and J. E. Jankowski, Jr., "The Real Price of Imported Oil", The Energy Journal, July 1980, pp 113-118.

Jankowski, J. E., Jr., "Future Energy Requirements for Implementing Basic Human Needs", University of Oklahoma's Review of Regional Economics and Business, October 1980, pp 28-33.

Noll, S., "A Minimum Benefits Analysis of Residential Passive Solar Sunspace Designs", technical paper, 5th National Passive Solar Energy Conference Proceedings, Amherst, MA, October 1980.

Noll, S., "Economic Analysis", Chapter H of the DOE Passive Solar Design Handbook, Volume 2: Passive Solar Design Analysis, Department of Energy Report DOE/CS-0127/2, January 1980.

Noll, S., J. F. Roach and L. Palmiter, "Energy Planning with Solar and Conservation: Individual Values and Community Choice", Proceedings, ISES Silver Jubilee Congress, Atlanta, Georgia, June 1979.

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This expertise has of course been greatly expanded in the area of LDC energy and policy analysis by the existing AID ARDEN Cooperative Agreement. ARDEN activities and reports are detailed elsewhere in this proposal. Also, as a result of this Cooperative Agreement, important working relationships have been established between RFF and institutions in developing areas. The institutions specifically involved in current RFF studies are indicated elsewhere in this proposal. In addition to such LDC institutions, RFF has established relationships with important international bodies in the field of energy, such as the Latin American regional energy institution OLADE, the Science Policy Research Institute at the University of Sussex (U.K.), the International Institute for Applied Systems Analysis (Laxenburg, Austria), the International Energy Agency (Paris), the Food and Agricultural Organization (Rome), among others.

Project Staff

The principal investigators on the project will continue to be Joy Dunkerley and William Ramsay. Their resumes are appended. Other RFF senior staff members may be utilized if and when appropriate.

Dr. Ramsay will be the RFF project officer and AID point of contact for the Cooperative Agreement.

Supplemental FY 81 Costs and Levels of Effort

The estimated project costs for the 5 months under FY 81 funding (June-October 1981) are given in detail in table 1 below. These requests include an additional level of effort of approximately 3.9 man-years. In addition, they call for a supplementary requests of \$73,500 for additional subgrants.

The costs are also broken down by project sub-area: Rural Electrification, Energy Demand and Conservation, Biomass, and General Support.

Details of some of the cost estimates are given in footnotes.

Table 2a gives an account of existing funds expended by subproject area through December 31, 1980, the last period for which full accounting is available. Estimated monthly in-house costs during the second and third quarters of FY 81 are also shown. Finally, a reconciliation of total funds spent and those still available are compared to outstanding subgrant obligations, giving the total presently available to defray costs in FY 81. According to these projections, FY 79-80 funding will last through about May 1981.

Table 2b projects the usage of supplemental FY 81 funds requested.

Table 3 recaps ARDEN expenditures so far and projected total costs for FY 79 through October 31, 1981.

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Table 1. Supplemental FY 81 Budget, By Subproject Area

Staff Member ^a and Other Expense Categories	FY 81 Rate per Person Month	Rural Electrification			Energy Demand and Conservation			Biomass			General Support			ARDEN Totals ^b		
		Z time	Person ^c Months	\$	Z time	Person ^c Months	\$	Z time	Person ^c Months	\$	Z time	Person ^c Months	\$	Z time	Person ^c Months	\$
Senior Fellows:																
William Ramsay	4,865 ^d	10	0.4326	2,105			40	1.7304	8,418	25	1.0815	5,262	75	3.2445	15,785	
Joy Dunkerley	3,641	40	1.7304	6,300						10	0.4326	1,575	50	2.1630	7,875	
Fellows:																
Douglas Barnes	2,136	80	3.4608	7,392			20	0.8652	1,848				100	4.3260	9,240	
Gunnar Knapp	1,942				70	3.0282	5,881	30	1.2978	2,520			100	4.3260	8,401	
Scott Woll	2,913				20	0.8652	2,520	20	0.8652	2,520	10	0.4326	50	2.1630	6,300	
Harry Broadman	2,330				80	1.3734	3,200	20	0.3433	800			100 ^e	1.7167	4,000	
Research (and Administrative) Assistants:																
Marilyn Meding	1,563				50	2.163	3,634				100	4.326	6,762	100	4.326	6,762
John Jankowski	1,680				20	0.8652	1,281	20	0.8652	1,281	20	0.8652	1,281	100	4.326	6,406
Stephen Polasky	1,481	40	1.7304	2,563	10	0.4326	588	10	0.4326	588	3	0.1298	176	33	1.438	1,940
Elizabeth Shue	1,359	10	0.4326	588				85	3.677	5,710				85	3.677	5,710
Julia Allen	1,553				33	1.428	1,836	33	1.428	1,836	50	2.1630	2,782	150 ^f	6.489	8,346
Secretaries: (2)	1,286	34	1.471	1,892												
Total Divisional Labor			9.2578	20,840		10.1556	18,940		11.5047	25,521		9.4307	19,098	40.3482	84,399	
Editorial Labor^g				425			1,275			850					2,550	
Total Divisional & Editorial Labor				21,265		20,215			26,371			19,098			86,949	
Total Benefits (23.1%)				4,912		4,670			6,092			4,412			20,086	
Total Labor plus Benefits				26,177		24,885			32,463			23,510			107,035	
Overhead (60.2%)				15,759		14,981			19,543			14,154			64,437	
Rent (8%)				2,094		1,991			2,597			1,881			8,563	
Computer Services				6,500		500			1,000						8,000	
Outside Consultants												2,000			2,000	
Conferences and Meetings^h												1,500			1,500	
Travelⁱ				2,324		2,386			1,100						5,810	
Publications^g				500		1,500			1,000						3,000	
Contracts^j				10,000		17,500			46,000						73,500	
Miscellaneous				300		300			300			900			1,800	
Total				63,654		64,043			104,003			43,945			275,645	

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Footnotes to Table 1

^a Staff member names are given here to show planned allocations of personnel, but this list does not guarantee exact personnel assignments or (except for Ms. Dunkerley and Dr. Ramsay) that specific staff members will be available for the project.

^b The total dollar amounts are derived by addition from the sub-project amounts, and may differ somewhat from amounts derivable by direct multiplication in the "Totals" column because of rounding errors.

^c Person months are figured on 10.30 billable months per year, i.e., in the 0.42 year supplemental FY 81 period, there are 4.326 billable person months.

^d Billed at the rate prescribed by the AID maximum, equivalent to the Federal Ceiling of \$50,112 p. a.

^e This staff member will not be available until later in FY 81, so his time is "100%" of a smaller total of months.

^f "150%" means 75% of the time of each of two secretaries.

^g Editorial and publications costs are based on 600 pages, and are costed at \$425 editorial labor per 100 pages and \$500 publications costs per 100 pages.

^h Conferences and Meetings expenses are for Advisory Committee meetings, at about \$750 per meeting.

ⁱ Additional travel for FY 81 is anticipated for necessary consultation with ongoing projects. Estimated expenses are as follows:

Travel:

Foreign

2 airfares @ \$1500	3,000
20 days per diem @ 80	1,600
100 miles ground transportation @ 22.5¢	225

Domestic

2 airfares @ 200	400
6 days per diem @ 60	360
100 miles ground transportation @ 22.5¢	225

\$5,810

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^j The list of subcontracts for which funds are asked are as follows:

FY 81

Tanzania--community forestry (A. Temu, M. Skutsch)	\$26,000
Upper Volta--community forestry (R. Winterbottom)	20,000
India--conservation (R. K. Pachauri)	12,000
India--rural electrification supplementary (Samanta)	10,000
Kenya--energy demand supplementary (L. Schipper)	<u>5,500</u>
	73,500

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Table 2a. ARDEN Expenditures, Actual and Estimated, From Existing FY 79-80 Funding

Period	Category	Subproject				Total
		Rural Electrification	Energy Demand and Conservation	Biomass	General Support	
Actual, Sept 1, 1979 - Dec 31, 1980:	Salaries, incl. Benefits	44,834.57	51,010.22	72,442.83	82,951.39	251,239.01
	Overhead, incl. Rent	19,959.94	22,711.50	32,316.02	37,013.15	112,000.61 ^a
	Travel	5,530.10	6,279.69	8,608.55	9,811.22	30,229.56
	Other	2,957.60	3,345.88	4,218.07	4,749.19	15,270.74
	Total In-House	<u>73,282.21</u>	<u>83,347.29</u>	<u>117,585.47</u>	<u>134,524.95</u>	<u>408,739.92</u>
	Subgrants	20,298.83	20,914.30	13,000.00	--	54,213.13
	Total	<u>93,581.04</u>	<u>104,261.59</u>	<u>130,585.47</u>	<u>134,524.95</u>	<u>462,953.05</u>
Projection of in-house costs only, for typical month in second and third quarters of FY 81:	Salaries, incl. Benefits	3,210	4,280	6,420	7,490	21,400
	Overhead, incl. Rent	1,444	1,926	2,889	3,371	9,630 ^b
	Travel	800	900	500	--	2,200
	Other	330	440	660	770	1,200
	Total Monthly In-House Costs	<u>5,784</u>	<u>7,546</u>	<u>10,469</u>	<u>11,631</u>	<u>34,430^c</u>
Reconciliation						
Original Grant	800,000.00					
Spent through						
Dec 31, 1980	<u>462,953.05</u>					
	337,046.95					
Subgrants						
obligated	<u>159,000.00</u>					
Remaining for						
In-house						
costs after	<u>178,046.95</u>					
Dec 31, 1980						

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Table 2b. Projection of FY 81 Expenditures From Supplemental (FY 81) Funds Requested

Category	Subproject				Total
	Rural Electrification	Energy Demand and Conservation	Biomass	General Support	
Salaries, incl. Benefits	26,177	24,885 ^d	32,463 ^d	23,510	107,035 ^d
Overhead, incl. Rent	17,853 ^d	16,972 ^d	22,140 ^d	16,035 ^d	73,000 ^d
Travel	2,324	2,386	1,100	--	5,810
Other	7,300	2,300	2,300	4,400	16,300
Subgrants	10,000	17,500	46,000	--	73,500
Total	<u>63,654</u>	<u>64,043</u>	<u>104,003</u>	<u>43,945</u>	<u>275,645</u>

1047

Table 2a. ARDEN Expenditures, Actual and Estimated, From Existing FY 79-80 Funding

Period	Category	Subproject				Total
		Rural Electrification	Energy Demand and Conservation	Biomass	General Support	
Actual, Sept 1, 1979 - Dec 31, 1980:	Salaries, incl. Benefits	44,834.57	51,010.22	72,442.83	82,951.39	251,239.01
	Overhead, incl. Rent	19,959.94	22,711.50	32,316.02	37,013.15	112,000.61 ^a
	Travel	5,530.10	6,279.69	8,608.55	9,811.22	30,229.56
	Other	2,957.60	3,345.88	4,218.07	4,749.19	15,270.74
	Total In-House	73,282.21	83,347.29	117,585.47	134,524.95	408,739.92
	Subgrants	20,298.83	20,914.30	13,000.00	--	54,213.13
	Total	<u>93,581.04</u>	<u>104,261.59</u>	<u>130,585.47</u>	<u>134,524.95</u>	<u>462,953.05</u>
Projection of <u>in-house</u> costs <u>only</u> , for typical month in second and third quarters of FY 81:	Salaries, incl. Benefits	3,210	4,280	6,420	7,490	21,400
	Overhead, incl. Rent	1,444	1,926	2,889	3,371	9,630 ^b
	Travel	800	900	500	--	2,200
	Other	330	440	660	770	1,200
	Total Monthly In-House Costs	<u>5,784</u>	<u>7,546</u>	<u>10,469</u>	<u>11,631</u>	<u>34,430</u>

Reconciliation

Original Grant	800,000.00
Spent through	
Dec 31, 1980	<u>462,953.05</u>
	337,046.95
Subgrants obligated	<u>159,000.00</u>
Remaining for in-house costs after	<u>178,046.95</u>
Dec 31, 1980	

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Table 2b. Projection of FY 81 Expenditures From Supplemental (FY 81) Funds Requested

Category	Subproject				Total
	Rural Electrification	Energy Demand and Conservation	Biomass	General Support	
Salaries, incl. Benefits	26,177	24,885 ^d	32,463 ^d	23,510 ^d	107,035 ^d
Overhead, incl. Rent	17,853 ^d	16,972 ^d	22,140 ^d	16,035 ^d	73,000 ^d
Travel	2,324	2,386	1,100	--	5,810
Other	7,300	2,300	2,300	4,400	16,300
Subgrants	<u>10,000</u>	<u>17,500</u>	<u>46,000</u>	--	<u>73,500</u>
Total	<u>63,654</u>	<u>64,043</u>	<u>104,003</u>	<u>43,945</u>	<u>275,645</u>

10/2/81

Footnotes to Table 2a and 2b

^aOverhead shown does not include the new RFF overhead rate for FY 81. For Oct 1 - Dec 31, 1980, additional overhead over that bid in the original ARDEN proposal was absorbed from RFF general funds. For the ARDEN project for Oct - Dec 1980, this additional amount totaled \$13,787.21.

^bAt the old overhead rate. The new rate would be \$4,960 more, making total typical monthly costs \$39,360.

^cAt this rate, existing funds will support in-house efforts through about May 31, 1981.

^dIncluding the new RFF overhead rate (see footnote "a").

Revised 5/15/79

AGENCY FOR INTERNATIONAL DEVELOPMENT

COOPERATIVE AGREEMENT

PROJECT TITLE: Energy Assessment & Policy Development

NO. AID/DSAN-CA-0179

PROJECT NO. 936-5703

AWARDED PURSUANT TO SEC. 297 and 635 OF THE FOREIGN ASSISTANCE ACT OF 1961, AS AMENDED

ISSUING OFFICE

Agency for International Development
Office of Contract Management
Washington, D.C. 20523

Cognizant AID Scientific/Technical Office

Office of Energy
DS/EY

RECIPIENT

Resources for the Future

Name

1755 Massachusetts Avenue, N.W.

Street Address

Washington, DC 20036

City

State

Zip Code

MAIL VOUCHERS (original and 3 copies) TO

EFFECTIVE DATE: September 1, 1979

EXPIRATION DATE: August 31, 1981

Agency for International Development
Office of Financial Management, FM/PAD
Washington, D.C. 20523

ACCOUNTING AND APPROPRIATION DATA

Amount Obligated: \$800,000

Appropriation No: 72-1191021.6

Allotment No: 946-36-099-00-20-91

PID/T No: 3698838

The United States of America, hereinafter called the Government, represented by the AID Grant Officer executing this Agreement, and the Recipient agree as follows: That the entire Agreement consists of: The Cover Page; the Schedule, consisting of 8 pages; Attachment "A", Program Description, and Attachment "B", Standard Provisions.

RECIPIENT Resources for the Future	UNITED STATES OF AMERICA AGENCY FOR INTERNATIONAL DEVELOPMENT
BY: (Signature of Authorized Individual) <i>[Signature]</i>	BY: (Signature of AID Grant Officer) <i>[Signature]</i>
TYPED OR PRINTED NAME <u>PRESIDENT TAMELY N. CISTLE</u>	TYPED OR PRINTED NAME Morton Darvin
TITLE <u>PRESIDENT</u>	TITLE AID Grant Officer
DATE <u>SEPT. 7 1979</u>	DATE <u>27 AUG 1979</u>

SEPTEMBER 1978 EDITION

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Table 3. ARDEN Recap: Total Subproject Costs Projected
(in \$ thousand, rounded)

Period	Type of Cost	Rural Electrification	Energy Demand and Conservation	Biomass	General Support	Total
FY 79-80, Actual as of September 30, 1980 (see table 2)	In-house	64.3	72.5	84.5	93.9	315.2
	Subcontracts	<u>16.3</u>	<u>18.3</u>	<u>10.5</u>	--	<u>45.1</u>
	Total	80.6	90.8	95.0	93.9	<u>360.3</u>
All Costs, Projected from Sept 1, 1979 - Oct 31, 1981 (see table 2)	In-house	159.2	174.1	225.1	234.2	792.6
	Subcontracts	<u>110.3</u>	<u>77.8</u>	<u>89.5</u>	--	<u>277.6</u>
	Total	269.5	251.9	314.6	234.2	<u>1,070.2</u>

Program Description

A. Purpose of Cooperative Agreement

The purpose of this Cooperative Agreement is to assist Resources for the Future (RFF) in developing and managing an energy studies project. This Cooperative Agreement will enable RFF to increase its capability to analyze developing country energy issues and the relationship between energy use and development. RFF, by developing and managing a multidisciplinary study of energy use and development, will contribute significantly to the design of well-conceived energy assistance programs of donor organizations; to the preparation of national and sectoral energy plans in developing countries; and to the exchange of information, analyses and research designs among academic and government institutions in developed and developing countries.

B. Specific Objectives

In developing and managing an energy studies project, the recipient will focus on the following objectives:

1. To provide data and analyses of specific energy concerns thus contributing to a greater understanding of energy and development issues in general.
2. To improve the quality and variety of background material on LDC energy issues available to energy decision-makers and project managers.
3. To increase understanding of interaction between rural and urban energy supply and demand scenarios.
4. To encourage interaction and information exchange among LDC energy analysts and their counterparts in donor countries.
5. To refine, strengthen, and broaden the expertise and capabilities of the recipient to participate in and contribute toward the resolution of energy problems of LDC's.

C. Specific Activities

To accomplish the above specific objectives RFF will develop, conduct and manage general studies relating to energy use and development. It is anticipated that up to eight general studies will be undertaken in the two years although each study may be composed of one or more mini-studies. The types of studies which will be done include, but are not limited to, the following:

- (a) Energy conservation in the LDC context.
- (b) Energy pricing policies (particularly kerosene and charcoal).
- (c) Supply strategies for traditional fuels
- (d) Charcoal production and utilization.
- (e) Implications of alternative development strategies for energy form and scale.
- (f) Investment needs for energy production.
- (g) Rural electrification and its role in development (including systems analysis as well as generating source).
- (h) Urban household and transportation energy alternatives.
- (i) Non-technical and non-economic aspects of energy systems (cultural, income distribution and institutional issues.
- (j) Implications of oil price increases for development strategies.

The conduct of these studies will require the close cooperation and involvement of LDC institutions. The recipient will issue grants for studies or parts of studies to these institutions, while maintaining overall managerial and supervisory responsibility. The effort will be divided into management and study tasks. At least fifty percent of the study effort will be provided by LDC institutions or independent investigators working in cooperation with such institutions.

D. AID-RFF Cooperation

The Office of Energy (DS/EY) in AID and the recipient will cooperate and collaborate closely in the developing and implementation of this project. The project manager in the appropriate technical office (DS/EY) will work closely with the project officer and other principal investigators in RFF and serve as the channel for AID guidance in this project. The AID project officer will be consulted on issues such as: selection of senior professional staff to participate in the project; selection of cooperating institutions in developing countries and elsewhere; selection of LDC energy issues to be studied. He will participate as AID's representative at RFF-sponsored conferences on the implementation of this project and be the principal liaison between AID and RFF.

E. Criteria for Approval of Projects

The recipient will create an Advisory Committee which will meet at least twice a year to review the work program and advise on future activities. The institution will follow its established outside peer review procedures before publishing studies.

The selection of the Advisory Committee will be the responsibility of the RFF Project Director who will consult with the AID Technical Office. The following general categories should be represented on the Committee (the exact number to be determined by the AID and RFF project officers):

- (a) AID (DS/EY and the Regional Bureaus)
- (b) Sociologist/social scientist
- (c) Development Economist
- (d) Physical Scientist
- (e) LDC Representative
- (f) Representative of foundations working on energy.
- (g) Representative of RFF Management Governing Board.

The Advisory Committee will be constituted and will meet shortly after the cooperative agreement is signed. At the initial meeting, the RFF project officer will present a proposed work plan for project years 1 and 2, including alternatives. The Advisory Committee will provide guidance on the priorities for various types of studies and establish the general work program.

The RFF project officer will then prepare detailed implementation plans for each study within the framework of the general work program, taking into account available resources and capabilities. These will be submitted to the AID Technical Office for approval under the criteria set forth in Paragraph G.

F. Project Subgrants

The recipient will make project subgrants to institutions and/or individuals in developing countries. These subgrants can fund individual studies or components of larger studies as approved by AID and the Advisory Council. Prior to making

any project subgrant over \$5000 the recipient shall submit the following information to the Project Officer, Office of Energy, for approval: (Approval will be based on criteria set forth in Paragraph G below.)

- (a) Subproject title.
- (b) Subproject description.
- (c) Duration of sub-project for which funding is proposed.
- (d) Name and address of the proposed sub-grantee and the location(s) where activities will take place.
- (e) Estimated cost of proposed subproject.
- (f) Qualifications of the subgrantee relevant to proposed subproject.
- (g) Resources which recipient proposes to contribute and inputs others will make to subproject, including dollar value.
- (h) Plan for carrying out proposed subproject.
- (i) Results expected from proposed subproject.
- (j) Data to be reported by subgrantee.

G. Criteria for AID Approval of Project Proposals

Upon completion of general work plan as reviewed by the Advisory Council, the RFF project officer will submit detailed proposals and implementation plans to the AID project officer for approval. The AID project officer, after consultation with AID technical and regional offices and USAID field missions, as appropriate, will provide final approval of those proposals in writing to RFF. Criteria for AID's approval of proposed projects are:

- (a) Consistency with AID's energy strategy.
- (b) Appropriate LDC government and AID regional and mission clearances.
- (c) Compatibility with general work plan.
- (d) Capability to undertake proposed activities as documented under Paragraph F. above.

H. AID Approvals

(1) Subgrant Approval

a. In accordance with Standard Provision (SP) No. 15 entitled "Subordinate Agreements," whereunder the recipient may not place a subordinate agreement without the prior written approval of the grant officer, the grant officer does, hereby, provide said approval for all subgrants to be placed with LDC Governments, LDC Institutions and LDC Individuals provided, however, that approval is obtained in writing from the Cognizant Technical Office of AID prior to placement of the subgrant.

b. One (1) copy of all executed LDC subgrants will be furnished to the grant officer.

c. Approval of a subgrant by the Cognizant Technical Office of AID does not relieve RFF from its responsibilities to comply with all other provisions of this grant.

(2) Consultants and Professional Personnel

Curriculum vitae of all consultants funded under this grant will be forwarded to the Cognizant Technical Office of AID for approval.

(3) International Travel

International Travel will be approved by AID in accordance with Standard Provision No. "6" - Travel and Transportation.

I. Staffing

(1) To carry out the objectives of this agreement, the recipient will assemble a staff to manage the energy studies program.

(2) The recruitment and selection of staff to be employed by RFF will be the recipient's responsibility and will be conducted in accordance with the recipient's regular personnel policies

J. Evaluation

AID and RFF will undertake an interim evaluation of the project six (6) months after the Cooperative Agreement is signed to obtain an early assessment of progress and to make any adjustments or accommodations that appear necessary. An intensive evaluation will be conducted by AID and RFF within 18 months after the Cooperative Agreement is signed which will:

(a) Be used as a basis for surveying the management activities and the progress of the technical and advisory services in achieving the stated objectives.

(b) Help AID determine whether the project should be extended, amended or terminated. FY 81-83 activities and funding will be determined after AID evaluation at end of second project year.

K. Reporting Requirements

RFF will prepare and submit to AID an interim program activity report for the first half of each performance year and a comprehensive annual program activity report on each anniversary date of the cooperative agreement which will summarize program and financial activities under the agreement for all previous program years, detail current program year information and propose activities for the next program year. Five copies of each report will be sent to the AID project manager and one copy to the grant officer. The annual reports should cover:

A concise but comprehensive assessment of the extent to which the purposes of the cooperation agreement are being achieved, including:

- a. How subgrants or activities undertaken in the major areas helped achieve the purposes of the cooperative agreement.
- b. Description of the subgrants completed.
- c. General problems encountered, requiring action.

L. Budget

The funds herein shall be used to finance the following items:

<u>Cost Element</u>	<u>Total Obligated Amount</u> <u>FR: 9/1/79 TO: 8/31/81</u>
1. Salaries including Fringe Benefits	\$318,969
2. Overhead	143,536
3. Travel	44,400
4. Other Direct Costs	23,095
5. Subgrants	270,000
TOTAL	<u>\$800,000</u>

The recipient may not exceed the total amount of the Budget. Adjustments among line items are unrestricted.

M. Standard Provisions

The following Standard Provisions contained in Attachment B do not apply to this Cooperative Agreement:

98. Payment - Periodic Advance

N. Overhead Rates

Pursuant to the provision of the Standard Provision entitled "Negotiated Overhead Rates - Non-Educational," a rate or rates shall be established for each of the Recipient's accounting periods during the term of the Cooperative Agreement. The rate for the initial period shall be as set forth below:

<u>Rate</u>	<u>Base</u>	<u>Period</u>
45.0% (Provisional subject to downward adjustment only)	Direct salaries and Fringe Benefits	FR: 9-1-79 TO: Until amended

Rates for subsequent periods shall be established in accordance with the term of the "Negotiated Overhead Rates - Non-Educational Institutions" clause of the Standard Provisions.

O. Title to Property

Title to all property purchased for use under this Cooperative Agreement vests in the U.S. Government.

P. Key Personnel

1. The key personnel which the Recipient shall furnish for the performance of this CA as follows:

Key Personnel

- | | |
|----------------|--|
| William Ramsay | Project Officer & Principal Investigator |
| Lincoln Gordon | CO-Principal Investigator |
| Joy Dunkerley | Co-Principal Investigator |

STANDARD PROVISIONS

1. DEFINITIONS

(a) "Administrator" means the Administrator or the Deputy Administrator of the Agency for International Development.

(b) "AID" means the Agency for International Development.

(c) "Consultant" means any especially well qualified person who is engaged on a temporary or intermittent basis to advise the Recipient and who is not an officer or employee of the Recipient who performs other duties for the Grantee.

(d) "AID Grant Officer" means the person executing this Agreement on behalf of the United States Government, and any other Government employee who is a properly designated AID Grant Officer; and the term includes, except as otherwise provided in this Agreement, the authorized representative of an AID Grant Officer acting within the limits of his authority.

(e) "Recipient Employee" means a person in the employ of the Recipient assigned to work under this Agreement.

(f) "Cooperating Country or Countries" means the foreign country or countries in or for which assistance is to be provided hereunder.

(g) "Cooperating Government" means the government of the Cooperating Country.

(h) "Economy Class" air travel (also known as jet economy, air coach, tourist class, etc.) means a class of air travel which is less than first class.

(i) "Recipient" means the institution entering into this Agreement with the Government.

(j) "Government" means the United States Government.

(k) "Mission" means the United States Mission to, or principal AID office in, the Cooperating Country.

(l) "Mission Director" means the principal AID officer of the Mission in the Cooperating Country, or his designated representative.

(m) "OMB Circular No. A-110" means the Office of Management and Budget Circular entitled Grants and Agreements With Institutions of Higher Education, Hospitals and Other Nonprofit Organizations: Uniform Administrative Requirements (41 CFR 32016; July 30, 1976), including any amendments thereto.

(n) "41 CFR 1-15.2" means the cost principles set out in Subpart 1-15.2 of the Federal Procurement Regulations.

(o) "Handbook 13" means AID Handbook 13, entitled Grants

(p) "Assistance Instruments" means either grants or cooperative agreements.

(q) "Subrecipient" means any organization other than the Recipient undertaking by subagreement hereunder program or project activities in fulfillment of the purposes or objectives of this Agreement.

(r) "AID Project Officer" means a designated representative of the cognizant AID Scientific/Technical Office named on the Cover Page.

2. ALLOWABLE COSTS AND CONTRIBUTIONS

(a) Allowable costs under this Agreement shall be limited to those costs which are reasonable, allocable, and allowable in accordance with the terms of (1) this Agreement, (2) any negotiated advance understanding on particular cost items, and (3) 41 CFR 1-15.2, in effect on the date of this Agreement.

(b) With regard to the allowability of non-Federal cost sharing contributions under this Agreement, Attachment E, "Cost Sharing and Matching", to OMB Circular No. A-110 (as set out in paragraph 1K of Handbook 13) shall also apply.

3. ACCOUNTING, AUDIT, AND RECORDS

(a) The Recipient shall maintain a financial management system in accordance with the requirements set forth in OMB Circular A-110 (as implemented in Paragraph 1L of Handbook 13). A copy of reports of audits resulting from this system as they concern this Agreement will be furnished to the Grant Officer.

(b) The AID Auditor General and the Comptroller General of the United States or their duly authorized representatives (see paragraph 16, Chapter 1 of Handbook 13) reserve the right to conduct an audit of the Recipient's books and records to determine whether the Recipient has expended AID's funds in accordance with the terms and conditions of this Agreement. The Recipient agrees both to make available any further information requested by AID with respect to any questions arising as a result of the audit and to include the requirements of this provision in any subordinate agreement, including subgrants, entered into with a U.S. institution.

4. NEGOTIATED OVERHEAD RATES - NON-EDUCATIONAL INSTITUTIONS

(This provision is applicable to non-educational institutions which do not have an acceptable indirect cost system).

(a) Pursuant to this provision, an overhead rate(s) shall be established for each of the Grantee's accounting periods during the term of this Grant. Pending establishment of a final rate(s), the parties have agreed that provisional payments on account of allowable costs shall be at the rate(s), on the base(s), and for the period(s), shown in the

Schedule to this Grant.

(b) The Grantee, as soon as possible but not later than ninety (90) days after the close of its accounting periods during the term of this Grant, shall submit to the AID Grant Officer with copies to the Office of Contract Management, AID, Washington, D.C. 20523 and to the Office of the Auditor General, AID, Washington, D.C., proposed final rate(s) for the period, together with supporting cost data. Negotiation of the final overhead rates by the Grantee and the AID Grant Officer shall be undertaken as promptly as practicable after receipt of the Grantee's proposal.

(c) Allowability of costs and acceptability of cost allocation methods shall be determined in accordance with 41 CFR 15.2 as in effect on the date of this Grant.

(d) The results of each negotiation shall be set forth in a written overhead rate agreement executed by both parties. Such Grant shall specify (1) the agreed final rate(s), (2) the base(s) to which the rate(s) applies, and (3) the period(s) for which the rate(s) applies. The overhead rate agreement shall not change any monetary ceilings, Grant obligation, or specific cost allowance or disallowance provided for in this Grant.

(e) Pending establishment of the final overhead rate(s) for any period, the Grantee shall be reimbursed either at a negotiated provisional rate(s) as provided above or at billing rate(s) acceptable to the AID Grant Officer, subject to appropriate adjustment when the final rate(s) for that period is established. To prevent substantial over or under payment, the provisional or billing rate(s) may, at the request of either party, be revised by mutual agreement, either retroactively or prospectively. Any such revision of negotiated provisional rate(s) provided for in this provision shall be set forth in a modification to this Grant.

5. SALARIES

All salaries, wages, fees, and stipends, which will be reimbursable by AID under this Agreement, shall be in accordance with both the Recipient's usual policy and practice and 41 CFR 1-15.2. To the extent that the Recipient's policy and practice conflict with 41 CFR 1-15.2, the latter shall prevail, unless the Schedule of this Agreement expressly provides otherwise.

Salaries and wages paid to Cooperating Country and Third Country Nationals may not, without specific written approval

of the Grant Officer, exceed either the Recipient's established policy and practice; or the level of salaries paid to equivalent personnel by the AID Mission in the Cooperating Country as determined by AID, paid to personnel of equivalent technical competence.

The compensation, leave and holidays, allowances and differentials, travel and transportation, and shipment of effects for Cooperating Country Nationals and Third Country National employees will be subject to AID's policy as set forth in Manual Order 1423.7.

6. TRAVEL AND TRANSPORTATION

(This provision is applicable when air travel or air shipment (both domestic and international) costs are reimbursable by AID under this Agreement.)

(a) Allowable costs of travel under this Agreement shall be in accordance with both the policy and practice of the Recipient and 41 CFR 1-15.2. To the extent the Recipient's policy and practice conflict with 41 CFR 1-15.2, the latter shall prevail unless the Schedule of this Agreement provides otherwise.

(b) The AID Grant Officer hereby approves international travel hereunder provided that the Recipient shall obtain written concurrence from the cognizant AID Project Officer prior to sending any individual outside the United States to perform work under this Agreement. For this purpose the Recipient shall advise the AID Project Officer at least 30 days in advance of any travel to be undertaken outside the United States. After concurrence is received the Recipient shall provide the cognizant Mission or U.S. Embassy advance notification (with a copy to the AID Project Officer) of the arrival date and flight identification of AID financed travellers.

(c) Travel to certain countries shall, at AID's option, be funded from U.S.-owned local currency. When AID intends to exercise this option, it will so notify the Recipient after receipt of advice of intent to travel required above. AID will issue a Government Transportation Request (GTR) which the Recipient may exchange for tickets, or AID will issue the tickets directly. Use of such U.S.-owned currencies will constitute a dollar charge to this Agreement.

(d) All international air travel and all international air shipments under this Agreement shall be made on United States flag carriers. Exceptions to this rule will be allowed in the following situations, provided that the Recipient certifies to the facts in the voucher and other documents

retained as part of the Agreement records required by Standard Provision 3 above:

(1) where the traveler, while enroute, has to wait 6 hours or more to transfer to a U.S. flag air carrier to proceed to the intended destination, or

(2) where a flight by a U.S. flag air carrier is interrupted by a stop anticipated to be 6 hours or more for refueling, reloading, repairs, etc. and no other flight by a U.S. flag air carrier is available during the 6 hour period, or

(3) where by itself or in combination with other U.S. flag or non-U.S. flag air carriers (if U.S. flag air carriers are "unavailable") it takes 12 hours or longer from the original airport to the destination airport to accomplish the Recipient's program than would service by a non-U.S. flag air carrier or carriers, or

(4) when the elapsed travel time on a scheduled flight from origin to destination airports by non-U.S. flag air carrier(s) is 3 hours or less, and services by U.S. flag air carrier(s) would involve twice such travel time.

NOTE: Where U.S. Government funds are used to reimburse Recipient's use of other than U.S. flag air carriers for international transportation, the Recipient will include a certification on vouchers involving such transportation which is essentially as follows:

CERTIFICATION OF UNAVAILABILITY OF U.S. FLAG AIR CARRIERS
I hereby certify that the transportation service for personnel (and their personal effects) or property by U.S. flag air carriers was unavailable for the following reason(s): (State appropriate reason(s) as set forth above; see 41 CFR 1-1.323-3 for further guidance).

(e) Travel allowances shall be reimbursed in accordance with 41 CFR 1-15.2 and the regulations contained in AID Handbook 22 entitled "Travel and Transportation"; however, if the Recipient's domestic and international travel allowance policies and procedures have been reviewed and approved by AID or another Federal department or agency pursuant to the applicable Federal cost principles, the Recipient may use its travel allowance system in lieu of that in Handbook 22 after it has furnished the AID Grant Officer with a copy of such approval. For travel outside the conterminous United States, reimbursement for each per diem locality will be the average cost of lodging rounded to the next whole dollar plus 50 percent of the maximum locality rate. Total reimbursement shall not exceed the maximum per diem for each locality,

unless specific approval is given in advance for actual expenses. Maximum locality rates shall be those established by the Department of Defense for non-foreign areas and by the Department of State for foreign areas. When staff house or other free or low cost facilities are used, the reduced rates indicated by footnote 1 on the per diem supplement (section 925) to the Standardized Regulations (Government Civilians, Foreign Areas) will be used directly as per diem. These low rates have already been computed on a basis similar to lodging plus, and no further computation is necessary.

(f) Fifty percent of all international ocean shipment made by the Recipient, to be financed hereunder, shall be made on U.S. flag vessels. Where U.S. flag vessels are not available, or their use would result in a significant delay, the Recipient may request a release from this requirement from the Transportation Support Division, Office of Commodity Management, AID, Washington, D.C. 20523, giving the basis for the request.

(i) When the AID Transportation Support Division makes and issues a determination to the Recipient that U.S. flag vessels are not available, the ocean shipment costs on foreign flag vessels, as named in the determination, will be eligible for reimbursement under the Agreement. In all instances Recipient vouchers submitted for reimbursement under the Agreement which include ocean shipment costs will include a certification essentially as follows: "I hereby certify that a copy of each ocean bill of lading concerned has been submitted to the Maritime Administration, Cargo Preference Control Center, Commerce Building, Washington, D.C. 20235, and that such bill(s) of lading state all of the carrier's charges including the basis for calculation such as weight or cubic measurement, and indicate the applicable A.I.D. Agreement Number."

(ii) Shipments by voluntary non-profit relief agencies (i.e., PYOs) shall be governed by paragraphs (a) and (b) above and by AID Regulation 2, "Overseas Shipments of Supplies by Voluntary Non-Profit Relief Agencies" (22 CFR 202).

7. PROCUREMENT OF GOODS AND SERVICES UNDER \$250,000

(This provision is applicable when the total procurement element, i.e., the sum of all purchase orders and contracts for goods and services, of this Agreement does not exceed \$250,000.)

(a) Ineligible Goods and Services

Under no circumstances shall the Recipient use AID funds to procure any of the following under this Agreement:

- (1) military equipment,
- (2) surveillance equipment,
- (3) commodities and services for support of police or other law enforcement activities,
- (4) abortion equipment and services,
- (5) luxury goods and gambling equipment, or
- (6) weather modification equipment.

(For a more detailed discussion of the subject, see AID Handbook 1, Supplement B, Chapter 4D.)

If AID determines that the Recipient has procured any of the ineligible goods and services specified above under this Agreement, and has received reimbursement from AID for such purpose, the Recipient agrees to refund to AID the entire amount of such reimbursement.

(b) Restricted Goods

The Recipient shall not use AID funds to procure any of the following goods or services from a non-U.S. source (i.e., other than AID Geographic Code 000) without the prior written authorization of the AID Grant Officer:

- (1) agricultural commodities,
- (2) motor vehicles,
- (3) pharmaceuticals,
- (4) pesticides,
- (5) plasticizers,
- (6) used equipment, or
- (7) U.S. Government-owned excess property.

(In addition to the foregoing rule regarding source, to be eligible for procurement with AID funds, the above commodities must meet the requirements contained in AID Handbook 1, Supplement B, Chapter 4C.)

If AID determines that the Recipient has procured any of the restricted goods specified above under this Agreement from non-U.S. sources without the prior written authorization of the AID Grant Officer and has received reimbursement from AID for such purpose, the Recipient agrees to refund to AID the entire amount of such reimbursement.

(c) Geographic Source and Order of Preference

Except as may be specifically approved or directed in advance by the AID Grant Officer under paragraph 7(b) above, all other goods (e.g., equipment, materials and supplies) and services, the costs of which are to be reimbursed under this Agreement by AID and which will be financed with United States dollars, shall be purchased in and shipped from only "Special Free World" countries (i.e., AID Geographic Code 935) in accordance with the following order of preference:

- (1) the United States (AID Geographic Code 000),
- (2) "Selected Free World" countries (AID Geographic Code 941),
- (3) the Cooperating Country,
- (4) "Special Free World" countries (AID Geographic Code 935).

(d) Application of Order of Preference

When the Recipient uses AID funds to procure goods and services from other than U.S. sources under the order of preference in 7(c) above, it shall document its files to justify each such instance. The documentation shall set forth the circumstances surrounding the procurement and shall be based on one or more of the following reasons, which will be set forth in the Recipient's documentation:

- (1) the procurement was of an emergency nature, which would not allow the delay attendant to soliciting U.S. sources,
- (2) the price differential for procurement from U.S. sources exceeded by 50% or more the delivered price from the non-U.S. source,
- (3) impelling local political considerations precluded consideration of U.S. sources,
- (4) the goods or services were not available from U.S. sources, or
- (5) procurement of locally available goods or services as opposed to procurement of U.S. goods and services, would best promote the objectives of the Foreign Assistance Program under this Agreement.

(e) The Recipient's Procurement System

(1) The Recipient may use its own procurement policies and procedures provided they conform to the geographic source and order of preference requirements of this provision and paragraphs 3 and 4, Attachment O of OMB Circular No. A-110 (as set forth in paragraphs 1U.3 and 1U.4 of Handbook 13).

(2) If the Recipient's procurement policies and procedures have been reviewed against the procurement requirements of paragraphs 3 and 4 of Attachment 0 to OMB Circular No. A-110, (as set forth in paragraphs 1U.3 and 1U.4 of Handbook 13) and have been approved by AID or another Federal department or agency, the Recipient shall furnish the AID Grant Officer a copy of such approval; otherwise the Recipient's procurement policies and procedures shall conform to the specified requirements of OMB Circular No. A-110. (See Handbook 13, Chapter 1, paragraph 1U.)

(f) Procurement Systems - Subrecipients

(1) U.S. subrecipients may use their own procurement policies and procedures provided the Recipient determines that they comply with the intent of paragraph 3 and the requirements of paragraph 4 of Attachment 0 to OMB Circular A-110 (as set forth in paragraphs 1U.3 and 1U.4 of Handbook 13).

(2) Non-U.S. subrecipients, who are located abroad and who do not perform in the U.S. under this Agreement, may use their own procurement policies and procedures provided that the Recipient determines that they comply with the intent of paragraph 3 of Attachment 0 to FMC Circular No. 73-8 (as set forth in paragraph 1U.3 of Handbook 13); Paragraph 4 of Attachment 0 to OMB Circular No. A-110 (as set forth in paragraph 1U.4 of Handbook 13) does not apply to such non-U.S. institutions.

(g) Small Business

To permit AID, in accordance with the small business provision of the Foreign Assistance Act of 1961, as amended, to give United States small business firms an opportunity to participate in supplying commodities and services procured under this Agreement, the Recipient shall, to the maximum extent possible, provide the following information to the Small Business Office, AID, Washington, D.C. 20523 at least 45 days prior (except where a shorter time is requested of, and granted by the Small Business Office) to placing any order or contract in excess of \$25,000:

- (1) brief general description and quantity of goods or services,
- (2) closing date for receiving quotations, proposals, or bids, and
- (3) address where invitations or specifications can be obtained.

(h) Ineligible Suppliers

AID funds provided under this Agreement shall not be used to procure any commodity or commodity-related services furnished by any supplier whose name appears on the List of Ineligible Suppliers under AID Regulation 8, Suppliers of Commodities and Commodity-Related Services Ineligible for AID Financing (22 CFR 208). The Recipient agrees to review said list prior to undertaking any procurement the cost of which is to be reimbursable by AID under this Agreement. AID will provide the Recipient with this list.

8. REFUNDS

(a) If use of AID funds results in accrual of interest to the Recipient or to any other person or entity to whom Recipient makes such funds available in carrying out the purposes of this Agreement, the Recipient shall refund to AID an amount equivalent to the amount of interest accrued.

(b) Funds obligated hereunder but not disbursed to the Recipient at the time this Agreement expires or is terminated, shall revert to AID, except for funds encumbered by the Recipient by a legally binding transaction applicable to this Agreement. Any funds disbursed to but not expended by the Recipient at the time of expiration or termination of this Agreement shall be refunded to AID.

(c) If, at any time, during the life of this Agreement, it is determined by AID that funds provided under this Agreement have been expended for purposes not in accordance with the terms of this Agreement, the Recipient shall refund such amounts to AID.

9A. PAYMENT - FEDERAL RESERVE LETTER OF CREDIT (FRLC) ADVANCE

(This provision is applicable when the total advances under all the Recipient's cost-reimbursement contracts and assistance instruments with AID exceed \$120,000 per annum and AID has, or expects to have a continuing relationship with the Recipient of at least one year.)

(a) AID shall open a Federal Reserve Letter of Credit (hereinafter referred to as an "FRLC") in the amount of this Agreement against which the Recipient may present payment vouchers (i.e., Form TFS 5401). The payment vouchers shall not ordinarily be submitted more frequently than daily and shall not be less than \$5,000 or more than \$5,000,000. Since the FRLC method enables the Recipient to obtain funds from the U.S. Treasury concurrently with and as frequently as disbursements are made by the Recipient, there need be no

time lag between disbursements by the Recipient and drawdowns from the U.S. Treasury by FRLC. Therefore, there is no necessity for the Recipient to maintain balances of Federal cash other than small balances.

(b) In no event shall the accumulated total of all such payment vouchers exceed the amount of the FRLC.

(c) If at any time, the AID Controller determines that the Recipient has presented payment vouchers in excess of the amount or amounts allowable in (a) and (b) above, the AID Controller shall advise the AID Grant Officer who may: (1) cause the FRLC to be suspended or revoked; (2) direct the Recipient to withhold submission of payment vouchers until such time as, in the judgment of the AID Controller, an appropriate level of actual, necessary and allowable expenditures has occurred or will occur under this Agreement, and/or (3) request the Recipient to repay to AID the amount of such excess. Upon receipt of the AID Grant Officer's request for repayment of excess advance payments, the Recipient shall promptly contact the AID Controller to make suitable arrangements for the repayment of such excess funds. Advances made by primary Recipients (those which receive payments directly from the Government) to secondary Recipients shall conform to the same standards applicable to advances made by the Government to the primary Grantee.

(d) Procedure for Recipient.

(1) After arranging with a commercial bank of its choice for operation under the FRLC and obtaining the name and address of the Federal Reserve Bank or branch serving its commercial bank, the Recipient shall deliver to the AID Controller 3 originals of Standard Form 1194, Authorized Signature Card for Payment Vouchers on Letters of Credit, signed by those official(s) authorized to sign payment vouchers against the FRLC and by an official of the Recipient who has authorized them to sign.

(2) The Recipient shall subsequently receive one certified copy of the FRLC.

(3) The Recipient shall confirm with its commercial bank that the FRLC has been opened and is available when funds are needed.

(4) To receive payment, the Recipient shall:

(A) Periodically, although normally not during the last five days of the month, prepare payment vouchers (Form TFS 5401) in an original and three copies.

(B) Have the original and two copies of the voucher signed by the authorized official(s) whose signature(s) appear on the Standard Form 1194.

(C) Present the original, duplicate and triplicate copy of the Form TFS 5401 to its commercial bank.

(D) Retain the quadruplicate copy of the voucher.

(5) After the first payment voucher (Form TFS 5401) has been processed, succeeding payment vouchers shall not be presented until the existing balance of previous payments has been expended or is insufficient to meet current needs.

(6) In preparing the payment voucher, the Recipient assigns a voucher number in numerical sequence beginning with 1 and continuing in sequence on all subsequent payment vouchers submitted under the FRLC.

(7) A report of expenditures (i.e., SF 269, Financial Status Report) shall be prepared and submitted not less than quarterly within 30 days of the end of the period to the AID Controller, AID, Washington, D.C. 20523. This SF 269 Report, submitted with Standard Form 1034, Public Voucher for Purchases and Services Other Than Personal, shall be in an original and 2 copies.

(8) The SF 269 report is reviewed against this Agreement's provisions, and any improper disbursement is disallowed. The Recipient is notified of the reason for the disallowance and is directed to adjust the next periodic report of expenditures to reflect the disallowance and to reduce its next payment voucher against the FRLC by the amount of the disallowance.

(9) In addition to the submission of the SF 269 and the SF 1034, the Recipient shall submit an original and 2 copies of SF 272, Federal Cash Transaction Report, as follows:

(A) For advances totaling less than \$1 million per year, the Recipient shall submit the SF 272 within 15 working days after the end of the reporting quarter.

(B) For advances totaling more than \$1 million per year, the Recipient shall submit the SF 272 within 15 working days after the end of each month, and

(C) The Recipient's cash needs for the ensuing period (i.e., quarter or month) shall be explained under the "Remarks" section of the SF 272.

(e) Refund of Excess Funds.

(1) If all costs have been settled under the Agreement and the Recipient fails to comply with the AID Grant Officer's request for repayment of excess FRLC funds, the Government shall have the right, on other contracts or assistance instruments held with the Recipient, to withhold reimbursements due to the Recipient in the amount of the excess being held by the Recipient.

(2) If the Recipient is still holding excess FRLC funds on a contract or an assistance instrument under which work has been completed or terminated but all costs have not been settled, the Recipient agrees to:

(A) Provide within 30 days after requested to do so by the AID Grant Officer, a breakdown of the dollar amounts which have not been settled between the Government and the

Recipient. (The AID Grant Officer will assume no costs are in dispute if the Recipient fails to reply within 30 days.)

(B) Upon written request of the AID Grant Officer, return to the Government the sum of dollars, if any, which represents the difference between (i) the Recipient's maximum position on claimed costs which have not been reimbursed and (ii) the total amount of unexpended funds which have been advanced under this Agreement; and

(C) If the Recipient fails to comply with the AID Grant Officer's request for repayment of excess FRLC funds, the Government shall have the right, on other contracts or assistance instruments held with the Recipient, to withhold payment of FRLC or other advances and/or withhold reimbursements due the Recipient in the amount of the excess being held by the Recipient.

9B. PAYMENT - PERIODIC ADVANCE

(This provision is applicable when total advances under all the Recipient's cost-reimbursement contracts and assistance instruments with AID do not exceed \$120,000 per annum or if total advances aggregate more than \$120,000 per annum but there is not a continuing relationship of at least one year.)

(a) Each month (or quarter, if the Recipient is on a quarterly basis) after the initial advance, the Recipient shall submit to the AID Controller an original and 2 copies of SF 272, Federal Cash Transaction Report, as follows:

(1) The Recipient shall submit the SF 272 within 15 working days after the end of the reporting period, and

(2) The Recipient's cash needs for the ensuing period (i.e., quarter or month) shall be explained under the "Remarks" section of the SF 272.

(b) Along with each SF 272 submission, the Recipient shall submit an original and 3 copies of SF 1034, Public Voucher for Purchases and Services Other Than Personal, each voucher shall be identified by this Agreement number and shall state the total actual expenditures for the reporting period.

(c) Each quarterly voucher (i.e., SF 1034) or third monthly voucher, if the Recipient is on a monthly basis, shall also be supported by an original and 2 copies of an SF 269, Financial Status Report. The SF 269 shall be submitted within 30 days after the end of the reporting quarter and may be submitted separately from the SF 1034 and the SF 272; however, the SF 269 shall cover the same period as the SF 1034(s) and the SF 272(s).

(d) Refund of Excess Funds.

(1) If all costs have been settled under this Agreement

and the Recipient fails to comply with the AID Grant Officer's request for repayment of excess advance funds, the Government shall have the right, on other contracts or assistance instruments held with the Recipient, to withhold reimbursements due to the Recipient in the amount of the excess being held by the Recipient.

(2) If the Recipient is still holding excess advance funds on a contract or assistance instrument under which the work has been completed or terminated but all costs have not been settled, the Recipient agrees to;

(A) Provide within 30 days after requested to do so by the AID Grant Officer, a breakdown of the dollar amounts which have not been settled between the Government and the Recipient. (The AID Grant Officer will assume no costs are in dispute if the Recipient fails to reply within 30 days.)

(B) Upon written request of the AID Grant Officer, return to the Government the sum of dollars, if any, which represents the difference between (i) the Recipient's maximum position on claimed costs which have not been reimbursed and (ii) the total amount of unexpended funds which have been advanced under this Grant; and

(C) If the Recipient fails to comply with the AID Grant Officer's request for repayment of excess advance funds, the Government shall have the right, on other contracts or assistance instruments held with the Recipient, to withhold payment of other advances and/or withhold reimbursements due the Recipient in the amount of the excess being held by the Recipient.

10. LIMITATION OF FUNDS

(This provision is applicable to all incrementally or partially funded Agreements.)

(a) It is estimated that the cost to AID for the performance of this Agreement will not exceed the estimated cost specified in the Schedule as being the estimated cost to AID for this Agreement, and the Recipient agrees to use its best efforts to carry out the program specified in the Schedule and all obligations under this Agreement within such estimated cost.

(b) The amount of AID funds presently available for payment and allotted to this Agreement, the items covered thereby, and the period of performance which it is estimated the allotted amount will cover, are specified in the Schedule. It is contemplated that from time to time additional funds will be allotted to this Agreement up to the full estimated cost to AID set forth in the Schedule. The Recipient agrees to carry out, or have carried out, the

program under this Agreement up to the point at which the total amount paid and payable by AID pursuant to the terms of this Agreement approximates but does not exceed the total amount actually allotted to this Agreement.

(c) (1) If, at any time, the Recipient has reason to believe that the costs which it expects to incur in carrying out the program of this Agreement in the next succeeding 60 days, when added to all costs previously incurred, will exceed 75 percent of the total amount of AID funds then allotted to this Agreement, the Recipient shall notify the AID Grant Officer in writing to that effect. The notice shall state the estimated amount of additional funds required to continue the program for the period set forth in the Schedule. (2) Sixty days prior to the end of the period specified in the Schedule, the Recipient will advise the AID Grant Officer in writing as to the estimated amount of additional AID funds, if any, that will be required for the timely carrying out of the program under this Agreement or for such further period as may be specified in the Schedule or otherwise agreed to by the parties. (3) If, after notification, pursuant to (c)(1) or (c)(2), above additional AID funds are not allotted by the end of the period set forth in the Schedule or an agreed date substituted therefor, the AID Grant Officer will, upon written request by the Recipient, terminate this Agreement pursuant to the provisions of the TERMINATION provision on such date. If the Recipient, in the exercise of its reasonable judgment, estimates that the funds available will allow it to continue to discharge its obligation hereunder for a period extending beyond such date, it shall specify the later date in its request and the AID Grant Officer, in his discretion, may terminate this Agreement on that later date.

(d) Except as required by other provisions of this Agreement, specifically citing and stated to be an exception from this provision, the Government shall not be obligated to reimburse the Recipient for costs incurred in excess of the total amount of AID funds from time to time allotted to this Agreement, and the Recipient shall not be obligated to continue carrying out the program under this Agreement (including actions under the TERMINATION provision) or otherwise to incur costs in excess of the amount of AID funds allotted to this Agreement, unless and until the AID Grant Officer has notified the Recipient in writing that such allotted amount of AID funds has been increased and has specified in such notice an increased amount constituting the total amount then allotted to this Agreement. To the extent the amount of AID funds allotted exceeds the estimated cost to AID set forth in the Schedule, such estimated cost to AID shall be correspondingly increased. No notice, communication

or representation in any other form or from any person other than the AID Grant Officer shall affect the amount allotted to this Agreement. In the absence of the specified notice, AID shall not be obligated to reimburse the Recipient for any costs in excess of the total amount of AID funds then allotted to this Agreement, whether those excess costs were incurred during the course of this Agreement or as result of termination. When and to the extent that the amount of AID funds allotted to this Agreement has been increased, any costs incurred by the Recipient in excess of the amount of AID funds previously allotted shall be allowable to the same extent as if such costs had been incurred after such increase in the amount of AID funds allotted, unless the AID Grant Officer issues a termination or other notice and directs that the increase is solely for the purpose of covering termination or other specified expenses.

11. USE OF FOREIGN CURRENCY

(a) Whenever the Recipient has a need to convert U.S. dollars into local currencies, such conversion shall be made through the U.S. Disbursing Officer. If the Disbursing Officer is unable to effect such conversion, the Recipient is free to use the facilities of any accredited financial institution.

(b) All expenditures of the Recipient in excess or near-excess currency countries, including per diem (subsistence) expenses, which will be reimbursable by AID under this Agreement, shall be funded from U.S. owned foreign currencies, unless otherwise authorized in writing by the AID Grant Officer .

12. AGREEMENT AMENDMENTS

This Agreement may be amended by formal written modifications to the basic document. Amendments may be proposed by either party to this Agreement; however, they must be acceptable to both parties before they become effective and binding.

13. DISPUTES

(a) Except as otherwise provided in this Agreement, any dispute concerning a question of fact arising under this Agreement which is not disposed of by agreement shall be decided by the AID Grant Officer, who shall reduce his decision to writing and mail or otherwise furnish a copy thereof to the Grantee. The decision of the AID Grant

Officer shall be final and conclusive unless within thirty (30) days from the date of receipt of such copy, the Recipient mails or otherwise furnishes to the AID Grant Officer a written appeal addressed to the Administrator, Agency for International Development, Washington, D.C. 20523. The decision of the Administrator or his duly authorized representative for the determination of such appeals shall be final and conclusive unless determined by a court of competent jurisdiction to have been fraudulent, or capricious, or arbitrary, or so grossly erroneous as necessarily to imply bad faith or not supported by substantial evidence. In connection with any appeal proceeding under this provision, the Recipient shall be afforded an opportunity to be heard and to offer evidence in support of its appeal.

(b) This DISPUTES provision does not preclude consideration of law questions in connection with decisions provided for in paragraph (a) above; provided that nothing in this Agreement shall be construed as making final the decision of any administrative official, representative, or board on a question of law.

14. TERMINATION

(a) For Cause. This Agreement may be terminated for cause at any time, in whole or in part, by the AID Grant Officer upon written notice to the Recipient, whenever it is determined that the Recipient has failed to comply with the conditions of the grant.

(b) For Convenience. This Agreement may be terminated for convenience at any time by either party, in whole or in part, if both parties agree that the continuation of this Agreement would not produce beneficial results commensurate with the further expenditures of funds. Both parties shall agree upon termination conditions, including the effective date and, in the case of partial termination, the portion to be terminated. The agreement to terminate shall be set forth in a letter from the AID Grant Officer to the Recipient.

(c) Termination Procedures. Upon receipt of and in accordance with a termination notice as specified in either paragraph (a) or (b) above, the Recipient shall forthwith take immediate action to minimize all expenditures and obligations financed by this Agreement, and shall cancel such unliquidated obligations whenever possible. Except as provided below, no further reimbursement shall be made after the effective date of termination, and the Recipient shall, within 30 calendar days after the effective date of such termination, repay to the Government all unexpended portions of funds theretofore paid by the Government to the Recipient

which are not otherwise obligated by a legally binding transaction applicable to this Agreement. Should the funds paid by the Government to the recipient prior to the effective date of the termination of this Agreement, be insufficient to cover the Recipient's obligations pursuant to the aforementioned legally binding transactions, the Recipient may submit to the Government within 90 calendar days after the effective date of such termination, a written claim covering such obligations, and subject to the limitations contained in this Agreement, the AID Grant Officer shall determine the amount or amounts to be paid by the Government to the Recipient under such claim in accordance with the applicable Federal cost principles.

15. SUBORDINATE AGREEMENTS

The placement of subordinate agreements (e.g., leases, options, agreements, or contracts) with other organizations, firms or institutions and the provisions of such subordinate agreements are subject to prior written consent of the AID Grant Officer if they will be funded by AID hereunder, unless the Recipient's procurement system has been reviewed and approved pursuant to the appropriate section(s) of Paragraph 1U of Chapter 1, Handbook 13. In no event shall any such subordinate agreement be on a cost-plus-a-percentage-of-cost basis. Subordinate contractors (including suppliers) shall be selected on a competitive basis to the maximum practicable extent consistent with the obligations and requirements of this Agreement.

16. PUBLICATIONS

(This provision is applicable to any agreement which produces any book, publication, or other copyrightable material.)

(a) If it is the Recipient's intention to identify AID's contribution to any publication resulting from this Agreement, the Recipient shall consult AID on the nature of the acknowledgement prior to publication.

(b) The Recipient shall provide the AID Project Manager with one copy of all published works developed under this Agreement. The Recipient shall provide the AID Project Manager with lists of other written work produced under this Agreement.

(c) In the event these Agreement funds are used to underwrite the cost of publishing, in lieu of the publisher assuming this cost as is the normal practice, any profits or royalties up to the amount of such cost shall be credited to this Agreement.

(d) The Recipient is permitted to secure copyright to any publication produced or composed under this Agreement in accordance with paragraph 178.b. of Chapter 1, Handbook 13; Provided, that the Recipient agrees to and does hereby grant to the Government a royalty-free, non-exclusive and irrevocable license throughout the world to use, duplicate, disclose, or dispose of such publications in any manner and for any purpose and to authorize others to use the work for Government purposes.

17. PATENTS

(This provision is applicable to any Agreement which produces patentable items, patent rights, processes or inventions.)

(a) The Recipient agrees to notify the AID Grant Officer in writing of any invention or discovery conceived or first actually reduced to practice in the course of or under this Agreement. The AID Grant Officer will determine the patent rights to be afforded the Recipient in accordance with the Presidential Memorandum and Statement of Government Patent Policy (36 FR 16889) and paragraph 178.a. of Chapter 1, Handbook 13.

(b) Nothing contained in this provision shall imply a license to the Government under any patent or be construed as affecting the scope of any license or other right otherwise granted to the Government under any patent.

18. INELIGIBLE COUNTRIES

Unless otherwise approved by the AID Grant Officer, no AID funds will be expended for costs incurred in countries ineligible for assistance under the Foreign Assistance Act of 1961, as amended, or under acts appropriating funds for foreign assistance.

19. EQUAL OPPORTUNITY IN EMPLOYMENT

(This provision is applicable to all Recipients, subrecipients and contractors under this Agreement who either perform work in the United States or who recruit personnel in the United States to do work abroad.)

(a) With respect to the employment of persons in the U.S. under this Agreement, the Recipient agrees to take all reasonable steps to ensure equality of opportunity in its employment practices without regard to race, color or

national origin of such persons and, in accordance with Title VI of the Civil Rights Act of 1964, when work funded by this Agreement is performed in the U.S., no person shall, on the grounds of race, color or national origin, be excluded from participation, be denied benefits, or be subjected to discrimination. In addition, the Recipient agrees to comply, in accordance with its written assurance of compliance, with the provisions of Part 209 of Chapter II, Title 22 of the Code of Federal Regulations, entitled Non-discrimination in Federally Assisted Programs of the Agency for International Development - Effectuation of Title VI of the Civil Rights Act of 1964.

(b) In addition, the Recipient agrees to take all reasonable steps to ensure equality of opportunity in its employment practices without regard to sex, religion, age, and handicap, in accordance with P.L. 92-261, P.L. 93-259, P.L. 93-112 and P.L. 93-508. When work funded by AID under this Agreement is performed in either the U.S. or overseas, no person shall on the grounds of sex, religion, age, or handicap, be excluded from participation, be denied benefits, or be subjected to discrimination.

20. U.S. OFFICIALS NOT TO BENEFIT

No member of or delegate to the U.S. Congress or resident U.S. commissioner shall be admitted to any share or part of this Agreement or to any benefit that may arise therefrom; but this provision shall not be construed to extend to this Agreement, if made with a corporation for its general benefit.

21. COVENANT AGAINST CONTINGENT FEES

The Recipient warrants that no person or selling agency has been employed or retained to solicit or secure this Agreement upon an agreement or understanding for a commission, percentage, brokerage, or contingent fee except bona fide employees or bona fide established commercial or selling agencies maintained by the Recipient for the purpose of securing business. For breach or violation of this warranty, AID shall have the right to cancel this Agreement without liability, or in its discretion, to deduct from the Agreement an amount, or otherwise recover, the full amount of each commission, percentage, brokerage, or contingent fee.

22. NONLIABILITY

AID assumes no liability with respect to any third party for any claims for damages arising out of the program supported by this Agreement.

23. TITLE TO, USE, AND CARE OF PROPERTY

(a) The term "property" as used in this provision shall mean Recipient purchased property the cost of which is reimbursed by AID under this Agreement. The term "non-expendable property" means property which is complete in itself; does not lose its identity or become a component part of another article when put into use; is durable, with an expected service life of two years or more.

(b) The Schedule of this Agreement shall specify (or the AID Grant Officer may direct) that title to specific property shall be vested in the Recipient, or in the Cooperating Country. When title is to be vested in the Recipient, paragraph (c) of this provision is applicable. When title is to be vested in the Cooperating Country, paragraph (d) of this provision is applicable. If specific direction regarding placement of title is not provided either in the Schedule of this Agreement, or by the AID Grant Officer, then title shall be vested in the Recipient, and paragraph (c) of this provision is applicable.

(c) Recipient Titled Property.

(1) The Recipient shall not charge for any depreciation, amortization, or use of property, title to which remains in the Recipient under this Agreement, or any other U.S. Government agreement, subagreement, contract, or subcontract.

(2) The Recipient agrees to use and maintain the property for the purpose of this Agreement in accordance with the requirements of paragraph 1T of Chapter 1, Handbook 13.

(3) With respect to nonexpendable property having an acquisition cost of \$1,000 or more, title to which vests in the Recipient, the Recipient agrees:

(i) To report such items to the AID Grant Officer from time to time as they are acquired and to maintain a control system which will permit their ready identification and location.

(ii) To transfer title to any such items in accordance with any written request therefor issued by the AID Grant Officer at any time prior to final payment under this Agreement.

(d) Cooperating Country Titled Property.

(1) Property titled to the Cooperating Country shall be under the custody and control of the Recipient until the owner of title directs otherwise, or completion of work under this Agreement, or its termination, at which time custody and

control shall be turned over to the owner of title, or disposed of in accordance with its instructions. All performance guarantees and warranties obtained from suppliers shall be taken in the name of the title owner.

(2) The Recipient shall prepare and establish a program to be approved by the Mission, for the receipt, use, maintenance, protection, custody, and care of the Cooperating Country titled property for which it has custodial responsibility, including the establishment of reasonable controls to enforce such program. The Recipient shall be guided by the requirements of paragraph 1T of Chapter 1, Handbook 13.

(3) Within 90 days after completion of this Agreement, or at such other date as may be fixed by the AID Grant Officer, the Recipient shall submit an inventory schedule covering all items of property under its custody, title to which is in the Cooperating Country or public or private agency designated by the Cooperating Country, which have not been consumed in the performance of this Agreement. The Recipient shall also indicate what disposition has been made of such property.

24. AUDIT AND RECORDS - NON-U.S. SUBRECIPIENTS

(1) The Recipient shall require non U.S. subrecipients (see paragraph L3, Chapter 1 of Handbook 13) to maintain books, records, documents and other evidence and accounting procedures and practices sufficient to reflect properly that funds provided by the Recipient were expended exclusively for the purposes of the subordinate agreement. Such records shall be maintained for three years following the expiration of the subordinate agreement.

(2) The Recipient shall ensure that, after each twelve months of a subordinate agreement with a non-U.S. institution, an audit is conducted on the subrecipient's records by an independent public accountant with a national certification similar or equivalent to a certified public accountant. If the Recipient determines that an audit is not possible or feasible it shall submit to the AID Grant Officer the reasons why such an audit is not possible or feasible, and present for the approval of the AID Grant Officer, alternatives which will achieve the objectives, set forth in this provision. The Recipient shall include in each subordinate agreement hereunder, a provision by which the Recipient asserts the right to audit if the independent audit does not take place or is unacceptable. The subordinate agreement shall also require that the subrecipient shall make available any further information as requested by the Recipient with respect to questions concerning the audit. The report of independent audit shall be submitted to the

Recipient and retained by it in accordance with the standard provision entitled ACCOUNTING, AUDIT AND RECORDS.

(3) The purpose of the independent audit shall be to determine the propriety and necessity of the subrecipient's expenditures in terms of the purposes for which the funds were made available, and the adequacy of the subrecipient's financial management. Notwithstanding the requirement for an independent audit, the AID Auditor General and the Comptroller General of the United States or their duly authorized representatives (see paragraph 16, Chapter 1 of Handbook 13) reserve the right to conduct an audit of the subrecipient's books and records to determine whether the subrecipient has expended AID's funds in accordance with the terms of this Agreement. The terms of this Provision are applicable only to subordinate agreements with non-U.S. institutions.

25. REGULATIONS GOVERNING EMPLOYEES OUTSIDE THE UNITED STATES

(This provision is applicable only to the Recipient's U.S. or third country national employees; it is not applicable to the Recipient's cooperating country national employees.)

(a) The Recipient's employees, when employed in work overseas, shall maintain private status and may not rely on local U.S. Government Offices or facilities for support while so engaged.

(b) The sale of personal property or automobiles by Recipient employees and their dependents in the foreign country to which they are assigned shall be subject to the same limitations and prohibitions which apply to direct-hire AID personnel employed by the Mission, except as this may conflict with host government regulations.

(c) Other than work to be performed under this Agreement for which an employee or consultant is assigned by the Recipient, no regular or short term employee or consultant of the Recipient shall engage directly or indirectly, either in his own name or in the name or through an agency of another person, in any business, profession, or occupation in the foreign countries to which he is assigned, nor shall he make loans or investments to or in any business, profession or occupation in the foreign countries to which he is assigned.

(d) The Recipient's employees, while in a foreign country, are expected to show respect for its conventions, customs, and institutions, to abide by its applicable laws and regulations, and not to interfere in its internal political affairs.

(e) In the event the conduct of any Recipient employee is not in accordance with the preceding paragraphs, the Recipient's chief of party shall consult with the Mission Director and the employee involved and shall recommend to the Recipient a course of action with regard to such employee.

(f) The parties recognize the right of the U.S. Ambassador to direct the removal from a country of any U.S. citizen or the discharge from this Agreement of any third country national when, in the discretion of the Ambassador, the interests of the United States so require.

26. NOTICES

Any notice given by any of the parties hereunder, shall be sufficient only if in writing and delivered in person or sent by telegraph, cable, registered or regular mail as follows:

TO THE: AID Grant Officer;

TO THE: Recipient at Recipient's address shown in this Agreement;
or to such other address as either of such parties shall designate by notice given as herein required. Notices hereunder shall be effective when delivered in accordance with this provision or on the effective date of the notice, whichever is later.

Commodity Exports, Economic Development and Policy

EXECUTIVE SUMMARY

The Commodity Problem, Goal Attainment, and Policies
in Developing Countries

by F. Gerard Adams and Jere R. Behrman

For:

AGENCY FOR INTERNATIONAL DEVELOPMENT

PROJECT NUMBER: 931-11-995-057

International Primary Commodity Markets and Economic Development Project

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INTRODUCTION

This memorandum summarizes the results of a series of studies on The Commodity Problem and Economic Goal Attainment in Developing Countries. This project, directed by F. Gerard Adams and Jere R. Behrman, has been carried out at Wharton EFA, Inc., and the Economics Research Unit of the University of Pennsylvania during the 1978-1980 period. The memorandum notes only the high points of the extensive research carried out. The details are summarized in six extensive studies. These studies will be published as books by Lexington Books, a division of D.C. Heath, and will include:

- T. Priovolos, The Commodity Problem and Goal Attainment: Ivory Coast and Coffee; (publication early 1981)
- M. Lasaga, The Commodity Problem and Goal Attainment: Chile and Copper; (publication early 1981)
- M. Nziramasanga and C. Obidegwu, The Commodity Problem and Goal Attainment: Zambia and Copper; (publication early 1981)
- G. Siri, The Commodity Problem and Goal Attainment: Central America; (report completed, volume in process)
- F. Gerard Adams and T. Priovolos, The Commodity Problem and Goal Attainment: Brazil and Coffee; (report in draft)
- F. Gerard Adams and Jere R. Behrman, The Commodity Problem and Goal Attainment in Developing Countries: An Integrated Econometric Examination of Basic Policy Issues; (volume in process)

In addition, preliminary papers from this study have been published in journals and books and presented at conferences, including:

- F. G. Adams, J.R. Behrman, R. Roldan, "Measuring the Impact of Primary Commodity Fluctuations on Economic Development: Coffee and Brazil," American Economic Review, 69:2, 1979, 164-168.
- F. G. Adams and R. Roldan, "Econometric Studies of the Impact of Primary Commodity Markets on Economic Development in Latin America," in W. Labys, M. Ishaq Nadiri, and Jose Nuñez del Arco, eds., Commodity Markets in Latin American Development: A Modeling Approach, (Cambridge: Ballinger Pub. Co. and NBER, 1980).

F. G. Adams, J. R. Behrman, and M. Lasaga, "Commodity Exports and NIEO Proposals for Buffer Stocks and Compensatory Finance: Implications for Latin America," in W. Baer, C. Longo, and M. Gillis (eds.) Trade Prospects Among the Americas: Latin American Export Diversification and the New Protectionism, Bureau of Economic and Business Research of the University of Illinois, 1981 (also in Quarterly Review of Economics and Business and Estudios Economicos).

Other studies have been distributed in xerox including:

Jon Manger, "A Review of the Literature on Causes, Effects and Other Aspects of Export Instability", (a report of Wharton EFA Inc., for the AID Project on Primary Commodity Stabilization and Economic Development, May 1979.)

We expect in the next few months to prepare additional papers for publication in recognized journals.

Section 1 Overview

In recent years, international concern has once again turned to the "commodity problem", the impact of fluctuations and trends of primary commodity prices on the developing countries. Despite the considerable progress of some of these countries, many of them are still largely dependent on primary commodity markets for their principal export earnings. Consequently, their economies may be affected broadly and in important specific ways by what happens in world commodity markets. The commodity problem is a primary focus of the discussions between the industrial countries and their less affluent suppliers of primary commodities as is best illustrated, perhaps, in the negotiations surrounding the UNCTAD Integrated Commodity Program proposal. It is also clearly an important issue in the planning of developing countries' economic policies: the resources which should be directed into primary commodity production, the way in which participation in primary commodity markets should be organized, and the other internal micro policies and the macro stabilization and growth policies that should be adopted in light of commodity developments.

It goes almost without saying that policy must be based on knowledge. Yet, despite the central role of the commodity problem in international and national policy dialogues, there is little widely accepted and solidly based information on the impact of the commodity problem. There remains considerable controversy even as to the direction of the impacts of commodity price fluctuations, though there is more consensus on the effects of the secular trends in the terms of trade of commodities. The need for information

exceeds broad generalizations about the direction of effects. Economic goals comprise a variety of targets, i.e., growth, price stability, balance of payments equilibrium, distributive equity, etc., and the effects of the commodity problem on the attainment of these goals may differ greatly depending on the weight given to each target. It is important, consequently, to know the impacts of developments in world primary commodity markets on the commodity producing sector itself, on various dimensions of macroeconomic performance, on the tax receipts of the government, on the distribution of income, on the linkages to other industrial and service sectors of the economy, etc. The channels of impact must be identified and their operation traced over time. The quantitative dimensions of the impacts must be evaluated. The potentials for policy to deal with the commodity problem or with its effects must be considered. Only with such information, is it possible to evaluate the significance of the commodity problem on goal attainment of the producer countries. And only with such information can successful policies be planned and carried out on the international level and on the level of the producer countries.

This report summarizes the findings of an extensive project designed to study the relationship between export earnings in international commodity markets, the producing sector in the developing economy, and the performance of the producer country economy. We have established a framework of analysis using integrated econometric models of the world commodity markets, the producer countries, and the commodity producing sectors. We have illustrated its potential by examining the case of the market for coffee and

the economies of the producing economies in Brazil, Ivory Coast, and Central America and the case of the copper market with respect to the economies of Chile and Zambia. The approach has been primarily empirical and econometric. After a conceptual discussion of the relevant issues and model structures, our concern has been with putting quantitative dimensions on the effects. We have also been concerned with evaluating the need for and the potential of policy to deal with the commodity problem and its impacts on goal attainment in the developing commodity producing countries. These extensive results are discussed in detail in six volumes--one on each of the five countries under examination and a summary volume by the directors--that are being published in a series by Lexington Books, a division of D.C. Heath and a number of reports to AID (see the Introduction).

In this report, we try to summarize the results of the project. Our concern is with the channels of influence from the commodity market to the producing country economy, the quantitative dimensions of these influences and the resulting implications of the movements in commodity markets on the multiple dimensions of economic goal attainment in the producing developing countries.

Section 2 The Channels of Influence from Commodity Markets to the National Economies

The impacts from primary commodity export market fluctuations and secular trends to the producing economies must be examined in a structural framework. Reduced form approaches have produced inconclusive results, suffer from severe data and theoretical limitations, and fail to recognize

clearly the channels through which the impacts from commodity exports operate. Thus, it is not very satisfying to note that fluctuations of copper prices influence the performance of the Zambian economy without being able to specify whether the effects are through foreign earnings, tax revenues, capital investment, or employment or through all of these.

We do not repeat in detail the model structures which we have used. But it is useful to review the principal channels of influence from commodity markets to national goal attainment and to evaluate their relative importance.

We begin first by considering the points of contact between the three principal "actors" in the analysis: the international commodity markets, the primary producing sectors (micro sectors) and the macro economies of producer country economies.

Subsection 2.1 World Primary Commodity Markets

Our approach to world commodity markets, specifically the markets for copper and coffee, is predominantly from the perspective of competitive markets where supply and demand (including speculative demands for inventories) determine a world market price. This does not exclude, however, the possibility of investigating the role of particular countries in influencing the world market price. This influence may be through externally determined shifts in supply, like a frost in Brazil or a mine disaster in Zambia for example, or through explicit supply or export tax policy, as for coffee in Brazil. Moreover, it does not exclude the use of the model system as the framework for buffer stock analysis.

The relation between the commodity markets and the country economies is in determining the world price of the commodity, a price to which the local export price and the producer prices are linked. In conjunction with the production of the primary commodity and the quantity available for export, the price then determines the foreign exchange earnings from commodity exports. In the case of the coffee model, the model also sets the quantity which is exported, though the system can also be operated with exports determined by Brazilian conditions or policy decisions.

An important aspect of the interaction between the commodity market and the producer economy is the simultaneity of price determination with commodity production. This simultaneity is important particularly for major producer countries (e.g., coffee in Brazil).

Subsection 2.2 Primary Commodity Micro Sectors

The micro sectors are the commodity producing sector models for coffee or copper which we have included in each of our country systems. These sector models directly link the international commodity markets to the national economy. They vary greatly in importance, copper being almost the only industrial sector in Zambia and providing over 90 percent of export revenue whereas coffee is a relatively small and relatively declining sector in Brazilian agriculture though it continues to provide 10 to 15 percent of Brazilian export earnings. The study finds great diversity among the specialized export sectors of the various countries reflecting not only the differences between coffee production, a labor intensive tropical tree crop, and copper production, a capital intensive

mining product, but also differences in the ownership, control, and objectives of the commodity producers. These elements have to be taken into account, particularly since nationalization and some land redistribution have changed the organization of these sectors in recent years.

The empirical analysis of the sectors points particularly to a number of important conclusions:

1. Output response to price. In all of the micro sectors, output is responsive to price in the world market, but with relatively low elasticities, particularly in the short run. This is not altogether surprising since the short-run output potential is limited in copper by mining capacity and in coffee by the acreage in mature trees. Moreover, the price obtained by the producer is frequently very different from the world market price. For example, in Ivory Coast where the producer coffee price is tied to the producer price of cocoa and in Brazil where export taxes apply. The empirical data suggest that even if we allow for the time necessary for the planting and maturing of trees and the construction of new mines, the supply responses are inelastic. There are, of course, also important variations in output associated with harvest failures, strikes, political upheaval, etc.

2. Linkage of employment to output. The short-run response of employment in the commodity producing sector with respect to output is relatively inelastic. This means that employment is less volatile than output, though the long-run response of employment to changes in output tends to come closer to a unit elastic one. There is no direct linkage between

variations in price and employment. Thus, variations in primary commodity earnings do not translate fully into variations in employment except to the extent that they involve changes in production, but even in that case the short-run response is not large.

3. Wage and non wage income. Wage rates are tied in some cases to primary commodity earnings, for example, in Zambia and Chile. But here too, the response of wage rates to earnings is relatively insensitive and has time lags. The consequence of this and of the previous point is that wage income is considerably less volatile than are the movements of the value of production or of the value of exports. This has important distributive consequences in that the non wage component of income, which includes returns to capital and taxes, shows considerable volatility with respect to the movements of price and value of production and exports.

4. Other direct linkage effects. The primary commodity sector has perceptible direct linkage effects with other sectors, particularly transportation services, trade construction and, in some cases, the domestic industrial sector. It is not clear from our empirical work that these linkages can be considered an important "engine" of economic development since the primary commodity sectors require only limited quantities of conventional inputs from the local economy. With respect to highly technical labor and materials, they draw on imports and sometimes expatriate workers (especially for production of some minerals).

5. Leading sector role. Particularly with respect to wages, the primary commodity sector may serve as a leading sector. This is apparent in

Zambia and Chile, where the wage decisions for copper influence wages in other sectors, and thereby the labor market and population movements.

6. Tax receipts and earnings of government owned industry. The empirical work shows the important role of the primary commodity sector as a source of government revenues. In part, this reflects the direct taxes imposed which appear in the public treasury; in part it represents the earnings of government from its ownership interest. In the case of Brazil and Chile, it used to include the exchange rate differential for coffee exports or domestic inputs for copper production. In more recent years it has included the receipts from the coffee fund "contribution quota" and the earnings of the coffee stabilization funds. All of these are highly sensitive to the revenues from the exports of coffee and so show considerable volatility.

7. Foreign exchange. In all cases considered, the primary sector is an important contributor to the total earnings of foreign exchange and to the instability of these earnings. Exports are determined by exportable production (after allowing for domestic consumption in the case of coffee) less public or private inventory accumulation. The value of foreign exchange earnings then depends on the quantity exported times the price, but the latter is itself influenced by production and exports if the system is linked with a world commodity model. The empirical results suggest that the role of primary commodity production as a supplier of foreign exchange is important in all the countries considered and that this sector's exports account for a good deal of the variation in aggregate foreign

receipts for all of the project countries. Furthermore, variations in foreign exchange cause related variations in the monetary base.

Subsection 2.3 macroeconomic Models

The macroeconomic models of the producing countries serve two purposes in the structure of this project:

1. to accept the linkages from the micro sector model and to translate these into macroeconomic impacts; and
2. to allow for the operation of general policies, both passive and active.

The models are designed to incorporate the structural characteristics of the producing economies. This means that the models encompass Keynesian demand-side elements as well as supply-side considerations which often dominate the simplified models of developing economies. This is particularly important since the micro sectors interact with the macro economy both from the demand and the supply sides. The macro model must also describe the effects on other aspects of goal attainment: the impacts on inflation and balance of payments equilibrium, and the effect on income distribution. At this juncture, it is important to note also that the models are able to capture feedback mechanisms, from the micro sector, to the macro economy, and subsequently, back to the micro sector.

With regard to policy, we make an important distinction. Passive policy is that response of government which represents a natural endogenous response to changes in conditions. Some typical examples are the expansion of government spending with an increase in government revenues or the

expansion of the money supply due to balance of payments surpluses. Active policy represents explicit policy actions intended to offset occurrences in the economy. For example, an increase in export taxes on coffee at a time when the world coffee price is rising. The distinction is arbitrary to some extent but, it is an important distinction since it is unrealistic to do policy simulations without including passive policies. On the other hand, it is useful to see what governments could do explicitly in the way of policy actions in the attempt to offset the deleterious aspects of the commodity problem. We have structured our models to encompass passive policies, and to allow "policy handles" for simulation studies of the potentials for active policy intervention.

The Channels of Influence

In surveying different commodities and different economies it is difficult to generalize the magnitude of the effects. Before we attempt to do so we will draw some generally applicable conclusions as to the principal channels through which the fluctuations and trend movements in the primary commodity markets are translated to the level of the developing country economy and its ability to attain its objectives.

A useful classification is to consider the direct effects and then the indirect effects. The direct effects represent the immediate impact on the micro commodity sector, and the indirect effects comprise the feedback and the passive policy responses. Active policy must be considered separately.

In all cases, it is essential to view the process of reaction to changes in the commodity market, and the transmission to the macro economy as occurring dynamically over time. The empirical evidence points to gradual adjustments and lag processes, which make the immediate effects altogether different from the adjustment over time in many cases.

Direct Channels

The direct channels of influence are not only dependent on the nature of the commodity, but also on the operation of the economy and on the linkages between the domestic sector and the world commodity market.

The initial linkage is the relationship between the world commodity market and the producer country primary commodity sector. Linkages are from the world price to the local export price to the domestic product price. Along this channel the impact may be amplified or reduced. In the case of Brazil, it appears that fluctuations in the world coffee market have an amplified impact on the price received by coffee producers, whereas in the case of Ivory Coast the management of the coffee producer price tends greatly to stabilize the fluctuations.

Of course, movements in price do not translate proportionately into changes in revenues, but in the absence of fluctuations in production in the producer country, movements in revenues are dominated by movements in price.¹ The direct impact of changes in revenues falls disproportionately on non wage income. This follows from the less than proportionate

¹The countries studies which are listed in the Introduction consider movements in production as well as price. We concentrate here only on price changes.

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adjustment of employment to output; indeed, if there is no change in production there is no change in employment. Moreover, in those cases where there is an impact of revenue or price on wages--for example, in Chile and Zambia--the effect is less than proportional and lagged. As a consequence, the dominant channel is through the revenue flow to non wage income, either profits of the producers or receipts of government or both.

Since the product is sold in world markets there is also a direct impact on earnings of foreign exchange.

There are also direct requirements for inputs of services, raw materials, and construction. The statistical analyses suggest that these impacts are more pronounced in the economies where the primary commodity sector obviously is dominant, for example, Zambia or to a lesser extent, the Ivory Coast. But they are also present in the larger, more diffuse economies.

Indirect Channels

The indirect channels comprise private demand responses, private supply responses, and the passive policy channels, which may have some demand and some supply consequences.

While the private demand effects are the most intuitively obvious channels, they are not always the most important. The typical demand linkage would be the flow of wage income into consumer demand. But as we have noted, the dominant impact of fluctuations in earnings from primary commodities is

likely to be, at least initially, on non-wage income. While the latter appears in the consumption function of several of the macro models, it carries a much lower propensity to consume than does the income from wages. Interestingly, the demand effect may be much higher to the extent that the non wage component of commodity earnings takes the form of government tax receipts. The concept of passive policy response in the form of public spending from public revenues suggests that the public propensity to spend is high, approaching unity. Some of the expenditure goes into public sector consumption, and some appears in the form of public investment. In Brazil, for example, induced public investment in infrastructure improves the supply potential of the agricultural sector. In any case, government receipts and expenditures represent an important channel of influence in most of the models.

Another important channel operates through the foreign balance. The countries differ somewhat in the response of imports to foreign exchange availabilities. In the relatively closed economies, Brazil or Chile in the period before Pinochét, foreign exchange availabilities represent a significant constraint on imports, so that foreign exchange earnings are quickly transformed into added imports. On the other hand, in the Ivory Coast, a very "open" economy, the foreign exchange constraint does not appear to be binding so there is little responsiveness of imports to foreign exchange earnings. But on the other hand, in the Ivorian case, there is a powerful link between income and imports. This has significant consequences not only for the foreign exchange balance itself, but also

for the monetary sector. The linkage here is through the effect of the balance on current account on the monetary base, assuming again a passive monetary policy. But the impact is not necessarily from an increase in the value of commodity exports to a more favorable balance on current account and an increase in the monetary base since the response of imports to greater foreign exchange availabilities may exceed the growth of exports. In other words, the linkage from changes in export revenue to money supply is clear, but the direction depends on the response of imports. The more constrained are imports by the availabilities of foreign exchange, the greater the likelihood of a significant import response and the less the impact on money supply.

A similar phenomenon occurs, but in the opposite direction, with respect to government expenditures. Increased government receipts may translate into a reduced deficit (or even a surplus) causing a reduction in the monetary base in the absence of active policy. But the impact may be largely offset by increased purchases. In that case, the Keynesian demand side effect is reinforced by the impact of the increased monetary base.

This discussion leads directly to the impact through the effect of money supply in these systems. This is an important channel in many of these models through the inflationary potential which originates in growth of the monetary base. Thus, in many of the countries considered, with the striking exception of the Ivory Coast, improvements in the value of commodity exports translate to a significant extent into accelerated inflationary pressures. Inflation is also influenced by labor costs

and exchange rates. While exchange rates are largely exogenous in the models considered here,² labor costs are an important ingredient. The linkage is not only through productivity, but also through the role of wages in the primary commodity sector on the other sectors of the economy. Wages in copper are a dominant factor in the determination of wages elsewhere in Zambia, for example, but not in the Ivory Coast where wages influence migration patterns.

Section 3 Impact of Commodity

Market Fluctuations on Goal Attainment

The main issue is how the movements in primary commodity markets affect the ability of the producer economies to achieve their economic objectives. The evaluation of this question in quantitative terms has been the purpose of our studies. We summarize and, if possible, generalize the results in this section.

The goals to be attained have been considered in detail in the project. They include not only the traditional focus in the developing world on economic growth, but also other considerations such as price stability, equity in the distribution of income, balance of payments equilibrium, and utilization of productive capacity. The attainment of these goals is influenced by the producer country's participation in primary commodity markets in various ways. The aim of our simulations of the world commodity market--micro--macro systems is to evaluate the impact of fluctuations and secular trends in commodity markets on the various dimensions of goal attainment.

²The impact of commodity export values on exchange rates may be a very important channel but determination of exchange rates is outside the scope of most of the models considered here.

Simulations were carried out assuming one-time changes in the commodity price as well as similar changes on a sustained basis. The results of these simulations are evaluated in comparison to a base simulation, lacking the price change. These simulations are conveniently summarized in Table 1. The table shows the impacts for the average of the first five years after the disturbance. In the first column are shown the consequences of a 10 percent one-time drop in the price of the commodity. In the second column are shown the results of offsetting the 10% drop with a 10% increase, in other words, the asymmetrical effect between a decline in price and an increase in price. Columns three and four show the impact of a sustained 10% drop in commodity prices, column 3 summarizing the average over the years 1 to 5 and column 4 for the years 6-10. While there is divergence between the countries and the commodities, we observe that there are some significant patterns of response. On the basis of Table 1 we summarize some of these patterns here.

Growth and Capacity Utilization Impact

Growth and capacity utilization of commodity price changes are in the direction anticipated in all but a few exceptional cases. A reduction in price reduces GDP and, almost but not quite symmetrically, increases in the commodity price tend to increase GDP. The macro economic impact, not surprisingly is closely related to the share of the primary commodity sector in the economy. Therefore, there is very little perceptible impact on Brazilian GDP in percentage terms, but not in absolute

terms, from the coffee sector. On the other hand, in Chile and Zambia where the dependence on copper is far greater, the impact on GDP is much more apparent. However, the effect of lower coffee price on the Ivory Coast appears remarkably small, considering the importance of the product, a result of the fact that the Ivorian coffee producers are substantially isolated from the world coffee market by a stabilization board.

A sustained price change has a substantially greater and growing impact than a one-time reduction. In part this simply reflects the nature of output determination in these industries, with output responses spread over a long time period after the change in price. Thus, a 10 percent lower price reduces GDP by 1.8 percent during the first five years and by 3.1 percent during the following five years in Chile. Impacts in Zambia and El Salvador are comparable. On the other hand, even in the long term, the impact on Brazilian growth is hardly perceptible and the effect on Ivory Coast GDP is small at first, rising to minus 1.0 percent during the second five years as the effect of low coffee prices on the stock of mature trees becomes effective.

There is no question that conditions in the world coffee market feed through to macroeconomic goal attainment. One time shifts in prices as well as secular shifts have effects on activity and growth beyond the sectors in which they occur. What is not so clear is whether there is potential or actual impact from fluctuations in commodity prices on growth. Some evidence is provided by the offset of symmetrical up and down price simulations. That evidence suggests that over a cycle the impacts on GDP tend to cancel out. Such a result was sustained in other simulations, reported in the specific country studies, which indicate

little if any impact of commodity price fluctuations on the growth aspect of economic performance, with the exception of the case of Ivory Coast. This should not be taken to mean, however, that price fluctuations impose no costs on the producing economies. There are clearly dislocation or adjustment costs other than those measured in the growth of aggregate GDP. And it can be argued that stable commodity prices would permit the development and implementation of investment plans in ways which our models, based on an unstable world, do not measure.

The results above are also supported by the impact of changes in commodity prices on investment. Again, the importance of the commodity in the economy and the dependence on investment on the commodity earnings are significant considerations. Again, the strongest effects, are apparent for the copper countries. Chilean investment is reduced by 0.9 percent with a one time 10 percent decline in copper prices. In Zambia the 2.5 percent impact is even greater. In these countries not only do copper earnings play an important role in investment, but there are direct linkages between activity in the copper market and investment. A noticeable asymmetry appears in the case of Chile where a reduction in price has a greater negative impact on investment than does the positive effect of an increase in price.

Over a long period, the effect of sustained changes in price is considerably greater particularly in the case of Zambia where a 10 percent reduction in the price of copper results ultimately in a more than 10 percent (13.9 percent in the second five years) decline in investment. This points to the dependence of Zambia on copper earnings and the magnified volatility of these earnings with respect to the price of copper. The impact on GDP growth is considerable, of course.

The coffee producing countries are again less affected, with very little aggregate impact on Brazil, a country which is not very dependent on coffee earnings for investment.

Economy-Wide Prices

The impact on overall prices operates through a number of channels as we have outlined above. There is, some diversity of impacts, particularly in terms of the dynamic adjustment over time. Initially, the effect of a reduction of the commodity price is negative, reflecting the translation of an adverse impact on the balance of payments on the money supply through the passive monetary policy channel. Note that the impact is much smaller in Ivory Coast because of the offsetting action of the stabilization fund. What is striking is that particularly for Chile and Ivory Coast there is a catchup--the initially lower prices are offset by higher prices in subsequent years and the long term effect of a reduction in commodity prices is not necessarily negative. Indeed, in Brazil and Chile, the effect is positive over a five year period. Part of the mechanism of explanation is the offset of the monetary impact by the structural, unit labor cost, phenomenon. The reduced GDP causes a decline in productivity with a consequent increase in labor costs since wages are sticky. With some time for adjustment, this effect in certain countries dominates the monetary impacts caused through the balance of payments and the public deficit. Another part lies in the great sensitivity of imports and of government deficits to the availability of foreign exchange and government revenues from the commodity sectors. If these are quite responsive, changes in them can dampen considerably the impact on the monetary base of the original movements in the international

commodity prices and earnings from commodity exports.

The effect of a sustained decline in commodity prices, tends to be negative (except in the case of Chile) so that repeated negative impacts on the balance of payments cumulate in reductions in the money supply and these tend to dominate other impacts on the price level. With respect to the question of asymmetry, it appears that the upward and downward price effects are similar except in the case of Brazil where it seems that the net effect of a positive and negative change in prices is a positive impact.

Income Distribution

Because of data inadequacies, we generally have not been able to surmount the difficulties in the related computable "general equilibrium" model literature regarding the representation of individual household income distribution effects. To represent the links through strong fixed coefficient assumptions as in most of this literature obviously is not very fruitful. Therefore, although we experiment with some more extensive representations in some models (e.g., Ivory Coast), we focus on the factorial dimension (e.g., wage versus non-wage income) and on the sectoral dimension (particularly regarding relatively poor agriculture).

The effect of a price reduction in commodities tends to be favorable to the wage share in the short run in view of the more volatile direct commodity production sector response of non wage income from commodity production in most cases. But there are offsets from the macroeconomic impacts. Thus, we find that in the short run the impact in Chile and Brazil is to increase the wage share of GDP though Zambia shows a somewhat sur-

prising reduction. In Ivory Coast, the offsetting operations of the stabilization fund account for the small effect. Since the focus of much of the immediate effect is in the commodity producing sector itself, the imperceptible short run effect in Brazil is not surprising given the relatively small size of the coffee sector.

Over the longer run however, the lower price of commodities, if sustained, reduces the activity in these major producing sectors and the consequence turns out to be lower employment and, in some instances, lower wage rates and a lower wage share. This is a very clear impact, particularly in Ivory Coast where a 10 percent lower coffee price ultimately reduces the share of wages in GDP by more than 10 percent. In Zambia, where copper is very important, we do not observe the effect as clearly. In the first five years of a sustained reduction in copper prices, the share of wages declines, partly as a consequence of effects on employment and partly as a result of the impact on the wage rate and on the non wage components. But over the longer term, the decline in other sectors of the economy is even greater so that the wage share is moderately increased, though, of course, out of a smaller aggregate GDP. We also note that although increases in the terms of trade generally improve goal attainment in the producing countries, the impact on the wage share generally is regressive (and vice versa).

The impact of price changes on the share of agriculture in GDP depends on the one hand, on the direct impact affecting the coffee sector or the copper sector, and on the other, on the effect on non-agricultural GDP. Both in Chile and Zambia, since the direct impact is on copper, a reduction in copper prices has a positive effect on the share of agriculture. This impact is moderate in the case of a one-year price change, but it

amounts to two or three percent in the case of a sustained change reflecting the downward adjustment of commodity production in response to price as well as the feedbacks which predominantly affect the non-agricultural sectors. Of course, in the coffee producing countries the dominant tendency is the opposite. A reduction in the coffee price tends to reduce the share of relatively poor agriculture despite some negative indirect effects on the rest of the economy.

Foreign Position

In all of the countries, a one time reduction in the international commodity prices reduces earnings from the commodity, overall export earnings, and foreign exchange reserves. However, the countries vary considerably in how vulnerable they are to such foreign exchange reductions. During the sample period, Chile and Brazil were relatively susceptible to foreign exchange cuts and Zambia was becoming increasingly susceptible. Generally, the more susceptible economies have policy responses that economize on foreign exchange utilization in such a case by, for example, increasing quantitative restrictions on imports. Of course, if the commodity price reductions are sustained long enough, even the more open and less foreign exchange constrained economies like Ivory Coast and El Salvador have to introduce policy measures to accommodate to the new relation of lower commodity export prices.

Other Simulations

This brief summary does little justice to the results of a large number of other simulations which are reported in the various studies comprising this project. Such simulations include a variety of studies measuring the impact of variations in production, one-time variations

attributable to the varieties in coffee yields or to labor troubles in copper and longer-term increases and decreases in production which may result from the development of coffee lands or the development of new mining investments. The simulations also include stochastic simulations measuring the impact of price variability over a period of time, calculations of the competitive impact of alternate crops such as cocoa in Ivory Coast, alternative scenarios on export determination for Brazil, mixed development strategy scenario simulations for Ivory Coast, Zambia and Chile, simulations related to the trade interactions between the countries of the Central American Common Market, etc. The reader is referred to the individual studies listed in the Introduction for the rich results from the variety of simulation exercises that were carried out as a part of this project. The reader should also refer to these underlying studies to find the basis for results reported above some of which may appear at first glance to differ from a priori expectations.

Section 4 Passive and Active Policy Impacts

The study shows that what we have termed passive policy provide an important channel, in many cases the most important channel, for the transmission of commodity market phenomena to the macro economy. The passive policy channels are not just simplifying behavioral assumptions of the econometric model. In many instances, behavior of the public sector does follow certain patterns which represent passive responses to changes in the economic environment. And these responses are incorporated in the passive policy channel. A most important one, of course, is the response of public expenditure to the loosening (or tightening) of the budget constraint as additional (less) tax revenue or public enterprise profits

are translated into public expenditures for consumption and investment. The money supply response to the public budget deficit (surplus) and to the foreign exchange deficit falls into the same category of passive policy, and a third category includes the foreign sectors policy response like those discussed above.

This is not to say that passive policy may not be offset at least partially by active policy steps. In many cases, active policy incorporates measures different from, and often opposite to, a passive policy. For example, neutralization of foreign exchange earnings from commodity exports represents a direct active response to offset the monetary expansion associated with the increased earnings from commodity exports.

Moreover, in principle at least, active policy need not be limited to such direct policy responses. More general policies may be used. For example, active fiscal, tax, and monetary policy may be used to offset the impact of movements in primary commodity markets on real and/or nominal stability. Likewise tax, income and price policies can be used to offset undesired distributional outcomes. And in theory, various policy instruments can be combined to more nearly achieve desired goals--although lags and uncertainty complicate actual policy formulation substantially.

Section 5 Policy Alternatives and Goal Achievement

Various policy alternatives are available to deal with the effects of commodity exports on the producer country economy. We cannot here, discuss in detail the various patterns of policy alternatives considered in the underlying country-commodity studies. We have considered three

types of policies: those focusing on the producing sector itself, those concerned with the international commodity market and those focusing on the macro economy. All these approaches offer potentials, but the quantitative dimensions of their effects vary depending on the economy, on the role of the commodity in that economy, and on the passive policies in force.

In this study we have been able to examine a limited selection of active policy measures, particularly of a broad macroeconomic type. On this basis we come to several important conclusions regarding the effectiveness of active policy.

First, the abilities of the various countries to make use of active policy vary greatly. This potential is quite limited in the open economies, for example, Ivory Coast and Central America, or in those dominated by the commodity sector, like Zambia, for example. Other economies, where commodity markets play a smaller role or where the economy has a great degree of self-containment, may be able to exercise effective active policy measures to offset the impacts of change originating in world commodity markets. Thus, possibilities for macro policies in Ivory Coast are limited, in view of the open character of that economy, whereas the passive policy of the stabilization board offsets some of the impact of fluctuations on the world coffee market. In Brazil, on the other hand, even though the fluctuations in the coffee market are amplified by micro policy, macro policies may effectively offset the impacts particularly since the coffee sector represents a small part of the economy.

Second, although general policies can be used to offset any one negative effect of changes in commodity markets, generally they also

change attainments of the other goals--and sometimes in negative respects. The set of policy instruments is not sufficiently great nor are the policies sufficiently available or powerful in most cases to permit simultaneous attainment of the various goals of the producer country economy. Their impact is complicated not only by the simultaneous nature of the economic systems but also by the lags in response. The net effect of using such active policies to try to offset the commodity market changes is difficult to anticipate and to control, even without external stochastic shocks.

Third, macro policy measures are not likely to effectively offset sustained secular shifts in the international commodity markets. Eventually, the deficits (surpluses) build up enough so that foreign exchange movements cannot be sterilized or that fiscal or monetary policy measures become counter-productive in respects to issues other than those concerned with their commodity market impacts.

With respect to policies on the commodity sector level, we can distinguish between those that can be carried out independently of a world market impact and those which do affect the world market, i.e., the ones where the producer country has market influence.

In the absence of a perceptible effect on the world market, the producer country policies can have substantial impact both in the stabilization of its revenues (or those of its producers) or in changes in supply potentials. In this study we have explored many of these possibilities. The effect of the price stabilization for coffee producers in Ivory Coast is particularly notable. The stimulus of increased producer price on production of coffee in Ivory Coast for example, is also notable, as are the effects of additional investment in the copper industry in Chile and Zambia. Of course, the potentials

of internal commodity sector policies are constrained by the availability of resources in the case of investment in copper mining for example, and by the competition of other uses of the land in the case of coffee.

The situation becomes considerably more complex when the country has market influence. In most of the cases studied we found that moderate fluctuations in the producer country had only moderate influence on the world market price, though such influence cannot be ignored. This is perhaps because policy simulations of commodity supply are not of the large magnitudes that would come from natural disasters such as mine collapses in copper or frosts in coffee. It would appear from the studies carried out here that only limited gains can be sought by the producer countries through impact on the relevant world commodity market, while substantial scope for domestic intervention into the commodity sector exists and such intervention has potentials both from the point of view of stabilization and from the point of view of secular trends.

Finally, with respect to the operation of the world commodity market stabilization, this study did not consider at length the operation of buffer stock stabilization schemes on a worldwide basis, though many simulations assumed stable prices and considered the impact on the countries' economies. As we have noted the impact of stabilization on growth itself is not clear but more stable commodity prices obviously have payouts in greater stability in the producer economy and in some instances in the level of investment. This would suggest that cost benefit calculations of participation in worldwide commodity price stabilization schemes need to take into account the broader impacts of stabilization on producer country goal attainment.

The management of macro and micro policies poses difficult challenges for the producer countries, particularly those which are dependent on one or two primary commodities for the bulk of their foreign exchange earnings. The potentials of these policies to ameliorate the undesirable impacts of instability and adverse secular trends vary greatly between the producer countries. Our work would suggest that policy responses embodying various kinds of policy must be considered in evaluating the impact of commodity markets on producer country goal achievement. Macro and micro policies must be taken into account in making recommendations for commodity policy management. Indeed, they must be part of an integrated policy program.

TABLE I

Some Summary Percentage Changes in Goal Attainment of Developing Country
Producers Induced by One Period and Sustained Changes in
International Commodity Prices^a

	<u>One Period Shock</u>		<u>Sustained Shock</u>	
	<u>10% Drop</u>	<u>10% Symmetrical</u>	<u>10% Drop</u>	
	(avg. yrs. 1-5)	(avg. yrs. 1-5)	(avg. yrs. 1-5)	(avg. yrs. 1-5)
<u>GDP</u>				
Chile	-.8	-.1	-1.8	-3.1
Zambia	-.4	.0	-1.9	-2.8
Brazil	.1	.1	-.0	-.2
Ivory Coast	-.1	.0	-.1	-1.0
El Salvador	-.6	.0	-2.0	-3.6
<u>INVESTMENT</u>				
Chile	-.9	-.5	-1.7	-2.8
Zambia	-2.5	-.1	-10.3	-13.9
Brazil	.1	.3	-.0	.0
El Salvador	-.4	.0	-1.2	-2.2
<u>PRICES</u>				
Chile	.9	-.2	-.7	7.9
Zambia	-1.2	.0	-5.1	-4.0

table 1 cont'd

	<u>One Period Shock</u>		<u>Sustained Shock</u>	
	<u>10% Drop</u> (avg. yrs. 1-5)	<u>10% Symmetrical</u> (avg. yrs. 1-5)	<u>10% Drop</u> (avg. yrs. 1-5)	(av. yrs. 1-5)
Brazil	- .4	- .9	- .8	-4.9
Ivory Coast	- .1	.1	- .4	-1.3
El Salvador	- .2	.0	- .8	- .9
<u>AGRICULTURAL SHARE IN GDP</u>				
Chile	.7	.1	1.6	3.2
Zambia	.3	.0	1.7	2.4
Brazil	.0	- .1	- .1	- .1
Ivory Coast	---	---	- .1	- .6
<u>WAGE SHARE IN GDP</u>				
Chile	.2	.7	- .5	-2.8
Zambia	- .4	.0	- .8	1.0
Brazil	.0	.1	.1	.2
Ivory Coast	- .1	.0	- .6	-11.0

^aThe percentages in this table are based on a comparison of base simulations without the price shocks and otherwise identical simulations with the price shocks.

International Primary Commodity Markets and
Economic Development: Analysis of Basic
Policy Issues - University of Pennsylvania

Dr. Thorbecke, who had been chairman of the RAC sub-committee that reviewed this project when it was considered by RAC, reported that the project appeared to be progressing well. However, he was concerned about linkages of the model to various commodity models. Additional materials are needed to evaluate this aspect of the project.

Dr. Keith Jay (IIA/EA) reported that his office has received about a dozen different documents which had been prepared in draft form for particular AID staff members. Such documents could be made available. Dr. Jay also expressed some concern over how the models would be linked to commodity models.

Dr. Smuckler requested that the materials be provided to Dr. Thorbecke who would review the progress of the project for the RAC.

International Primary Commodity Markets and Economic Development

Dr. Smuckler reminded the RAC that approval of the project, International Primary Commodity Markets and Economic Development, had been delayed until a revision of the proposal that took account of issues raised by RAC could be prepared. The RAC also wanted assurances that the manner in which the time of one of the principal investigators was divided between this project and other AID projects, the Rockefeller project, and other projects in the Commodity Market proposal was acceptable to the AID Contract Office.

Dr. Rechcigl reported that the Contract Office had provided a memorandum stating that the problem of the division of the principal investigators time was settled to their satisfaction.

Dr. Thorbecke, who was not present at the RAC meeting, had sent a letter to Dr. Lorenzo Perez, AID/FPC stating that he felt all of the issues raised by RAC about this project had been taken into account. He was also satisfied that the budgeting of the principal investigators is acceptable to the AID Contract Office. He was still concerned that Dr. Behrman may be overcommitted with projects.

Dr. Thorbecke suggested the possible alternative of approving the first nine or twelve months of the proposal and calling for a critical review of achievements at the end of this first stage before approving the next stage.

Dr. Montgomery thought it was important that RAC have assurances that the project could not be done without this additional funding from AID.

Dr. Long added that Dr. Behrman was involved in a mixture of activities but that he must work for AID on that part that AID pays

Dr. Michalopoulos explained that his office had carefully examined the division of Dr. Behrman's time on the various projects that he was involved in and concluded that this was truly an additional project. He felt AID need only make sure inputs are made and outputs are what we ask for. Behrman is one of several people working on the project. He hoped RAC would approve the entire project. Otherwise it would be necessary to split the project and assume an extra management workload within AID.

Dr. Swanson stated that he too had reviewed the revision and felt sure the issues raised by RAC had been taken into account. He thought some RAC monitoring of the project might be useful.

Dr. Montgomery suggested that a PPC report discussing the working arrangement with Behrman was all that was necessary.

The following motion was proposed and seconded:

Motion: That the project be approved as revised. AID staff is to report to RAC at its next meeting on the additive nature of the project's separately funded elements.

Vote: The motion carried unanimously.

Dates for Next RAC Meeting

The Committee approved November 30 and December 1 as the dates for the next RAC meeting.

Arrangements for Site Visits

The chairman recognized Dr. Schweigert who discussed some observations from a recent site visit to a project in Guatemala.

Dr. Schweigert described briefly the visit and then made the point that such trips probably would be more objective if the AID technical offices were not involved in the reviews. He suggested that the arrangements for site visits probably should be made by the Research Office (TA/RES).

Dr. Wishik, who was also on the site visit, added that former participants in projects should not be invited to go as official reviewers. Reviewers also should not include other business on site visit trips since this often interferes with the primary purpose of the trip and is disruptive to other reviewers.

Dr. Long pointed out that there are two types of site visits. Some visits are for technical evaluation of projects while others are for the purpose of monitoring the project by the technical office. He added that Dr. Schweigert's comment was a topic discussed in the executive staff meeting. In Dr. Long's opinion, it was difficult to get RAC involved in such visits often enough. AID people may go along on technical evaluation trips but should not be official reviewers. Dr. Schweigert agreed that some AID personnel should accompany technical reviewers.

Application of Radio Teaching In Elementary Mathematics in Nicaragua

A memorandum from Dr. Rechcigl concerning a change in the minutes from the March 31-April 1 meeting was brought to the attention of RAC by the chairman. The Office of Education and Human Resources did not agree with the minutes as they had been approved at the May 18-19, meeting.

A motion was made and seconded that the minutes for the project, Application of Radio in Teaching Elementary Mathematics in Nicaragua be changed to show the first part of the motion that was approved on May 18-19 to read as follows:

Motion: That the proposal be approved with the recommendation that

- (1) the essentials of the original design be reinstated and
- (2) efforts be concentrated on grade 1-4 with possibility of outlines for grades 5 and 6.

Vote: The motion was passed unanimously.

Minutes of the March 31-61-April 1, 1977 RAC Meeting

International Primary Commodity
Markets and Economic Development: An Integrated
Econometric Analysis of Basic Policy Issues -
Wharton Econometric Forecasting Associates, Inc.
- University of Pennsylvania

Dr. Thorbecke, Chairman of the subcommittee composed of Drs. Heady, Montgomery, and Swanson, reviewed the project as follows:

The questions which must be raised in connection with the proposal fall under the following headings:

- (1) The importance and relevance of this research topic to AID and the development community in general.
- (2) The substance, methodology and critical merits of this proposal - including the competence of the principal investigators to obtain useful results.
- (3) Considerations regarding the budget and the research management of this proposal.

Appropriateness of Research Area to AID and the Development Community

AID has a role to play in advising the U.S. government on commodity trade issues. Better insights of the effects of commodity exports on LDC's domestic economies would help AID fulfill its role. Therefore, the subcommittee is convinced that, the research area is appropriate to AID.

Other organizations, particularly the World Bank are involved in research activities in the broad area of commodity models and their linkage to national macro-economic models. However, the area is not saturated and high social benefits are likely to accrue

from more research on the subject, particularly from additional research on the linkages between commodity exports, the disaggregated pattern of employment and the factorial and household income distribution.

Substance and Methodology of the Proposal and Evaluation of Likely Results

The investigators intend to analyze and obtain better insight on "the direct and indirect effects of primary commodity output and exports on income, employment and income distribution in the LDC's, through the linkages between the commodity - producing sectors and the rest of the economy of developing countries". They will also research the impact of various aspects of commodity markets on national development goals. The researchers argue strongly for more disaggregation in commodity models and in the national macroeconomic models to which they are attached so that an analysis of alternative national and international policies can be studied. Further disaggregation would permit an analysis of the intranational effects of exports.

The proposal can be broken into two distinct but interrelated parts:

- (1) The modeling of international commodity markets for specific products and of national macroeconomic models for selected countries. The national macroeconomic models would be linked

to the international commodity model.

(2) The design of disaggregated country models which are linked to the corresponding national macroeconomic model.

The first part would permit one to follow the effects of changes in the international markets on key macroeconomic variables for specific countries. This part would also permit the analysis of the reverse, i.e., of the effects of changes in aggregate national commodity exports on international commodity markets. The second type of linkage is essential to exploring the effects of national macroeconomic changes on policy objectives, such as income distribution, which can only be analyzed in a meaningful way within the context of a disaggregated national model.

The two principal investigators have been at the forefront of building international commodity models and linking them to aggregate national macroeconomic models (the first linkage). However, it is the second linkage, where little research has been done, that is essential in determining the effects of changes in commodity exports on national policy objectives. The principal investigators have provided almost no discussion regarding the design and specification of disaggregated country models within which this second linkage could be analyzed. Since the investigators comparative advantage lies in macro-modeling, a discussion of what they intend to do at a higher level of disaggregation is much more important. In the absence of such discussion,

the subcommittee feels that more information on the proposed methodology is essential before the project can be endorsed.

Two lines of research ^{1/} relating to the second linkage has been explored in detail. Both research avenues rely heavily on policy oriented disaggregation of the major variables, such as a detailed Social Accounting Matrix.

Budget and Related Matters

Two questions on the budget and related matters were raised. First, the two principal investigators are involved in at least a half a dozen other projects which appear to be highly related. The members of the subcommittee would like to be reassured that AID is "buying" a research project that does not duplicate other projects which are already financed and underway.

Secondly, the overall budget of \$372,000 over two years seems high. The principal investigators as consultants are budgeted for 40 days during

^{1/} As an example, see Irma Adelman and Sherman Robinson, "A Wage and Price Endogenous General Equilibrium Model of Developing Country: Factors Affecting the Distribution of Income in the Short-run", applied to South Korea and, as an example of the second type, Graham Pyatt and Erik Thorbecke, Planning Techniques for a Better Future, (Geneva, 1976) and, by the same authors, Planning for Redistribution, Employment and Growth, (unpublished manuscript, 1976).

the summer and 36 days during the academic year. The charges during the academic year are difficult to justify. The justification, that this avoids payment of overhead to Wharton Associates and does not significantly change AID costs, warrants some discussion.

Dr. Heady asked if one day per week of the principal investigators time was enough for him to be effective. He wondered just how the allocation of the principal investigators time would be made. He also raised the question of whether the project should not include additional commodities in countries other than those in which the principal investigators were already working.

Dr. Montgomery questioned whether the proposed work was sufficiently different from other Berman projects to be considered a separate project. He pointed out that the project would be considered successful if everything that was proposed were actually carried out. Analysis at the country level was carried out. He felt that it would be advisable to limit the project to country analysis.

Dr. Swanson thought that there was a need for greater detail on the international part. How would it be done? Perhaps through mathematical modelling? Where would the data come from for time series analysis for Zambia and the Ivory Coast?

Dr. Anderson asked whether the principal investigators were really knowledgeable in the theory on which they must rely to carry out the project.

Dr. Peterson asked how the results could be used.

Dr. Thorbecke remarked that the competence of the investigators was unquestioned. He felt that if the proposed methodology would make it possible to say anything about changes in patterns of income distribution that were the impact of new policies than the research would certainly be useful.

Dr. Black wondered if the proposed model could predict the price of coffee.

Dr. Thorbecke replied that some models of commodity markets had done well at predicting world prices. Cocoa was cited as an example.

Dr. Heady pointed out that at present such models could not predict distribution of income. When commodity prices are increased who benefits, the growers, the middlemen?

Dr. Perez explained that to understand the nature of the problem one must realize that the idea is to look at commodity prices and their relation to the rest of the economy. The principal investigators would try to link an international model to national models. They have been trying to link household, international commodity markets and country models.

With econometrics a behavioral equation can be specified. The econometric model would be the main tool supplemented with other tools such as accounting methods.

Berman and his colleagues do not wish to down play the difficulties in the project. They are willing to disaggregate the national model to capture effects on income distribution. It is a question of availability of resources to study all these issues.

Dr. Perez also pointed out that it is very difficult to measure additionality of their project to other Berman projects. For example, the Rockefeller model is very disaggregated and in all likelihood Berman will not construct another model but will make the Rockefeller model available for this project.

The principal investigators have developed a plan for University of Pennsylvania faculty with pertinent experience on the project. A research team will be formed in this manner.

Dr. Perez is sure that the work can be done with the data base. Berman is certain in the case of the data for Latin America, Central America, and Asia, but is not certain about Africa where he may not have a data base.

The fact that Berman's name appears as a consultant can be explained. AID wrote an in-house proposal and requested two consultants to review the proposal and discuss it at a seminar. Berman was one of the consultants. After the seminar, proposals were requested.

Dr. Heady pointed out that it would be useful to explain the use of the principal investigators as consultants on the project, not as researchers.

Dr. Perez explained that ultimately Berman is the principal investigator although he and Adams will work as a team on methodology. They will hire the University of Pennsylvania faculty as consultants. They also want to hire 1 full-time economist for 2 years and some country experts.

Dr. Montgomery asked that it be put into the record that AID is reminded that double payments from more than one project is serious. The University is not responsible alone. AID should be very careful to establish that this project is in addition to the principal investigators other projects. This is not a criticism because it is appropriate and should be clear.

Dr. Michalopoulos reiterated the point that AID would benefit from paying the consultants directly rather than through the University. The University's overhead would cost more than consultants fees. Dr. Berman can't take off to do the research himself because of University restrictions. He will take some leave and will pay other faculty to do some of the work.

Dr. Anderson asked what could be done if some impropriety were discovered regarding double payments for the same work.

Dr. Michalopoulos said it would be a serious offense but he did not know what could be done.

Dr. Long remarked that he was disturbed about the implications of impropriety. The contract office would have to sort out any problems before the contract is awarded. The only retroactive action would be a lawsuit.

Dr. Perez did not think it appropriate to question whether the Rockefeller Foundation and AID would pay for the same work. The project has a budget. Criticisms could be made that not enough time would be available for the project, but it would be inappropriate to suggest impropriety at this stage.

Dr. Thorbecke stated that he had checked with Drs. Long and Rechcigl and the project manager on three different occasions. Originally he had considered asking for some modification and that the project be re-submitted to RAC. Upon reflection and after talking with Drs. Long and Rechcigl, it was decided that it would speed things up to have the project resubmitted to the subcommittee.

Motion: Dr. Thorbecke moved that the proposal be resubmitted to the RAC subcommittee after taking into account the questions and reservations which were raised in the subcommittee report. The revised proposal would require, in particular, the following items: (1) A detailed discussion of the methodology which would be used to explore the link between a national macroeconomic model and disaggregated changes within countries. In this connection, it is essential that the principal investigators indicate how they expect to treat the whole income distribution question; (2) The relationship and the division of labor between the present proposal and other related projects in which the principal

investigators are engaged, be clarified and be made much more explicit. A stronger assurance must be given that funds for this project will pay for a truly additional output; and (3) Some assurances are obtained from the project manager and the management of AID that the way the principal investigators' time is budgeted (as consultants on a daily rate basis) is appropriate.

Dr. Heady seconded the motion.

Discussion: Dr. Smuckler asked if there were any procedural problems with resubmitting the project to the subcommittee rather than the entire RAC.

Dr. Schweigert asked if this is not contrary to the Smuckler policy.

Dr. Long pointed out that in the past all manner of relationships between RAC and its subcommittees had been established. When there are methodological flaws it is quite appropriate, if RAC so wills it, for the project to be resubmitted to the subcommittee.

Dr. Smuckler (after the motion passes unanimously) ruled that the subcommittee will review the project again after the changes given in the motion were made and would report back to RAC.

Dr. Long expressed the hope that the subcommittee would be active with the AID staff and that the subject of impropriety would be

International Fertility Research Program (IFRP) International
Research Program

I. SUBCOMMITTEE REPORT

Dr. Connell, chairman of the subcommittee, that also included Drs. Linder Montgomery and Wishik reported as follows:

IFRP was established on July 1, 1971. Its goal was to conduct comparative field trials on new means of fertility regulation primarily in developing countries. In order to accomplish this, IFRP developed an international network of more than 250 collaborating investigators working in over 30 countries. Data collection was preceded by the establishment of standard methods of gathering and reporting clinical data. The earliest work by IFRP documented the short-term safety and effectiveness of a number of fertility regulation methods. It also pointed out the relative appropriateness of the different methods and procedures for people living in a variety of cultural and medical environments. Finally, IFRP disseminated the information generated by these trials, and helped to improve developing-country research capabilities.

IFRP has conducted straight and comparative field trials in six major areas: 1) systematic contraception including oral preparations, 2) intrauterine contraception, 3) menstrual regulation, 4) pregnancy termination, 5) male sterilization, and 6) female sterilization. The emphasis has shifted from time to time, but support to continue work in these same general categories is currently being sought. In March, 1977, a renewal proposal was reviewed by RAC. A number of serious issues were raised and funding was not approved pending further study. In September, 1977, a RAC subcommittee visited IFRP. During the site visit, the subcommittee looking into the validity and reliability of methods of data collection and analysis. It concluded that while the data were generally satisfactory, they had not been subjected to more than rudimentary analysis.

The subcommittee also considered proposals which would have expanded IFRP's functions to include studies of alternative community-based delivery systems, program evaluation, program administration and implementation strategies. In the view of the subcommittee, IFRP's capability for doing this type of research was extremely limited and thus it felt that these activities would require procedures and skills quite different from those which IFRP had developed to date and thus should not

be undertaken. Moreover, such a change, it concluded, would take IFRP away from its original and unique role in research on family planning technology. At the conclusion of its site visit, the RAC subcommittee made a number of recommendations which included the following.

1. IFRP should set a ceiling on the number of participating centers, the amount of data to be generated, and the duration of data collection.
2. IFRP should be more selective in maintaining its repository of data for future use; it should develop more complex study designs and use more sophisticated analytical techniques in processing existing data.
3. IFRP should reassess its staffing pattern and the makeup of its consultative groups.
4. IFRP should consider the addition of a senior staff member with wide prior training and experience in basic research in reproductive biology regulation techniques.
5. IFRP should continue to place its greatest emphasis on clinical trials and the services needed for the implementation of the various fertility regulation techniques.
6. IFRP should not attempt to cover the much broader administrative community and social aspects of planning program development.
7. In the future, only the research components of the IFRP program should be reviewed by RAC.

Following receipt of the report of the RAC subcommittee and consultation with AID staff, a number of changes were made by IFRP in both staffing and administrative procedures, a number of them recommended by RAC. Those areas which contained primarily the research elements of the program were identified and a new contract was written to cover them. Thus, IFRP has support to continue their biomedical research in the area of fertility regulation as well as related research in the social sciences.

IFRP also applied for and received funds under a new grant which allowed it to carry out certain other programmatic activities. Under the grant, a considerably broader range of

activities are funded in the area of institutional development for fertility research and programmatic support for family planning services.

In September, 1980, at the request of AID, an American Public Health Association (APHA) Evaluation Team was selected. It consisted of Dr. Elizabeth B. Connell, Chairman, Dr. Donald Lauro, Sociologist/Demographer, REsearch Associate, Columbia University Center for Population and Dr. Nicholas Wright, Epidemiologist, Chief of the Contraceptive Evaluation Branch, NICHD, NIH. The team first reviewed a number of documents including annual contract and grant reports for 1978, 1979, and 1980, the current IFRP table of organization, project descriptions, research forms procedure, a publications list, minutes of the Board meetings, and copies of trip reports. After reading this material, the team made a site visit to IFRP where interviews were carried out with members of the various divisions and with individual staff members to hold more in-depth discussions relevant to their particular areas of interest. The team then met on several occasions to discuss their findings. Notwithstanding the considerable differences in the academic backgrounds and work experiences of the various team members, there was complete unanimity of opinion on all of the major issues under review and the report sent to the RAC members represents the unanimous conclusions and recommendations of all its members.

Following this extensive review and deliberation, the team concluded that IFRP was continuing to make progress in dealing with the recommendations of the RAC subcommittee but that there were areas in organization, staffing and research which could be further strengthened. Therefore, a series of recommendations were made dealing with the following areas:

1. Structure: IFRP is currently being funded by AID under two mechanisms, a contract and a grant. This has had both advantages and disadvantages for the overall IFRP program. At the present time, projects funded under the contract are almost entirely in the research category. However, there are a number of studies supported by grant monies which are also research in nature but are not reviewed by RAC. Additionally, there are a number of small projects which the team felt were handled as research projects but were best called program introductions.

2. Focus: The team felt that more attempts should be made to move beyond short-term clinical trials into field and clinical studies of the long-term impact of fertility regulation techniques in developing countries. Moreover, it is believed that grant activities, though necessarily at times deviating significantly from contract efforts, should focus in the same general directions as the contract-supported work. It felt that to use the flexibility built into the grant as a mandate to go off in a number of new directions would be a mistake.
3. Staff: The team noted the changes in staffing over the past year, particularly the shift to lower level positions. It, like RAC, recommended the early recruitment of a senior scientist with broad biostatistical and epidemiological training. The team further recommended the establishment of contractual links with local universities, including the UNCSPH, to increase the skills necessary to IFRP's research program. It felt that the presence of scientists from neighboring institutions might help to re-establish prior links with these academic groups.
4. Projects: The team made a number of specific technical recommendations regarding individual projects, as noted in their final report.
5. Technology Transfer and Training: The team believed that IFRP's interest in providing mini-computers and the necessary software packaging and training required for their effective use to LDC's was worthwhile and recommended that IFRP conduct more short-term formal, training courses.
6. Technical Advisory Committee: The team felt that, as presently constituted and utilized, the TAC did not sufficiently serve the needs of IFRP for systematic review of particular research proposals and projects, providing only an overview function. Thus, it strongly recommended that all research proposals be subject to thorough and rigorous review by a revitalized and re-oriented TAC.

This is a large, complicated and expensive project.

II. SUBCOMMITTEE COMMENTS

Dr. Linder thought there were many points about the project that could be criticized. However, at this point he preferred not to discuss the details of the project in depth. He personally thought it was a poor project of low grade science. He would go along with this review committee providing that the review is not a whitewashing of what is really being done at IFRP.

Dr. Linder pointed out that the relationship with University of North Carolina which was described in the project documents doesn't exist. It is quite clear from the previous reviews that the IFRP is professionally isolated.

Dr. Montgomery asked with so many flaws in the program, why should the PARFR program continue? Or better yet, why not continue only those activities in the program that are essential to AID's Population program? Even though the data base is not what it should be the information that has been provided by IFRP has been valuable and if this organization did not exist it would have to be invented because such an organization is necessary.

Portions of the portfolio are supervised and standardized. There is some research done in that portion. There are documents describing the program, one is describing research and one describing a technical assistance component.

Dr. Montgomery could not distinguish between the two in terms of the activities that were undertaken. He pointed out that there were reports of tension among the staff between those in research and those on technical assistance activity. Research activities are reviewed; the technical assistance activities are not. Why is IFRP in research? The leaders in the program say they need to establish credibility among scientists in the Population field. Dr. Montgomery was not sure that they added to their credibility with statements of this type.

Dr. Wishik commented that there was a need for a thorough review. Even if there were no technical reservations about the program the fact that it is carried out at great expense to AID would warrant a special indepth review. The review, in Dr. Wishik's opinion, should include an overseas trip to observe activities in LDCs.

Dr. Wishik thought the intensive review should include a statistical look at the material in the data file at North Carolina. He was wondering if just any type of data can be put into the IFRP data bank.

II. OTHER COMMENTS

Dr. Carter asked why are there no other donors to the IFRP? The program presently is almost totally funded by AID. Second, he wondered why it wasn't associated with the University. During an earlier review there was some suspicion because the IFRP printed their own journal.

Dr. Carter pointed out that it appeared to him that an enormous amount of funding was being spent and the management of that funding was somewhat out of control.

Dr. Connell did not think that the IFRP was such a monster. She pointed out that IFRP had, itself sold the journal that it published. For economic reasons there are elements of the program that were out of control but not irreversibly so.

Dr. Anderson asked how did we get into this program? Did not RAC want to look at the project carefully?

Dr. Moss said the project was extremely difficult to deal with. He asked for some of the RAC members to point out some of the excellent things that the project had accomplished.

Dr. Connell remarked that there were enough good points. There are many projects that are well conceived and well carried out. The problems in many of the areas of the IFRP program were not insurmountable.

Dr. Carter asked if IFRP had a continuing relationship with outsiders.

Dr. Montgomery pointed out that when the project was site-visited the last time it came to RAC the review committee looked at some new research proposals. New research was discouraged then because the staff expertise at IFRP was not sufficiently strong. He did not think that the IFRP had structured a process for continual appraisal of research since then.

Dr. Wishik asked what are the merits of the program? He pointed out that the breakthrough in contraceptive techniques was accomplished after the disassociation of contraception and sex. Contraceptive technology which the IFRP is field testing

can't breakthrough some of the obstacles to these family planning methods. There are only two organizations, WHO and IFRP carrying out research on contraceptive methods. WHO does not subject new methods to Phase III testing, IFRP does.

Dr. Wishik pointed out that IFRP has made sufficient contributions but it is necessary to learn more about what these contributions are. The program needs to be narrowed and tightened up and controlled better. He felt that the ongoing relationship with a RAC subcommittee was necessary. Finally, Dr. Wishik thought that the Population Office should act to set up a strategy plan for contraceptive research.

Dr. Elmendorf remarked that as a field worker who has worked for a large organization such as IFRP, she feels that this program is important. Better organization is needed but the importance of the work is beyond question. With regard to the data bank, Dr. Elmendorf felt the great need was for following through on new prospective contraceptive techniques rather than obtaining quick results. She pointed out that the IFRP had no women on its research staff.

Dr. Speidel, of AID staff, remarked that the project plays a key role in AID's population program. As an example, IFRP has provided a reliable set of guidelines on more than one occasion which were used to make decisions on the purchase and distribution of IUDs and other contraceptive devices. However, he also felt some frustration since the program was not living up to its initial promise. It has not become the outstanding field research unit that it was intended to be. Steps had been taken to resolve problems in the organization management of IFRP but the reforms were not fully realized.

Dr. Speidel pointed out that the University relationship with IFRP didn't really work out as it was planned. Initially, the field work was to be carried out with IFRP being located within the University of North Carolina. This did not work out and it was necessary to establish a separate unit. As for the funding of IFRP it was true that most of their funds come from AID. However they do have one \$600,000 grant from the National Institutes of Health. The organization is being encouraged to seek diversified support.

With regard to the research versus the technical assistance or grant part of the AID support for IFRP, Dr. Speidel remarked that AID was keeping the relatively rigorous research under contract. Grants had proven to be better for the other type of

work that the IFRP does for AID. As for a contraceptive stragtey program, Dr. Speidel did not wish to address that issue off the top of his head.

Dr. Shelton, also of AID staff, pointed out that apparently everybody agrees that some good is coming from IFRP as are some accomplishments that are not so good. He personally thought that the contributions of the IFRP are very good. It's true that almost every decision the Agency has made in the population field has relied very heavily on IFRP data. Perhaps we are making decisions on not so good data but there has been good correlation with other work. He agrees that they lack some senior people and that they need to improve the role of the technical advisor. He didn't agree with the report on the differences between the grant and research. There are definitely differences in the type of activities carried out under each. He did not think the organization was out of control. There are only 99 people working and the yearly budget is smaller than previously (2.0 million last year as compared to 2.3 million for the previous years.) A lot of these issues were much worse in 1977 when the project came to the RAC and progress has been made in resolving the problems.

III. MOTION

That the proposal be approved with a three-year authorization but only a one year approval for financing at the present time; (2) That the chairman of RAC appoint a RAC subcommittee which will have an overview function for the duration of the project; (3) That a review team be constituted. The team should have representation from RAC, AID and the IFRP Board of Directors. The team should have expertise in management, biostatistics, medicine, epidemiology and demography. Selection of the team members should be carried out jointly by the AID Population Office and RAC; (4) That the team conduct an in-depth review of the entire IFRP program funded by AID, both the grant and the contract. This on-site review should encompass the activities in North Carolina and a representative number of the overseas projects; (5) That the team write a comprehensive report of its findings and make a series of concrete detailed recommendations; (6) That this report be completed as rapidly as possible, consistent with thoroughness, but, in any event, within one year of this date; (7) That a status report be sent to RAC by the Population Office prior to its fall meeting in 1981; (8) That a final report covering the team's recommendations be made available to RAC prior to its winter

meeting early in 1982; (9) That decisions regarding recommendations for future funding and program content beyond one year be made at the time of that winter RAC meeting.

Dr. Wishik wanted to make a friendly amendment to the motion. He thought the scope of the review should include both the grant and research under activities under IFRP.

Dr. Rechciql asked about the role of the contractor in the review.

Dr. Speidel pointed out that the contractor will work with the review team.

Dr. Montgomery asked about a continuing relationship between IFRP and the RAC.

Dr. Connell reported that she did not see this as a single time involvement of the RAC. She felt that the relationship between the RAC and IFRP would continue.

Dr. Wishik pointed out that there were two ways of having a continuing relationship with the RAC, one would be to have a subcommittee within RAC to review the project continuously, the second approach would be to have outside experts plus a RAC subcommittee that would continue for the life of the project.

Dr. Anderson asked Dr. Linder why wasn't the project eliciting a collegial relationship with the University of North Carolina? Why aren't other professionals talking to them? How much of their work shows up in publication, etc.?

Dr. Linder sketched the history of the relationship between the University of North Carolina and IFRP. Originally, the project was in the medical school of the University. There were constant conflicts between the project and the University administration. The project was impatient with administrative procedures at the University. The IFRP engaged in human experiments using devices that may or may not have had the necessary approval of the FDA and others. The University was afraid of reactions to this. They did not have a formal relationship with the Biostatistics Department of the University of North Carolina. Finally, the relationship dissolved because the IFRP did not accept the advice of the members of the statistical faculty and others in the University. It was a messy sort of divorce. However, this did not prevent consultation from members of the University faculty to IFRP sponsored projects.

IV. VOTE

The motion was approved with two abstentions.

The chairman of the RAC interpreted the motion to mean that a permanent subcommittee of the RAC would be established to work with IFRP in keeping the program under review.

Dr. Smuckler will advise the Committee of the permanent chairman who would be appointed shortly.

Minutes of the April 30-May 1, 1979 RAC Meeting

Progress Report - International Fertility Research (IFRP)
- International Fertility Programme, Inc.

Dr. J. Shelton (DS/POP), was recognized and highlighted the contents of the following written report:

Since this project was last presented to RAC in March of 1978, IFRP has continued to implement the changes which grew out of the RAC sub-committee and A.I.D. evaluations of September, 1977. The major changes have included:

1. Concentration on a smaller number of higher priority research areas.
2. Simplifying the machinery for identifying what these higher priority research areas should be, including close collaboration with A.I.D. staff.
3. Through the use of task forces for each of the seven study areas, simplifying and improving the machinery for overall study plans within each area.
4. Streamlining IFRP's overall organizational structure under the leadership of the new Executive Director, Malcolm Potts.
5. Consolidating the number of research centers and focusing more on centers able to carry out higher quality research.
6. Improving the quality of the analysis and promulgation of the data.

We have observed these changes taking place, and are quite pleased with the results. To illustrate the success of these changes, we have selected two specific research studies to describe briefly in more detail.

The first study concerns IFRP's "postpartum IUD," of which the most prominent example is the "suture loop" (Figure 1). It is a standard Lippes Loop D IUD with biodegradable chromic sutures affixed to the upper crossarm. The IUI is intended for insertion immediately postpartum. Data are now available for 341 insertions from three countries (Philippines, Bangladesh and Egypt) and are presented in Tables I and II which are from an upcoming article in the Lancet. As can be seen from the tables, the expulsion rate has been remarkably low, 5.3% at six months. Expulsion of IUDs which have been inserted postpartum has been a major programmatic problem in the past. A decade ago the Population Council reported expulsion rates of 26.5% at three months when the IUDs had been inserted in the first 24 hours. An IUD which can be inserted immediately postpartum and which will be well retained would have considerable programmatic value in developing countries.

The second study is an analysis of female sterilization failures. It is an example of how IFRP's "rich data base" is quite valuable and unique. Since pregnancy following a sterilization is a fairly rare event, a very large data set is required to evaluate it. The data base was 13,439 women who were "healthy" and "fecund" before their sterilization, which the operator considered complete and successful, who had an intact uterus and ovaries after the sterilization and had been followed up at least six months after the procedure. Confirmed clearcut sterilization failures occurred in 118 women. "Cases" of sterilization failure were matched to two sets of controls. One set of controls was matched on provider variables; the other set of controls

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was matched on certain patient characteristics. Analysis was by matched-pair technique. The major findings were:

1. Laparoscopic patients whose tubes were occluded using Hilka's spring-loaded clips had higher pregnancy rates than those whose tubes were occluded by the falope ring.
2. Culdoscopic patients where tubes were occluded using the tantalum clips likewise had a higher pregnancy rate than for the tubal rings.
3. There seemed to be a higher risk of ectopic pregnancy in interval laparoscopic patients whose tubes were divided using the cauterization/division technique.
4. Patients which characteristics related to higher fecundity (younger, lower parity, not breastfeeding) had a higher risk of sterilization failure.

Discussion

Dr. Carter asked for clarification of the difference in the IFRP research program and a grant that IFRP has from AID for similar work.

Dr. Shelton explained that of the total grant of about \$4.5 million, more than half would go for national fertility research programs within LDCs.

Dr. Connell asked about overlap among AID fertility projects and between the AID program and the programs of other international donors.

Dr. Speidel remarked that there is some overlap which DS/POP is continuously trying to eliminate. He mentioned some overlap between IFRP and a recent proposal from Johns Hopkins University as an example.

Dr. Tanter asked if there was anything to report on the interaction of user characteristics and contraceptive practices or on the cost benefits of the different techniques IFRP was field testing. Drs. Speidel and Shelton provided some examples of contributions by IFRP on both areas.

Minutes of the March 30-31, 1978 RAC Meeting

International Fertility Research Program -
(IFRP)

The report of Dr. Connell, Chairman of the subcommittee that included Drs. Montgomery, Tanter, Carter and Linder was summarized as follows:

The current project statement reflects attempts on the part of IFRP to respond to the recommendations made by both AID and RAC following their respective site visits. For example, changes have been or will be made in the type of research to be carried out, the number of participating centers, the overall structure and staffing pattern of IFRP and the utilization of a larger percentage of more complicated study designs. Within the past week additional information was made available to the RAC subcommittee indicating that IFRP plans a personnel reduction of 22% and has created a new office, that of Executive Director.

The subcommittee believes that the IFRP general protocols for the various activities which it carries out are appropriate and useful. Data gathered in the past have been of considerable value in providing guidance as to what would constitute the best technology for family planning programs. IFRP's claim of cost-effectiveness vis a vis other programs is probably justified, primarily due to its central data collection and analysis capabilities.

There are still some concerns that the subcommittee has expressed and additional information on these areas would be helpful. A question was raised by one of the

subcommittee members as to when this particular type of project could reasonably be expected to terminate. He further questioned whether the large residual number of LDC centers and investigators is really necessary. The division of centers into two levels of expertise seems to be somewhat arbitrary and possibly calculated to give the impression of a decrease in the overall number of centers, whereas in actuality it is relatively unchanged. Second, the work plan for the coming three years lists a very large number of projects. Many of these are still straight evaluations of a single contraceptive technique. For example, 34 IUD studies are listed as ongoing; plans for the future project an even greater number. The subcommittee wonders whether certain of these projects should not more properly contain a larger number of devices within each study in order to get better comparative data. Also, there is serious concern as to whether this number of studies can be adequately evaluated and utilized. Finally, one of the reviewers stated that he felt that even this revised version of the proposal portrayed a very elaborate automated organization still requiring an inordinately high budget.

At the original review of the project's extension in March 1977, a recommendation was made that RAC only evaluate the research components of the program and the current project statement reflects that request. However,

the subcommittee wonders whether those projects such as the community-based delivery system, the expansion of the publication activities, and the development of the maternity record system, all of which were considered to be in appropriate for IFRP to carry out, were perhaps merely moved into that portion of the program which is not currently being presented to RAC.

The current subcommittee, including two members who were part of the site visit team, are generally in favor of IFRP as restated. One of the new subcommittee members has recently carried out an additional site visit. Specific recommendations will therefore not be made until the other members have had a chance to review his report.

Dr. Montgomery added that the site visit which he and two other RAC members and AID staff made to IFRP was very informative and reassuring although it may have appeared threatening to the IFRP to have so many reviewers there at one time.

The research strategy pursued at IFRP provides linkages for some comparison of contraceptive techniques. It appears though that there are diminishing returns to trying new techniques with marginal variation over those already tested. It would at the same time not be wise to say we've learned enough about some of these contraceptive methods. Probably, though, some more important studies

could be made. In particular, there is a need for well designed comparative studies of delivery systems and the social science aspects of contraception. AID and the RAC should be receptive to proposals that get analytical work in this area started. Survey techniques are now available in almost every country. Analytical techniques are not.

Dr. Tanter made a special site visit to IFRP so he could speak about the project from information. He wanted to look at the IFRP project from the point of view of: (1) its impact on population policy and (2) the value of the immense data base that has accumulated at the IFRP home office. Several of the contraceptive methods might interact with user characteristics to cause a larger impact than others on demographic change. Adolescent women users is an example of a special group about which more should be known. Attention should also be given to the cost benefit of new family planning techniques. This should be considered along with risk.

Dr. Tanter found the group strong on studies that impact on policies. He thought that IFRP was a unique institution that has stimulated growth of LDC research and family planning programs.

The storage of massive amounts of data on computer tapes for use by others was impressive. The group is engaged primarily in the secondary analysis of aggregate

data and in monitoring tests of a single technique of contraception. The data base would be more valuable if it could be used in comparative studies of contraceptive techniques. Dr. Tarter thought the staff biomedical statistician was well trained for analytical work.

Dr. Montgomery added that the RAC should not discourage the work of IFRP but that IFRP should not enter totally new areas of research without a research protocol.

Dr. Connell agreed that the question is not one of cost effectiveness but should IFRP carry out research without a protocol or should they carry out some activities at all. How will their research be done?

Dr. Carter reminded the RAC of the \$10,500,00 cost of the project. He also questioned how IFRP could find 55 research centers of excellence while Northwestern University can only find a few to do work on the PARFR project.

Dr. Carter pointed out that the project statement allows a lot of leverage for emphasis. RAC and AID should be able to guide IFRP more effectively. They seem to be emphasizing IUD work, oral and systemic contraception, barrier contraception and pregnancy termination. WHO feels that the pill would be off the market by the year 2000. If there are now 34 different IUD's could at least one that is free from complications be developed?

Dr. Linder noted that the subcommittee report suggests several areas where IFRP ought to stop or slow down. It's

not clear from the project statement that these suggestions had been acted upon. It is also difficult for RAC to evaluate the complicated multi-faceted budget that was presented. Costs by activities can't be identified.

The organization chart appears to show everybody as a chief. IFRP appears to be overorganized.

The subcommittee report indicated that the research was too mechanized, without intellectual input. There also appears to be some conflict about the competence of the bio-statistician. The experimental designers at the University of North Carolina could not agree with IFRP on their statistical design.

Dr. Anderson asked if AID was not putting an enormous amount of money on a small problem. Are not societal changes more important to controlling population growth. Both Dr. Linder and Dr. Connell responded that there were people at both extremes, technological changes and societal changes. Most knowledgeable people had opinions somewhere between the two extremes.

Dr. Moss asked how a 22 percent staff reduction resulted in only a 3 percent reduction in the budget. In addition, why is it necessary for IFRP to subsidize a professional journal at the rate of \$200,000 per year. He is personally involved with five professional journals, none of which receive support for items such as printing and binding.

Dr. Carter agreed that the budget should be shown in greater detail for effective evaluation.

Dr. Speidel of the AID staff went over some of the accomplishments of the IFRP Program and how it fits into AID's total population program. Generally, the IFRP program is a means of evaluating new technology and provides the Agency and others with a much needed source of data on new contraceptive techniques. He covered some other points raised by RAC members.

When will the Program end? As long as substantial progress is being made on the population problem and meaningful changes are taking place, AID will need a program such as this one.

Number of Research Centers - The number of centers has been cut and not just made to appear that way. PARF does a different type of research, (biomedical research) that requires a higher level of competency than in the IFRP Program. Thus more centers are available for this type of work.

What's going on outside research? IFRP is not yet into health care delivery, library services, and a number of other things that could be included in the program. In Brazil there is a contraceptive prevalence survey, for example.

Operations Research - Dr. Tanter asked about the Population Offices operations research. These are projects where user technique tests are being done. The Population Office has done work on IUDs etc. that takes user characteristics into account.

Control of IFRP Research - The Office of Population reviews all new activities. Thus control over the project is maintained. Dr. Speidel agreed that the number of research activities in the IFRP is large and thought they should concentrate more on high priority items in the future.

Budget and Organization of IFRP - IFRP is reorganizing after the recent site visits. This reorganization should result in a more simplified organization chart. The budget presented in the project paper does not have all the detail, but this detail is available. DS/POP summarized the budget so that RAC would not have to dig through the detail. There has been a significant reduction in the budget as compared to the one originally requested.

Journal Support - The journal is the official journal of FIGO and IFRP and DS/POP considers it a legitimate expense of the program. It is important to disseminate IFRP information and the journal cannot expect many subscriptions from LDC where its main audience is found.

Dr. Carter clarified his remarks on control of the IFRP Program. Perhaps it would be in the best interest of DS/POP to have some outside group (NAS, for example) to prepare a report on what the needs will be in the future.

Dr. Speidel remarked that the IFRP biomedical data would be presented at a conference on April 25th. Representatives of major organizations in this field should provide input of the type Dr. Carter is suggesting.

Dr. Anderson asked two questions: (1) Is the journal a house organ? and (2) how are the priorities of projects determined? The AID staff replied that the journal is essentially a house organ and that priorities are primarily judgment decisions by IFRP and AID.

Dr. Montgomery asked how is the research designs used in the IFRP reviewed if it is not done by RAC. If a grant is made to develop research with no specification of methodology, the review process is circumvented.

Dr. Gillespie explained that the major obstacle in carrying out this work is not the lack of access to U.S. statisticians but is the availability of people in LDCs to get the work done. So the experimental design is kept very simple and reviewing it is no problem. For this reason, most operations research projects are also not sent to RAC for review.

Dr. Smuckler wanted to know if IFRP received financial support from outside AID.

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Dr. Speidel replied that a few private drug companies and foundations provided a little support. Almost all financial support is from

Dr. Connell presented the following motion which was seconded and approved unanimously.

Motion:

That the IFRP proposal be approved as submitted. It is further recommended that the AID staff prepare a report on IFRP activities for RAC in approximately one year with major emphasis on:

1. IFRP administrative structure
2. IFRP staffing patterns
3. research projects
 - a. areas of study
 - b. funding levels
4. IFRP research centers
 - a. classification
 - b. numbers
5. IFRP investigators

Minutes of the Nov. 30 - Dec. 1, 1977 RAC Meeting

PROGRESS REPORT

International Fertility Research Program
(IFRP) - North Carolina University

Dr. Connell, Chairperson of the site visit team, reported to the RAC on the findings and recommendations that came out of the September site visit to IFRP in North Carolina. At the last RAC, comments were made concerning the rapid growth and overall size of IFRP as well as the research, institution building, and service components of the program. As a result of the RAC discussion, a sub-committee site visit was recommended. The site visit was to focus on IFRP's program. A group from the Population Office accompanied the RAC team and made an independent review, which resulted in a report very similar to the RAC sub-committee.

Briefly, the RAC team felt IFRP to be a hard working ambitious group. Their main objective has been to collect large scale data on various fertility control techniques and to that end, they have been quite successful. They have established an excellent outreach program and have established lines of communication throughout the world. The sub-committee, however, felt that IFRP could better exploit the data they have already collected and that IFRP should not divert its international venture into too many activities, such as fertility control research, maternal-child health, community based delivery systems, etc. In addition, the RAC felt the Medical Advisory Committee and Senior Management could be strengthened by the addition of individuals with a solid medical research background.

The library facilities appeared to be good and should not be extensively expanded . The RAC felt IFRP should reassess its staffing patterns to focus more on the primary mission of IFRP. In general, the site-review team recommended that: 1) the ceiling on the number of international centers be respected, 2) IFRP should limit their institution building activities, 3) IFRP should terminate projects when sufficient data has been collected, 4) less effort be made to keep extensive amounts of old data, 5) better experimental design and more sophisticated data analysis be employed on individual projects, and lastly, 6) IFRP more restrict their activities to stage III field trials.

Overall the sub-committee would recommend continued but reduced funding to enable IFRP to meet their primary contracting responsibilities as initially designed.

Dr. Wishik wished to add a few additional comments. He felt IFRP should focus on contraceptive technology rather than on large individual country programs in many aspects of fertility control. In addition, he felt IFRP has a tremendous repository of data and that this data should be added to and then re-examined. IFRP has done very well with individual comparative studies but there could be an infinite number of such studies and comparisons possible. It would be more useful to design a complex analytical system to examine multiple techniques which would be a more appropriate level of activity for the technical staff to undertake.

Dr. Gillispie (DSB/POP) discussed the report that was prepared by the staff of POP/R that accompanied the RAC site-review team. He indicated that the office agreed with the majority of the RAC comments and that the forthcoming proposal from IFRP would reflect the comments of both RAC and the Office of Population.

Dr. Smuckler thanked all the parties involved and accepted the report for the RAC.

International Fertility Research
Program (IFRP) - International
Fertility Research Programme, Inc.

Dr. Connell, Chairman of the Subcommittee composed of Drs. Linder, Weller, and Wishik, reported as follows:

As noted in the previous review, the IFRP works in six major areas--pregnancy termination, menstrual regulation, intrauterine contraceptive devices, male and female sterilization, and systemic contraceptives, including oral contraceptives by carrying out comparative field trials with their network of more than 250 investigators in more than 30 developing countries. In order to be able to do this effectively, IFRP has developed standardized protocols with instructional manuals and common data collection forms. It has thus built up a large volume of information, leading to improvements in both medical technology and program management. Data on 200,000 cases are currently in the IFRP data bank. More than 288 scientific papers have been written and disseminated by IFRP staff and contributors. As a result of its earlier work, a number of the investigators in the LDC's are now carrying out their own semi-autonomous research.

Under the proposed extension, IFRP will develop record systems to evaluate both clinic-based and community-based family planning programs, including a Maternity Record System.

In addition, a small physician training course in the newer surgical techniques is proposed.

In addition to the programs that the IFRP has been carrying on which have been reviewed previously and again today, a new program related to maternity care has recently been added. A study was recently carried out to see if it would be feasible to expand the total program to include a section on maternity care. Somewhat to the surprise of the staff, the response was overwhelmingly positive. In fact, some concern has been expressed that this segment of the program should not be allowed to enlarge at the expense of the other programs, since it appears to have the potential for great popularity. It has been known for many years that there are certain areas of the world where family planning programs per se are not welcome, being perceived as acts of genocide or political subversion. However, when these services are offered as part of a maternal and infant care program, they are viewed in a very different and much more favorable light. Therefore, it would appear to be expedient to add this new element to the total IFRP program.

Review of the proposal by the subcommittee has raised certain questions. Concern was expressed by one member about the change from a primary analytical evaluative focus to an operational status. The rapid increase in program requiring

expansion of both staff and space and the continued enlargement of the number of collaborators pose managerial questions as to whether this could be effectively carried out. The functions of the Medical Advisory Committee and the Ethics Committee were not felt to be sufficiently well outlined. Furthermore, the new relationship with the Journal of the International Federation of Gynecology and Obstetrics was not well described as to how this will function.

Another subcommittee member felt that the proposal was not basically a request for funding of research but rather a request to fund a whole institution. It was therefore very hard to look at any specific research in any of the areas which would be covered. He also expressed concern, similar to the other subcommittee members, about the sustained increases in funding, whether they could be well utilized and whether A.I.D. ever foresaw an end to the requests for continued support.

As noted in the previous review by RAC, this program has been in existence for six years and the present proposal would extend it to eleven years with a total expenditure of \$51,069,973. At first glance, this would appear perhaps to be an excessive amount of time and money to give to one organization. However, when looked at in greater detail, the IFRP has and continues to serve a rather unique function in the

population field. In order to evaluate both contraceptive technology and programs, it is necessary to develop common protocols to be used by a variety of investigators throughout the world. These data must be carefully analyzed by a group skilled in statistical evaluation. These functions have been carried out by IFRP for the past six years. Although certain other groups perform some of these same activities, no single group has built up such a widespread international network of clinical services, reporting data to a central statistical unit. The need for such data collection and analysis continues and is actually growing. Therefore, since the mechanism for doing this work is already in place and functioning well, it would appear to be appropriate to continue to fund it as requested.

Dr. Thorbecke noted that this project accounts for 23% of all research funds in the population program, and that the IFRP is totally an A.I.D. appendage. Does this imply that work can be conducted better outside the A.I.D. structure, more objectively, more independent, more decentralized? Mr. Ludington stated that he had participated in the earlier review of the project and was now committed to this corporation approach. However, there is too little information for a meaningful judgment. Dr. Heady noted that the request was for 5 years in contrast to the usual limit of a 3 year approval.

Dr. Joe Speidel, PHA/POP, stated that the budget was based on figures supplied by the IFRP and that he estimated they could be reduced by \$1-2 million per year. He commented that in terms of 1972 dollars the plan represented a reasonable growth in actual operational activities. Clinical work is expensive. It is estimated that it normally requires from \$5 to 15 million to conduct the required tests for the introduction of a new drug. There is no absolute answer as to the minimum number of cases needed for a test, although probably such a value is in the neighborhood of 1000 minimum for drug tests and 5000 for female sterilization tests. When do we know when to stop? This is a matter of both statistical and clinical judgment based on the cumulative evidence. Why a single organization? It is important to have continuity and to avoid gaps after the research in shifting to program services. IFRP provides an evaluation and supervisory role in monitoring the complexity of ongoing activities. This project is essentially a program rather than a research project. It is difficult to predict when specific approaches will be ready for clinical trials. Possibly some form of RAC participation in program review and planning would be of direct value. Overall the quality of the IFRP data has been good.

Dr. Connell said that there is need to continue test activities even if there are no new methods. There is some unhappiness with the current technology, and it is recognized that it may take \$50 million to get a new technique ready for the market. She stated that she had great respect for Dr. Speidel. The only alternative to a project of this type would be to set up a new organization to do the same thing. She supported the proposal.

Dr. Peterson asked about the procedure of requesting more than one plans to accept. He understands the procedure, but finds it confusing. A prudent course would be to have an outside advisory committee. Dr. Anderson asked Dr. Speidel what he thought were the three weaknesses in the program. Dr. Speidel said that they would be (1) too big, (2) too fast, and (3) research information coordination. Dr. Moss commented on the importance of mission funding with hard dollars for research. However, the budget item of \$8 million overhead for an organization which is 100% A.I.D. funded requires explanation; also the \$50,000 per year for consultants.

Dr. Schweigert stated that the key appeared to be to support the mission with control of detail in planning.

Dr. Connell summarized by indicating three options:

(1) forget it, which is not the sense of the RAC, (2) request more analysis for a subsequent meeting of the RAC, which does

appear to be a productive course, and (3) recommend cutting back the budget to levels suggested by Dr. Speidel, with provisos that (a) approval be limited to 3 years, (b) RAC participation in the advisory group be established, (c) advisory committee meetings be increased to 2-3 per year, and (d) that reports be made to the RAC.

Dr. Weller urged concentration on the research components of the program. He also expressed concern about the policy affecting the whole program.

Dr. Speidel indicated that the Subcommittee had access to some 38 pages of Budget specifications in Appendix C. The overhead rate of 20% is better than that of most contracts with universities. Delay in acting on this proposal would pose serious problems operationally. Hence he would opt for the third alternative as posed by Dr. Connell.

Motion: That the RAC approves extension of the program with the provisos: (1) the budget be reduced by \$2 million per year, (2) the approved period be reduced from 5 to 3 years, (3) a RAC subcommittee meet with the Medical Advisory Committee of the IFRP, (4) that Advisory meetings be increased to 3 per year, and that (5) reports be made to the RAC.
Moved by Dr. Connell, seconded by Dr. Schweigert.

Discussion: Mr. Ludington stated his preference for a 1 year extension. Dr. Weller concurred as a basis for better understanding. Dr. Wishik pointed out that the proposed research reference services represented movement into a well established area already financed by A.I.D. and, hence, involving possible duplication. The motion should indicate new directions.

Dr. Smuckler stated that since a revised motion could probably not be put together within a few minutes, he suggested the subcommittee review their position for a post luncheon action.

Dr. Connell withdrew her motion.

New Motion: That the project be extended for one year and funded at the level of \$3.7 million for continuation of the current research. RAC recognizes that programatic activity by IFRP can be carried out by A.I.D. with other funds. Furthermore, a RAC evaluation is to be carried out during this year leading to recommendations on both research projects and the nature of the continuing relationship to RAC.

Moved by Dr. Connell, seconded by Dr. Schweigert.

Vote: Unanimous approval;

Dr. Speidel commented that the discussion had helped clarify how the RAC likes to look at complex projects, and this will set a precedent on how to present components within

a total context in the future. Dr. Wishik pointed out that how the RAC carries out the review process is up to Drs. Smuckler and Long.

INNOVATIVE SCIENTIFIC RESEARCH

Listing of Sub-Projects

Research area/Title		Purpose
<u>Terrestrial Resources</u>		
Program for the Assessment of Azolla Use in Tropical LDCs	150	Further develop a non-petroleum based source of nitrogen for rice farming systems in tropical LDCs
Grantee: University of Hawaii in conjunction with network of LDC institutions		
A Multi-use Resource: Agronomy of Orbignya Species	185	To improve the domestication of the babassu tree which is an important resource for subsistence farmers.
Grantee: New York Botanical Garden in conjunction with Instituto Nacional de Pesquisas da Amazonia		
Sesbania as an LDC Agro-forestry Resource	105	Assess the potential of this important species as economically efficient multiple use nitrogen-fixing trees suitable to low income farms.
Grantee: University of Hawaii		
Food from Wood: A New Enzymatic System for Degradation of Lignocellulose	180	To identify the mechanism by which one animal is able to use enzymes to derive nourishment from the lignin in wood fibre, and to investigate the usefulness of this process to food production.
Grantee: University of Idaho		
Potentially Useful Plant Resources of the Eastern Andes	150	To identify unexploited plants native to Peru which might have significant economic or environmental value.
Grantee: Missouri Botanical Garden in conjunction with Universidad Nacional de Amazonia Peruana		

Research area/Title	Amount (\$000)	Purpose
Tissue Culture Improvement of Plantain: (Musa Species), An Important Rural Food Source Grantee: Centro Agronomico Tropical y Investigacion y Ensenanza (CATIE)	175	To develop tissue culture techniques needed to increase the yields of this important food source.
A.I.D./OAS Program for Research Cooperation in Latin America and the Caribbean on Tropical Plant Resources Grantee: Organization of American States	400	To involve U.S. and Latin American scientists in jointly prioritizing and organizing a research program of small grants on a few key development problems.
<u>Marine Resources</u>		
Food from the Sea: Mariculture of Tridacnid Grantee: University of California, Santa Barbara in conjunction with Palau Marine Research Institute	198	To acquire data on the biology of Tridacna relevant to understanding how to increase this food source.

Research area/Title	Amount (\$000)	Purpose
<u>Physical Sciences/Energy</u>		
AID/AAES Program for Engineering Technology in the Service of Development	145	To stimulate inventions through inter- national competitions to produce specific technology, in cooperation with the American Association of Engineering Societies.
Grantee: American Association of Engineering Societies		
<u>Health Sciences</u>		
Biological Control: Effect of Insect Pathogens on the Ability of Mosquitoes to Transmit Malaria	193	Develop a biological control mechanism to control malaria transmitting mosquitoes.
Grantee: University of Florida, Gainesville		
Immunization Against Trypanosomiasis: Potential of a Non-Glycoprotein Surface Component Approach	170	To examine an alternative approach to the development of a vaccine against trypanosomiasis.
Grantee: University of California in conjunction with International Laboratory for Research on Animal Diseases (Kenya)		
Comparative Study on Vector Competence of Simulium Damnosum Complex in the Rain Forest of Liberia and the Savanna Region of Sierra Leone	240	Study of the ecology of transmission of river blindness disease carried by man- biting black flies
Grantee: Johns Hopkins University in conjunction with Govt. of Sierra Leone and Liberian Institute for Biomedical Research		

Research area/Title

**Amount
(\$000)**

Purpose

Other

**Small Grants Cooperation with the
International Foundation for Science**

450

This grant will permit the U.S. to participate through the National Academy of Sciences in this important multi-donor sponsored research grant program.

**Grantee: National Academy of Sciences
as funding mechanism for the
International Foundation for
Science**

TOTAL 2,741

**STRENGTHENING SCIENTIFIC AND TECHNOLOGICAL
CAPACITY IN LDCS
LISTING OF SUB-PROJECTS**

<u>Research Area/Title</u>	<u>Amount</u> (<u>\$000</u>)	<u>Purpose</u>
<u>Terrestrial Resources</u>		
A.I.D./Chemrawn Practical Workshop In Agricultural Research	250	To draw up an agenda of agriculture research priorities which combine the scientific opportunities revealed by the CHEMPAWN Conference with the priority needs of LDCs. CHEMPAWN will be held in the Philippines in December 1982 and will review the state of the art of chemical knowledge relevant to the problems of food production and consumption.
Grantee: National Academy of Sciences in conjunction with Chemical Research Applied to World Needs (Chemrawn)		
Training Course on Plant Tissue Culture Methods and Applications in Agriculture		To introduce younger Asian Scientists to modern techniques of growing plant tissue in a laboratory setting and to discuss their relevance to improving agricultural crops in Asia. Course will be held at Los Banos October 7 - November 7, 1981
Grantee: International Cell Research Organization of UNESCO in con- junction with the University of the Philippines at Los Banos		
<u>Marine Resources</u>		
Technical Training Center for Aquaculture in Costa Rica	152	To expand the knowledge and industry of aquaculture (particularly fresh water shrimp) through support to a training center in conjunction with the private sector.
Grantee: Corporacion Costarricense de Desarrollo (CODESA)		

Research Area/Title

Amount
(\$000)

Purpose

Physical Sciences/Energy

A.I.D./NIH Cooperative Project
in LDC Repair and Maintenance of
Scientific Equipment

300

To assist LDCs establish training
facilities for scientific and/or
medical equipment repair personnel.

Contractor: Fogarty International
Center in conjunction
with two LDC institutions

Low Cost Microcomputer Technology
for Less Developed Countries

200

Assess potential of microcomputer
to meet LDC statistical processing
needs and to develop appropriate
statistical software.

Contractor: U.S. Bureau of the
Census with focus on
African statistical in-
stitutions

Health Sciences

LDC Capacity Strengthening
in Food Safety Control

100

To provide technical assistance
to help Tunisia determine the
frequency and levels of pesticide
residue and heavy metal con-
tamination of foods in the Tunisian
diet and to help establish a
technically based food surveillance
system.

Contractor: Food and Drug
Administration in
conjunction with
the Institut
National de Nutrition
et de Technologie
Alimentaire

Research Area/Title

Amount
(\$000)

Purpose

OTHER

A.I.D./OAS Cooperative Project
in Science Base Development

150

To encourage the development of
Latin American and Caribbean
science education at the high
school levels.

Grantee: Organization of
American States

TOTAL

1.156