PD-AAT-481 Tab 8

AGENCY FOR INTERNATIONAL DEVELOPMENT

PROJECT DATA SHEET

1. TRANSACTION CODE: A (A = Add, C = Change, D = Delete)

Amendment Number

DOCUMENT CODE: 3

COUNTRY/ENTITY: Bureau for Science and Technology

3. PROJECT NUMBER: 936-4178

4. BUREAU/OFFICE: Office of Agriculture — 10

5. PROJECT TITLE (maximum 40 characters): Improved Animal Vaccines thru Biotech

6. PROJECT ASSISTANCE COMPLETION DATE (PACD): MM 09 DD 30 YY 90

7. ESTIMATED DATE OF OBLIGATION (Under 'B.' below, enter 1, 2, 3, or 4)

A. Initial FY 86 B. Quarter 4 C. Final FY 86

8. COSTS ($000 OR EQUIVALENT $1 =)

A. FUNDING SOURCE	FIRST FY 86 B. FX	C. L/C	D. Total	LIFE OF PROJECT E. FX	F. L/C	G. Total
AID Appropriated Total	870		870	870		870
(Grant)	(870)	( )	(870)	(870)	( )	(870)
(Loan)	( )	( )	( )	( )	( )	( )
Other U.S. 1.						
Other U.S. 2.						
Host Country						
Other Donor(s)						
TOTALS	870		870	870		870

9. SCHEDULE OF AID FUNDING ($000)

A. APPROPRIATION	B. PRIMARY PURPOSE CODE	C. PRIMARY TECH. CODE 1. Grant	2. Loan	D. OBLIGATIONS TO DATE 1. Grant	2. Loan	E. AMOUNT APPROVED THIS ACTION 1. Grant	2. Loan	F. LIFE OF PROJECT 1. Grant	2. Loan
(1) FN	111 I	078				870		870	
(2)									
(3)									
(4)									
TOTALS						870		870	

10. SECONDARY TECHNICAL CODES (maximum 6 codes of 3 positions each): 119 | 080 | 041

11. SECONDARY PURPOSE CODE: 121

12. SPECIAL CONCERNS CODES (maximum 7 codes of 4 positions each)

A. Code	R/AG	XII	R/ED
B. Amount	$870	$390	$156

13. PROJECT PURPOSE (maximum 480 characters).

The purpose of the proposed research is to develop an effective infectious vaccinia virus recombinant vaccine to eradicate rinderpest.

14. SCHEDULED EVALUATIONS

Interim MM 02 YY 88 MM 02 YY 89 Final MM 10 YY 89

15. SOURCE/ORIGIN OF GOODS AND SERVICES: X 000 ☐ 941 ☐ Local ☐ Other (Specify)

16. AMENDMENTS/NATURE OF CHANGE PROPOSED (This is page 1 of a ____ page PP Amendment.)

17. APPROVED BY

Signature

Title: Anson R. Bertrand, S&T/FA

Date Signed: MM DD YY 30 MAY 1986

18. DATE DOCUMENT RECEIVED IN AID/W, OR FOR AID/W DOCUMENTS, DATE OF DISTRIBUTION

MM DD YY

PROJECT AUTHORIZATION

Country: Interregional

Project: Improved Animal Vaccine thru Biotech

Project No. 936-4178

1. Pursuant to Section 104 of the Foreign Assistance Act of 1961, as amended, I hereby authorize the centrally funded project, Improved Animal Vaccine thru Biotech, involving planned obligations not to exceed $870,000 in grant funds over a three-year period from the date of authorization, subject to the availability of funds in accordance with the A.I.D. OYB/allotment process, to help in financing foreign exchange and local currency costs for the project.

2. The purpose of this project is to: develop an effective infectious vaccinia virus recombinant vaccine to eradicate rinderpest.

3. The agreement which may be negotiated and executed by the officer to whom such authority is delegated in accordance with A.I.D. regulations and Delegations of Authority shall be subject to the following terms and conditions, together with such other terms and conditions as A.I.D. may deem appropriate.

4. The Cooperative Agreement shall contain a clause requiring the cooperator to implement this research activity in accordance with appropriate Federal guidelines and regulations for research involving recombinant DNA work and testing of genetically engineered organisms.

5. Source and Origin of Commodities, Nationality of Services

a. Commodities financed by A.I.D. under the project shall have their source and origin in the cooperating country* or the United States, except as A.I.D. may otherwise agree in writing. Except for ocean shipping, the suppliers of commodities or services shall have the cooperating country or the United States as their place of nationality, except as A.I.D. may otherwise agree in writing.

b. Ocean shipping financed by A.I.D. under the project shall, except as A.I.D. may otherwise agree in writing, be financed only on flag vessels of the United States.

*Each country where research, training, technical, or other assistance takes place under the project shall be deemed to be a cooperating country for the purpose of permitting local cost financing of goods and services for the activity being conducted in such country. Such activities may be undertaken in any country included in A.I.D. geographic code 935.

Anson R. Bertrand
Agency Director
for Food and Agriculture

Clearances:

S&T/AGR,	Tejpal Gill		Date 5/29/86
	Lloyd Frederick		Date 29 May 86
	Kerri-Ann Jones		Date 5/29/86
	Charles Mullenax		Date 29 May 86
	Harvey Hortik		Date 29 May 86
S&T/PO	Betty Roche		Date 5/29/86
	Gerald F. Gower		Date 5/30/86
GC	Stephen Tisa		Date

Drafted:MMozynski:5/12/86VacAcMem

Tab A

Improved Animal Vaccines thru Biotechnology

Certification of the Procurement Plan

I certify that the procurement plan for this Project Paper (936-4178) was developed with full consideration of maximum involvement by minority and women-owned firms, historically Black colleges and universities and minority controlled PVOs in the provision of goods and services, and that the Project is not appropriate for minority or Gray amendment contracting. We know of no minority institutions with an on-going program of the type required, or the requisite faculty and facilities needed to implement this project. However, to ensure consideration of minority organizations as defined in the Gray Amendment, we will work with the Office of Procurement, and the Office of Small and Disadvantaged Businesses, to identify all potential organizations and institutions which may be available to work with WSU in the area of vaccinia virus vaccine.

Anson R. Bertrand
Agency Director,
for Food and Agriculture

WANG:4135g

MAY 29 1986

ACTION MEMORANDUM FOR THE AGENCY DIRECTOR FOR FOOD AND AGRICULTURE, BUREAU FOR SCIENCE AND TECHNOLOGY

FROM: Tejpal Gill, Acting Office Director S&T/AGR

SUBJECT: Improved Animal Vaccine thru Biotechnology

Action: Your approval is required to authorize a three-year project, Improved Animal Vaccines thru Biotech (936-4178) which has an authorized life of project cost of $870,000 from the Agriculture, Rural Development and Nutrition account, Section 103 of the Foreign Assistance Act of 1961, as amended.

Discussion: Animals are an integral part of the world's agricultural system. However, the overall production potential from livestock is limited by the incidence of animal diseases. At the request of the Office of Agriculture, Bureau for Science and Technology (S&T/AGR), the National Science Foundation (NSF) convened a Panel in June 1985 on "Vaccine Development for Tropical Animal Diseases." The panel was comprised of research scientists with experience in vaccine development, veterinary medicine, and international development. This panel was convened to help S&T/AGR identify the disease or diseases that AID can have major impact on, given our limited resources.

The panel supported AID's interest in using biotechnology to address the problems of animal disease, indicating that the methods of biotechnology are especially appropriate for the development of a vaccine suitable for the developing world. In particular, the panel stated in its report that: "A special kind of bioengineered vaccine appears to be tailored-made to meet the special needs of tropical LDCs - Vaccinia vectored vaccine." This approach involves the use of vaccinia virus to carry the immunogenic genes into the host. The panel also produced a list of target diseases based on the following criteria:

1. Diseases causing the greatest losses in a large number of LDCs and for which cheap and effective control strategies are not available;

2. Diseases on which other agencies are not likely to support research at the level required for success;

3. Diseases in which early success can be reasonably predicted.

Rinderpest, the target disease in this proposed project, was on that list.

936-4178

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Rinderpest is an acute, febrile, and highly contagious viral disease of ruminants. It is found in Africa and parts of Asia, and is capable of killing 90 percent of susceptible animals. An effective live attenuated virus vaccine is available, but a successful eradication program has not been maintained. The vaccine requires a level of control not found in developing countries (e.g., cold storage, sterile conditions, and syringes). Development of vaccinia vectored virus will overcome these problems. Additionally, there is much information available on rinderpest virus since it is closely related to human measles virus and canine distemper. The possibility of an early success in the development of a rinderpest vaccine is very good.

On April 1, 1986, S&T/AGR invited the Washington State University (WSU) to submit a proposal to develop an improved vaccine for rinderpest using vaccinia vector technology. (Letter to WSU attached as Tab D). This request was based on the unique capability of WSU and Dr. Tilahun Yilma and his collaborators/co-workers who have been making great advances in use of vaccinia virus in vaccine development. (Justification for non-competitive assistance award to WSU is attached as Tab B).

The proposal has been received. It has been reviewed by the Project Design Committee in S&T/AGR and by Dr. Philip Harriman, Program Director, Prokaryotic Genetics at the National Science Foundation (NSF). Dr. Harriman has recommended funding with the highest enthusiasm (copy of his letter attached Tab E).

This new project is consistent with the objectives of AID's Food and Agriculture Policy and Strategy to enable LDCs to become self-reliant in food and to assure food security. One clear way to achieve these objectives is to decrease the incidences of cattle losses due to rinderpest and increase the efficiencies of animal production systems. The use of vaccinia virus to develop an improved rinderpest vaccine is truly a research "target of opportunity." Vaccinia has been engineered to produce vaccines against a number of diseases. The time is now ripe for this technology to focus on the development of vaccines for important diseases in the developing world. The existence of an improved vaccine for rinderpest would clearly increase food production and agricultural productivity.

The specific objectives of this proposal are to: 1) clone the rinderpest genes responsible for the production of immunogenic proteins (hemmagglutinin (HA) and fusion (F)); 2) construct an

infectious vaccinia virus recombinant that optimally expresses the two immunogenic genes of rinderpest virus; 3) measure immunity and protection against viral challenge in cattle vaccinated with this recombinant vaccinia vaccine up to 12 months after vaccination; and 4) develop more effective promoters and improved techniques for enhanced expression of immunogenic genes in vaccinia virus recombinant vectors. At the end of this project a recombinant vaccinia virus vaccine for rinderpest will have been developed and small scale laboratory testing will be have completed. The rinderpest vaccine will be ready for the next step toward utilization, large scale field testing (outside of a containment facility), which is beyond the scope of this project.

A panel of scientists from the International Centers and related A.I.D. projects will be established to provide advice to the S&T/AGR project. It will consist of representatives from the Small Ruminant CRSP, ILRAD, and ICIPE. Additionally, an external review committee comprised of experts in vaccine development and molecular biology will be established to conduct in depth reviews and evaluations of this project.

Method of Implementation: This project will be implemented under a cooperative agreement. WSU contributions will include support for the services of the principal investigator, Dr. Tilhahun Yilma and 25% of the salary of Dr. Yilma's research technician. WSU has already made substantial investment in work on vaccinia virus, in terms of laboratory equipment and facilities and these will be available for use in this project.

S&T/AGR will provide funding ($870,000) and project management which will include technical and administrative support and oversight. An A.I.D. direct hire will serve as project manager and spend 60% of his/her time managing the activities to assure that the objectives are achieved, facilitate acquisition of mission clearance, monitor project operations, approve certain administrative actions within the project, and maintain close liaison with the grantee.

Environmental Considerations: As this project will produce a genetically engineered vaccine for a highly infectious animal virus, there are specific safety issues which must be addressed. These fall into 3 major categories: 1) rinderpest work, 2) recombinant DNA work and, 3) small scale testing of the recombinant vaccine (in containment facilities). The agencies which determine the regulations for these issues are currently the National Institutes of Health and the Animal and

Plant Health Inspection Service of the USDA. All appropriate regulations, including international regulations, will be followed and agencies will be kept informed in order that the movement of the vaccine to large scale testing will proceed as smoothly as possible.

Facilities and procedures in Kenya will adhere to all Kenyan regulations. Facilities and procedures will be reviewed to ensure that they are adequate based on United States and Kenyan standards. The A.I.D. environmental coordinator will assist S&T/AGR in developing the Kenyan containment facility certification team and participate in this review process. The government of Kenya, appropiate ministries, will be requested to approve, in writing, the work in Kenya and will be kept informed of all project activities in Kenya.

Agency Policy: The project will be implemented in accordance with relevant Agency policies, including those on food and agriculture, development of human resource and institutional capabilities, and expanding the role of LDC scientists and institutions.

Justification to Congress: An Advice of Program Change is being cleared and will be sent to the Congressional Committees shortly.

Funding: The FY 86 OYB has $870,000 to fully fund this three-year project. Funds for future evaluations of the project have been transferred to the agriculture research and development project (936-4109). This project was originally included in the Project Identification Document (PID) of the umbrella project "Improved Plant and Animal Tolerance to Stress" and was approved by the Senior Assistant Administrator for Science and Technology on March 20, 1986. It was decided that an umbrella approach was not appropriate at that time and that the development of the Improved Animal Vaccine thru Biotech initiative should proceed as a separate project. The cost estimates are appropriate for the planned level of effort described in the project paper (PP).

Certification of the Procurement Plan: Certification that the procurement plan for this project was developed with full consideration of maximum involvement by minority and women-owned firms, historically Black colleges and universities and minority controlled PVOs in the provision of goods and services, and that the project is not appropriate for Minority or Gray amendment contracting is attached as Tab A.

Recommendation: That you sign the attached Project Authorization (Tab B) approving the three-year, $870,000 "Improved Animal Vaccine thru Biotech" project.

Attachment:
- Tab A - Certification of the Procurement Plan
- Tab B - PAF
- Tab C - Project Paper
- Tab D - Letter to WSU requesting proposal
- Tab E - NSF Letter

Clearances:

S&T/AGR,	Tejpal Gill	date	5/29/86
	Lloyd Frederick	date	29 May 86
	Kerri-Ann Jones	date	5/29/86
	Charles Mullenax	date	29 May 86
	Harvey Hortick	date	29 May 86
S&T/PO,	Gerald Gower	date	5/30/86
GC,	Steve Tisa	date	

Drafted by:S&T/AGR/RNR:KJones:michelle:5/23/86:4135g

APPROVED

DISAPPROVED

DATE 5/31/86

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

L. Frederick
7a6 D

APR - 1 1986

Dr. James H. Henson, Director
International Program Development
Washington State University
Pullman, Washington 99614

Dear Dr. Henson:

The Agency for International Development (A.I.D.) places high priority on reducing animal diseases in the developing world. Livestock are an important component of agricultural production systems in developing countries and provide food, fiber, fuel, fertilizer, income, and also, opportunities for investment. Animal disease losses can have a catastrophic impact on the nutrition, income, and well-being of large segments of developing country populations and overall national economies. Other adverse long-term economic impact, such as closure of export markets, also occurs following outbreaks of disease. Replacement of herds and recovery of markets is frequently a long-term and costly process. For example, an outbreak of rinderpest in Guinea required 4 to 5 years of calf production to replenish the losses.

Within the last decade, rinderpest, a highly contagious and usually fatal cattle disease, has once again assumed epidemic proportions in Africa, and today threatens livestock populations in the developed as well as the developing world.

In this regard, we understand that Washington State University (WSU) has an ongoing research program using advanced biotechnology that has shown considerable promise in the development of animal disease vaccines using the vaccinia virus as a vector. A.I.D. wishes to explore the possibility of assisting WSU to expedite the use of this technology to facilitate early development and testing of an improved rinderpest vaccine that is effective and cost-efficient for use in developing countries. We understand that vaccinia-vectored vaccine technology promises the advantages of safety, ease of production, and heat stability, all of which are essential for effective vaccines in developing countries.

The unique capability of WSU in this technology is manifested by the research results already obtained by Dr. Tilahun Yilma and co-workers, who are making good progress toward perfecting the vaccinia virus as a vector for vaccines.

We have been informed that:

-- The group has developed a vaccine for vesicular stomatitus virus and is ready to begin field testing.

-- The group has available probes (DNA) for working on rinderpest. This is a critical step in developing a vaccine, as a probe is needed to identify the gene or genes which will be used to make the vaccine.

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-- Dr. Yilma has had experience working in Africa in vaccine development programs and has professional linkages with scientists there.

-- Dr. Yilma has a professional relationship with USDA/Plum Island, having worked there for several years. Because rinderpest is an exotic disease which cannot be studied in the U.S., this project requires collaboration with Plum Island in the isolation and testing of the DNA fragment(s) needed for vaccine production at WSU.

-- Dr. Yilma has established relationships with several biotechnology industrial firms which could be helpful in speeding the development of a new vaccine.

We know that adequate animal facilities and research laboratories exist at WSU and are staffed with experienced scientists and technicians already working on the development of vaccines and related technologies. We also understand that WSU has established an ongoing research network with several of the developing countries (e.g., Kenya and Sudan), and that many of the WSU's scientists have had considerable experience working with tropical diseases in Kenya, Sudan, Senegal, Gambia, Nigeria, and elsewhere.

Because it appears that A.I.D. and Washington State have mutual interests in expediting the ongoing research efforts on vaccinia virus vectored vaccine for rinderpest development, A.I.D. is willing to receive a proposal in this area for possible FY 1986 funding which could provide assistance to the University for a period of two to three years.

This letter is not to be construed as a commitment of any kind between WSU and A.I.D., nor does it commit the Government to pay any costs related to the preparation and submission of a proposal.

If you are interested in submitting a proposal, it should follow the guidelines attached. We will be looking forward to hearing from you regarding this research.

Sincerely,

/s/

Anson R. Bertrand
Agency Director for
Food and Agriculture
Bureau for Science and Technology

Enclosure: Guidelines for Proposals

S&T/AGR:Frances Li:3/24/86:WANG 1134C (ON AID WANG)

Rev. 3/27/86:lsl:Rev. 3-31-86:S&T/AGR, F. Li:mdp

Clearance:	S&T/PO, V. Anderson	/s/	date 4/1/86
	SER/AAM, J Frame	(Draft)	date 3/31/86
	S&T/AGR/RNR, T. Gill	(Draft)	date 3/28/86
	S&T/AGR/RNR L Frederick	(Draft)	date 3/28/86
	S&T/AGR/AP, H. Hortik	(Draft)	date 3/28/86

GUIDELINES FOR PROPOSALS

I. Characteristics of a Suitable Proposal

The proposal submitted by Washington State University (WSU) should contain the following information in order to permit consideration in an objective and timely manner:

A. Basic Information

1. Name and address

2. Type of Organization; e.g., educational

3. Identification of any proprietary data which the WSU intends to be used by the Agency for evaluation purposes only. NOTE: If the WSU wishes to impose a restriction on the use of data in the proposal, the title page shall be marked with the following legend:

"USE AND DISCLOSURE OF DATA

The data in this proposal shall not be disclosed outside the Government and shall not be duplicated, used, or disclosed in whole or in part for any purpose other than to evaluate the proposal; provided, that if a grant is awarded as a result of or in connection with the submission of these data, the Government shall have the right to duplicate, use, or disclose the data to the extent provided in the grant. This restriction does not limit the Government's right to use information contained in the data if it is obtainable from another source without restriction. The data subject to this restriction are contained on pages _____."

Each page within the proposal to be restricted must be marked with the following legend:

"Use or disclosure of proposal data is subject to the restriction on the title page of this Proposal."

4. Date of submission; and

5. Signature of an authorized representative of WSU authorized to contractually obligate the University.

B. Technical Information

1. A concise title and an abstract (approximately 200 words) of the proposed research;

2. A reasonably complete discussion of the objectives of the effort or activity, the research techniques and amount of effort to be employed, the anticipated results, time frame, and how the work to be accomplished will advance A.I.D.'s objective to control the outbreak and spread of rinderpest.

3. The names and brief biographical information on the offeror's key personnel, (including alternates, if desired), who would be involved. For each individual involved in the various required disciplines (including microbiology and molecular biology, immunology, biochemistry and epidemiology), include the amount of time each will devote to the program activities. Provide biographical sketches and pertinent bibliographies on all professional personnel, both technical and managerial, to be assigned to this project. Indicate the total staff which will be required and explain how you propose to administer funding provided by A.I.D.

4. The assistance expected from A.I.D., i.e. facilities, equipment, materials, or personnel resources.

5. Description of the collaborative relationship between the University and A.I.D.

C. Supporting Information

1. A cost estimate or budget for the proposed effort sufficiently detailed by elements of costs for meaningful evaluation;

2. Describe any organizational conflicts of interest, and environmental impact;

3. Information with respect to all contracts, grants or cooperative agreements involving similar or related projects over the past three years funded by A.I.D. and other organizations (both commercial and Governmental);

4. Brief description of the offeror's previous work or experience in the field of vaccinia virus as a vector for vaccines; and

5. Facilities to be utilized for the work.

D. The proposal shall contain a signed statement from an official authorized to contractually obligate the offeror, that the proposal as submitted represents an original effort by Washington State University.

II: Cost Sharing

Define what costs the University will underwrite.

III. Procedures for Submission and Evaluation of Proposals

A. WSU's proposal is to be submitted to Dr. Anson R. Bertrand, Agency Director for Food and Agriculture, Room 409 SA-18, Bureau for Science and Technology, Agency for International Development, Washington, D. C. 20523. Receipt of the proposal will be acknowledged promptly; the proposal will then undergo a preliminary examination to determine that it:

1. Contains sufficient technical and cost information.

2. Has been approved by a responsible representative of WSU authorized to contractually obligate the University.

B. If the proposal does not meet these requirements, WSU shall be given the opportunity to provide the required data.

C. In evaluating WSU's proposal, A.I.D. shall consider the following:

1. Unique, innovative, or meritorious methods, approaches, or ideas which originated with, or were assembled by WSU;

2. Overall scientific, technical, and socio-economic merit of the proposed effort or activity;

3. Potential contribution which the proposed effort is expected to make to A.I.D.'s mission to reduce the incidence and severity of animal disease in the developing world;

4. Capabilities, related experience, facilities, or techniques, or combinations thereof, which the offeror possesses and offers, which are considered to be integral factors for achieving the objectives of the proposal; and

5. Qualifications, capabilities, and experience of the proposed principal investigator, team leader, or other personnel considered to be critical in achieving the objectives of the proposal.

S&T/AGR:FLi:mdp:revised 3-21-86:WANG 3301e:revised 4/1/86:S&T/PO, V. Anderson

15

Tab E

Proposal on "An Infectious Vaccinia Virus Recombinant Vaccine for Rinderpest", T. Yilma Principal Investigator.

I find it hard to imagine a proposal on vaccine development that would have a higher chance of success than this one. Drs. Yilma, Moss, Callis, etc. have the backgrounds that are necessary to insure that an effective recombinant vaccine will be developed and be used in an effective way. There is no reason to think that the techniques that Dr. Moss has used successfully in the past to incorporate genes for other antigens into vaccinia virus will not work in this case. The other scientists involved have excellent training and experience in molecular biology and/or vaccine applications and all should be able to collaborate in producing and testing an effective vaccine.
If all that is proposed can be carried out with the $917,858 requested the AID will be getting a bargain. I recommend funding with the highest enthusiasm.

Philip D. Harriman, Ph.D.
Program Director, Prokaryotic Genetics
National Science Foundation.
May 15, 1986

Tab C

IMPROVED ANIMAL VACCINES THRU BIOTECHNOLOGY

PROJECT NUMBER 936-4178

5/29/86

17

Improved Animal Vaccine thru Biotechnology

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407lg:Revised 5/28/86

Improved Animal Vaccine thru Biotechnology

Executive Summary

The Office of Agriculture, Directorate for Food and Agriculture is proposing a three-year project "Improved Animal Vaccine thru Biotechnology" at a cost of $870,000. This project will fund research in the development of recombinant vaccinia virus vaccine for rinderpest. We are proposing that the project be implemented under a cooperative agreement with Washington State University, which has predominant capability in this area. The FY 1986 OYB for S&T/AGR contains $870,000 which will be obligated for this three year effort.

Animal losses due to diseases in the developing world have been estimated to reduce potential production by 30 - 60 percent depending on the country. One of the most devastating of these diseases is rinderpest, a highly contagious viral disease of ruminants. Approximately 30 years ago an effective vaccine against rinderpest was developed, using traditional methods. Using this vaccine, a major international eradication program was conducted between 1962-1968 which was quite successful. However, the requirements for use of this vaccine proved too complex for conditions in the developing world (e.g., refrigeration and sterile inoculation conditions) and the program could not be maintained. Rinderpest is again rampant in Africa.

Recent advances in biotechnology have revolutionized vaccine research and this project will apply these advances to the development of an improved vaccine for rinderpest. The methods of biotechnology, specifically recombinant DNA technology, allow the development of vaccines which are well defined (specific genes are moved around) and well suited for the conditions found in the developing world. In particular, vaccinia virus will be used in the development of the rinderpest vaccine. This approach holds great potential for a number of reasons. Vaccinia virus has already been used in the successful development of vaccines for vesicular stomatitis virus (VSV) bluetongue, and a number of human diseases. Such vaccines are heat stable and easily administered through scarification. Additionally, vaccinia offers the potential for developing one vaccine for multiple diseases, since several different genes can be inserted into the virus.

This project will take the available vaccinia vector technology and apply it to the development of the improved rinderpest vaccine. The

scientific team to work on this research and development includes two world experts on vaccinia virus, experts in international vaccine programs, African scientific collaborators, and USDA/ARS scientists at Plum Island. An additional strength of this team is that two of the participants, Drs. T. Yilma (the Principal Investigator) and R. De Tray, took part in the earlier rinderpest eradication program in Africa.

Dr. Yilma, the principal investigator, at WSU has worked extensively with vaccinia virus. His laboratory has developed a vaccine for vesicular stomatitis virus using recombinant DNA technology and vaccinia virus. This proposed project will support research to develop a vaccine for rinderpest using these methods. The major output of this project will be the development of a recombinant vaccinia virus vaccine for rinderpest which, on the basis of animal trials in the laboratory, will produce resistance to challenge by virulent rinderpest virus. At the end of the first year the recombinant vaccine will be developed. Animal testing of the vaccine will be conducted at Plum Island (Animal Disease Center of the USDA/ARS) during the second year of this program and animal tests in Kenya will be conducted during the third year. This animal testing will demonstrate that the rinderpest vaccine is ready for large scale field testing, which is outside the scope of this project.

This project will contribute to the goal of increasing stability and efficiency of food production by alleviating some of the constraints which limit agricultural production systems in the developing world. Additionally this project conforms with the S&T/AGR CPSS, which outlines the role of S&T/AGR. Specifically, this project satisfies S&T/AGR's mandate to: 1) identify research "targets of opportunity" in agricultural research and technology development for the Agency; and 2) formulate and manage a portfolio of research programs to develop technologies to increase food production and to increase the productivity of agricultural resources. The use of vaccinia virus to develop an improved rinderpest vaccine is truly a research "target of opportunity". Vaccinia has been engineered to produce vaccines against a number of diseases. The time is now ripe for this technology to focus on the development of vaccines for important diseases in the developing world. The existence of an improved vaccine for rinderpest would clearly increase food production and agricultural productivity. It is anticipated that this rinderpest project will be the first step of a larger AID initiative utilizing biotechnology to develop animal vaccines.

Genetic engineering of animal vaccines warrants high priority for technology development and transfer. This technology will be transferred to Africa by the participation of African scientists in the vaccine testing stage and the training of postdoctoral African scientists (2) in molecular biology through the project. The training will be conducted in Dr. Yilma's laboratory. The postdoctoral scientists will participate in all phases of the research from bench work to animal trials. These scientists will return to Africa with skills in vaccinia virus vector technology which can be applied to the development of vaccines for other important African diseases.

The Office of Agriculture has considered all the criteria outlined in the CPSS which must be addressed when considering a project which is predominantly research oriented. An NSF scientific panel was convened on the subject of high priority research areas in animal health; its recommendations have served as the guidelines for this project. The project addresses regional problems; it has the potential to increase agricultural production; expected payoff is beyond near term; related research programs have been considered; there is no need for regional bureau or mission buy-ins; and adequate performance for continued support will be measured by progress against the research plan.

The project also conforms with the Title XII mandate in identifying Washington State University at Pullman, a land grant institution, as the lead institution. Additionally it draws upon the expertise of the USDA, through Agricultural Research Service (ARS) at Plum Island.

The workplan of this project calls for developing the vaccinia vector expressing the rinderpest gene (the vaccine) during the first year. The immune responses of cattle to the recombinant vaccine will be assessed (at Plum Island and in Kenya) during the remaining 24 months.

WANG:Vaccine

1.0 Goal, Purpose, Description, Objectives and Background

1.1 Goal - The goal of this project is to increase stability and efficiency of food production by alleviating some of the constraints which limit agricultural production systems in the developing world.

1.2 Purpose - The purpose of the proposed research is to develop an effective infectious vaccinia virus recombinant vaccine to eradicate rinderpest.

1.3 Project Description - Rinderpest is an acute, febrile, and highly contagious viral disease of ruminants, particularly cattle and buffalo, manifested by a rapid course and greater than 90 percent mortality. The scientists at WSU have achieved a number of accomplishments in vaccinia virus recombinant technology; e.g. they have expressed more than a dozen heterologous genes in this system. Under this project, the scientists will initially clone the entire coding sequence of the two immunogenic genes (Fusion and Hemagluttinin) of rinderpest virus and then express them in a single vaccinia virus recombinant. The immune response of cattle to the vaccine will be assessed by humoral immune response and by challenge with a virulent strain of rinderpest virus.

A tissue culture vaccine for rinderpest was developed using traditional methods approximately 30 years ago. This vaccine was effective, but was not easily adapted to the conditions in Africa and eradication programs have been unsuccessful.

Unlike this tissue culture vaccine for rinderpest, the production and use of a vaccinia vector vaccine would not require refrigeration, trained personnel, or expensive tissue culture facilities. The recombinant vaccine could be produced in the same way as smallpox vaccine was produced during the WHO smallpox eradication campaign. Alternatively, cattle owners could use the cutaneous scab from an animal vaccinated with the recombinant vaccine to produce vaccine for other animals, and administer the vaccine themselves. The use of a vaccinia virus recombinant vector is the best technology available for the eradication of rinderpest, particularly in developing countries where previous attempts to eradicate the disease have failed.

It is believed that vaccination of cattle and other animals with a vaccinia virus recombinant expressing the hemagglutinin and fusion protein genes of rinderpest virus will provide protection against rinderpest for the economic life of the animal. Furthermore, such vaccination is practical under conditions prevailing in developing countries and could lead to eradication of rinderpest worldwide, as has been achieved with smallpox in Africa and Asia under similar circumstances. Eradication of rinderpest, a devasting plague for many centuries, using a vaccinia virus recombinant will clearly demonstrate the potential of this new generation of vaccine to control the major infectious diseases that inflict suffering and severe economic hardship on man and animals worldwide.

1.4 Objectives - The specific objectives of this proposal are:

1. To clone the hemagglutinin (HA) and fusion (F) protein immunogenic genes of rinderpest virus.

2. To construct an infectious vaccinia virus recombinant that optimally expresses the two immunogenic genes of rinderpest virus.

3. To measure immunity and protection against viral challenge in cattle vaccinated with this recombinant vaccinia vaccine at intervals up to 12 months after vaccination.

4. To develop more effective promoters and improved techniques for enhanced expression of immunogenic genes in vaccinia virus recombinant vectors.

1.5 Background

1.5.1 Development Assistance Background

The limiting effects that animal diseases have on agricultural production in the developing countries have long been recognized and the international community has made a number of efforts to overcome these diseases. Presented below are some examples of the ongoing programs.

1) International Programs

International Laboratory for Research of Animal Diseases (ILRAD) in Nairobi, Kenya. This international agricultural research center was founded in 1973 with a mandate to develop effective control measures for livestock diseases which seriously limit food production. ILRAD specifically focusses on trypanosomiasis and East Coast Fever. Funding is provided by the Consultative Group for International Agricultural Research (CGIAR). The U.S. provides approximately 25 percent of the centers funding through AID. The program at ILRAD consists of basic research, training, and outreach programs.

- Australian Center for International Research is funding a project in Sri Lanka entitled "Control of Tick Borne Diseases of Ruminants, with Particular Reference to Babesiosis and Anaplasmosis". The project is funded for three years with total support level of $588,000.

- International Centre of Insect Physiology and Ecology (ICIPI). This international center is working on a tick vaccine to control the vector of East Coast Fever. Project title: Host Resistance/Integrated Tick Control Research.

2) AID Funded Projects

a. Mission projects

Zimbabwe - A cooperative program was signed in June 1985 for three years at a total support level of $2 million between the Veterinary Research Laboratory of Zimbabwe and the University of Florida. The purpose of the project is to develop a vaccine for heartwater disease. Efforts will be made to develop a traditional vaccine by first developing a highly sensitive and specific serological test and expidemiological models.

India - A five-year $1.5 million project is in the process of being approved to develop vaccines for blood diseases in cattle. The project is part of the mission's Agricultural Research Umbrella and is an agreement with the International Center for Agricultural Research in India. Winrock International is the U.S. contractor.

Niger - The mission is funding a rinderpest vaccine project collaboratively between Tufts University and Plum Island. Dr. Solad, the principal investigator, has indicated that the project is predominantly focused on improving the heat stability of the current rinderpest vaccine by the addition of different reagents. The project originally planned to develop a new vaccine through genetic engineering (possibly using vaccinia virus). The Tufts group has no experience in vaccinia work and this appears to be a future direction for their research. They are not working on it presently and they have not established a collaborative agreement with Plum Island yet. Plum Island has also indicated to the Turts group that developing a rinderpest vaccine using vaccinia is a low priority.

b. Science Advisor's Grants

These grants are distributed on a competitive basis to scientists from developing and developed countries for innovative research which is applicable to problems in the developing countries. These grants are for 2-3 years for a maximum of $150,000. Listed below are the titles of some relevant projects and the countries involved:

- Preparation of a Live Vaccine against Caprine Arthritis-Encephalitis Virus-Israel.

- New approaches to the Control of Bovine Babesiosis - University of Missouri-Mexico.

- Development of Modified Live Vaccine against Bovine Babesiosis-University of Missouri.

- Control of Ticks and Tick Transmitted Diseases by Vaccination of the Host-Dominican Republic.

c) Small Ruminant Collaborative Research Support Program

Kenyan Ministry of Agriculture and Livestock Development and the SR-CRSP have developed a traditional vaccine for contagious caprine pleuropneumonia. Additional work is being done at Washington State University to develop an engineered vaccine. A candidate antigen has been identified.

1.5.2 Future Development Assistance

Development assistance in vaccine development will continue as this is a critical area where any improvement could have a major impact on agricultural production. ILRAD has just begun working on a ten-year research strategy which will be completed in 1994. Also, it is likely that region-specific projects will continue to develop. The proposed animal vaccine development will interact with ongoing projects in vaccine development and serve as a resource for them. This subactivity will focus on the state-of-the-art methods of biotechnology, specifically vaccinia virus technology.

1.5.3 Research Background

It took almost 200 years and a sustained international disease control campaign to eradicate smallpox using the vaccinia virus vaccination technique popularized by Edward Jenner in 1796. The scientists at WSU propose that a similar effort could eradicate rinderpest in a decade. Although this may seem to be a bold and perhaps unbelievable statement, the technology to achieve this goal is now available.

Essentially, WSU scientists propose to eradicate rinderpest by using the following steps: 1) construction of the vaccinia vector vaccine for rinderpest; 2) testing of this vaccine in cattle under experimental conditions; 3) testing of the vaccine in the field; and 4) an international campaign to use the vaccine and apply disease control measures in countries where rinderpest exists.

These scientists believe that a vaccinia vector vaccine for rinderpest can be constructed because they have already made vaccinia vectors for vesicular stomatitis and bluetongue, and one of the scientists (Dr. Moss) has made vaccinia vectors for human hepatitis B, rabies, influenza, and malaria. They know that rinderpest can readily be prevented by vaccination because conventional vaccine technology (using Plowright's approach) does work - the problem has been in the effective delivery of this protection under conditions prevailing in deceloping countries, a problem that vaccinia vaccines would overcome. Elaborate and advanced molecular biology techniques and facilities, which exist only in a few research centers in the

world, will be necessary to create the rinderpest vaccinia vector. Once the vector has been constructed, there are existing protocols and facilities to test the vaccine experimentally and in the field - including centers erected specifically for this purpose by AID and other international organizations at Bomako, Mali; Muguga, Kenya; and Vom, Nigeria.

This project will bring together a unique group of collaborators: the leading research group on vaccinia vector vaccines for animals at WSU; the originator of vaccinia technology at the National Institutes of Health (NIH); the former director of the USAID JP 15 rinderpest eradication program; the Plum Island Center for exotic animal disease research of the U.S. Department of Agriculture, Agricultural Research Service (USDA/ARS); one of the world's leading private-sector genetic engineering companies, California Biotechnology, Inc. (CBI); and the Veterinary Services of the Governments of Kenya, Mali and Nigeria. A stronger team to eradicate rinderpest using a vaccinia vector vaccine could not be assembled.

1) Rinderpest and attempts at its control

Rinderpest is an acute, febrile, highly contagious viral disease of ruminants, particularly cattle and buffalo, manifested by a rapid course and high mortality. The disease is characterized by inflammation, hemorrhage, necrosis, and erosion of the gastronintestinal tract accompanied by bloody diarrhea, wasting, and death (Plowright 1968).

History. Rinderpest is one of the most important and devastating diseases of cattle. It came to be recognized as a distinct disease during the great European epizootic of AD 376-386 (Plowright 1968) In the 18th century alone, an estimated 200 million cattle in Europe fell victim to the disease (Curasson 1932, 1942). The most spectacular expizootic of recent times was kindled by the introduction of Indian cattle to Somaliland during the Italian invasion of Ethiopia in 1889 (Lugard 1983, Hutcheon 1902). Since the disease quickly afflicted the entire cattle population of Ethiopia and the mortality rate exceeded 90 percent, the epizootic is commemorated on the Ethiopian calendar - 'The Year of Yekebit Elkit (the Annihilation of Cattle'. Shortly thereafter, the disease engulfed the entire continent of Africa, killing hundreds of millions of cattle.

The urgent need to combat rinderpest resulted in the establishment of the first veterinary school in Europe and convening of the First International Veterinary Congress at Hamburg in July 1863. More recently, rinderpest was responsible in large measure for the founding of the Office Internationale des Epizooties in Paris (Vittoz 1963). In addition, the largest disease eradication program in the history of veterinary medicine, the JP 15 program to eradicate rinderpest, was initiated in 1963 in West Africa and later extended to East Africa. During a period of three years (1970-1972), Dr.

Tilahun Yilma, the principal investigator of this proposal, participated in the vaccination of over 40 million cattle in East Africa as part of the JP 15 program, which was headed by Dr. Donald DeTray of USAID, who is a collaborator in the proposed research to be funded under this project.

For the most part, prophylactic measures against rinderpest in Africa before 1960 were carried out without cooperation between neighboring countries in regard to timing, methodology, or other factors. The only hope of eradicating rinderpest depended upon a program of joint campaigns to be put into effect across frontiers and coordinated by an international organization.

The Inter-African Bureau of Animal Health (IBAH) took the initiative and proposed a joint campaign against rinderpest in Central and Western Africa. This proposal was approved in principle by the Commission for Technical Cooperation in Africa (CCTA)/CSA in 1960. The project was later approved and named "Joint Project No. 15" (JP 15) of the CCTA, which applied for the necessary financing to European and American organizations for aid to developing countries. The vaccination program commenced in September 1962, and ended in 1968 with a total of 81,484,126 cattle vaccinated and a dramatic drop in the number, size, and location of rinderpest outbreaks. Funding for the execution of JP 15 in Nigeria, Niger, Chad, and Cameroon was provided by the European Economic Commission (EEC) and AID.

After the successful campaign in West Africa, the JP 15 program was extended to six countries in East Africa (Kenya, Tanzania, Uganda, Somalia, Ethiopia, and the Sudan), and again AID was one of the major contributors of equipment, transportation, vaccine, and operating capital for the eradication program. After just 3 years, a total of 43,615,328 cattle had been vaccinated in the six countries.

In the JP 15 campaign, it was decided that animals were to be vaccinated with the tissue-cultire-adapted Plowright vaccine, which had been demonstrated to provide protective immunity in cattle for at least 7 years -- literally, the economic life of the animal. In addition, the IBAH was delegated to implement the following protocols in each participating country (these protocols are spelled out here because similar agreements will be needed to use the vaccinia vector vaccine to be constructed under the cooperative agreement with WSU.

- To vaccinate with an effective rinderpest vaccine the annual calf crop at least once per year in two successive years. These animals were marked or ear punched so that any animal over two years old had two such ear or identification marks.

- To vaccinate and mark, or ear punch, all adult cattle with no evidence of previous vaccination.

- To keep neighboring countries informed of the status of rinderpest through IBAH and by means of annual scheduled meetings convened by the Scientific Technical and Research Commission (STRC)/IBAH. In particular, each country notified all concerned by the most rapid means possible of any outbreaks of rinderpest, stating the number of animals involved and the geographical grid location of the outbreak.

- In the case of an outbreak of rinderpest, to implement the accepted control measures as quickly as possible and under professional veterinary supervision. The etiology of any rinderpest-like infections was verified. Recognized control measures included strict quarantine of all infected herds and in-contacts, the slaughter of all infected animals where practicable (with the payment of compensation), and vaccination or re-vaccination of all amimals within a radius of 10 miles of the outbreak.

- To have veterinary authorities maintain strict surveillance over the movements of cattle furnished with veterinary health certificates.

- To provide adequate legislation to permit the undertaking of the requirements of paragraphs 1 - 5 above.

These measures were implemented to prevent the recrudescence of rinderpest to the pre-JP 15 level. It was also realized, however, that implementation of annual vaccination, in the absence of rinderpest, would not be an easy task in Africa or anywhere else where animal owners could not see the benefits of continuous vaccination.

Because of failure of continuous surveillance, vaccination, and implementation of the control measures that were instituted during the JP 15 eradication program, rinderpest is again rampant in Africa. For example, in Nigeria alone there were over 71 outbreaks up to the end of 1982 involving over 13,000 cattle and with mortality exceeding 50 percent. The source of this epizootic was traced to infected cattle that originated in the Sudan and entered Nigeria via Chad (Lamorde & Kumar 1983). Rinderpest also has re-emerged in East Africa (Rossiter 1983) to kill large numbers of buffaloes in Northern Tanzania, for example, Although no field outbreaks of rinderpest were reported in Kenya, there is serological evidence for the presence of the virus in nonvaccinated sheep, goat, and wild ungulates.

Livestock are an important component of agricultural production systems in developing countries, providing food, fiber, fuel, fertilizer, income, and opportunities for investment. Animal disease losses can have a catastrophic impact on the nutrition, income, and well-being of large segments of developing country populations as

well as entire national economies. Other adverse long-term economic impacts, such as closure of export markets, also occur following outbreaks of disease. Replacement of herds and recovery of markets is often a long and costly process. For example, 4 to 5 years of calf production were required to replenish the losses caused by an outbreak of rinderpest in Guinea. Epidemics of diseases as devastating as rinderpest pose a threat to livestock populations in the developed world as well.

Many of the reasons for recrudescence of rinderpest in Africa are related to problems in vaccine production, preservation, and delivery. Developing countries do not have the technical resources to manufacture the Plowright tissue culture vaccine, nor do they have the foreign currency to purchase this relatively expensive product from abroad. In the arid, hot desert regions of much of Africa, it is difficult to keep tissue culture vaccine potent and efficacious without reliable refrigeration facilities. The continuous movement of nomadic hersmen and their animals also make it difficult to assemble groups for vaccination. Many countries do not have the financial resources to buy syringes and needles, distribute vaccine, and continue to implement control measures.

The vaccinia virus recombinant vaccine for rinderpest that we propose to develop will overcome all the drawbacks to the use of tissue culture vaccine in developing countries. The failure of conventional vaccine technology to control rinderpest was not due to any lack of efficacy of the vaccine. The real problems were in sustained manufacture and delivery of vaccine in the field in Africa - lack of refrigeration, the cost of syringes and needles, vaccine instability, and the costs of purchasing vaccine with scarce foreign exchange or of making it with inadequate technical resources. The scientists at WSU know most of these problems can be overcome with vaccinia vectors because developing countries have already demonstrated that they can manufacture and deliver vaccinia for smallpox vaccine. The strength of this proposal on rinderpest is that the most difficult practical part of controlling rinderpest - that which involves participation of developing countries, perhaps without continuing technical assistance from international agencies - has been shown to be possible.

2) <u>Definition of Terms</u>

Presented here is a list of some scientific terms used in this project paper (PP). This is not a complete list of the technical terms, only a list of those terms which will aid the non-science reader to gain a general understanding of the work. Throughout the text additional technical terms are indicated with an "*" and the definitions of these can be found in the expanded glossary - Appendix 2.

<u>Adjuvant</u> - any of many foreign materials introduced with an antigen to enhance its immunogenicity (i.e., the ability to stimulate an immune response).

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Antigen or immunogenic protein - a substance (protein) which is recognized by host's immune system as non-self (foreign) and therefore results in an immune response.

DNA - deoxyribonucleic acid - a large double stranded molecule which consists of four repeating bases (adenine-A, guanine-G, thymine-T, and cytosine-C. This is the genetic material of the cell. It carries information by means of its linear sequence.

Gene - a segment of DNA which codes for one protein.

Genome - the entire collection of DNA in a given cell; i.e., one set of genetic material.

Messenger RNA - ribonucleic acid - a large single stranded molecule which consists of four repeating bases (adenine-A, guanine-G, uracil-U, and cytosine C). This molecule serves as a messenger to carry from the DNA the code needed to make a protein to the site of protein synthesis in the cell.

Plasmid - circular pieces of DNA which are found in bacteria; these circles are often used to carry foreign DNA.

Promoter - a segment of DNA which is responsible for the increased expression of a gene. An increased gene expression results in increased protein production. If this protein is an antigen, the immune response is also increased.

Recombinant - any organism or plasmid which contains DNA from another source (foreign DNA) as well as its own DNA.

Sequence - the sequential arrangement of the four bases (see DNA above) which comprise DNA. This sequence of bases in the DNA determines what protein will be produced by the cell.

Vector - any organism (viral or bacterial) or plasmid which is used to carry foreign DNA.

3) Rinderpest virus Rinderpest virus is a member of the family Paramyxoviridae and is classified in the genes Morbillivirus, along with measles virus of humans and distemper virus of dogs. In fact, all three viruses are antigenically related and confer cross-immunity in experimental animals (Barrett & Underwood 1985).

The virus is enveloped and has a spherical morphology with a helical nucleocapsid*. The virus has a single-stranded RNA genome with a minus polarity, and with it are associated three tightly bound polypeptides (L.P. NP), which constitute the RNA-dependent RNA polymerase*. Like other paramyxoviruses, morbilliviruses are composed of six structural proteins: the large (L) protein, polymerase (phospho P) protein and nucleoprotein (NP) inside the virion, and the hemagglutinin (HA), fusion (F), and matrix (M) proteins in their envelope (Norrby et all 1985). With the exception of the L protein, which has not been characterized antigenically, cross-reactivity among the other proteins has been demonstrated between rinderpest, measles, and distemper viruses (Norrby et al, 1985). Although Provost and Borredon (1968) suggested that rinderpest virus may hemagglutinate* erythrocytes* of the monkey Erythrocebus patas, no hemagglutinating activity for rinderpest viral proteins has been unequivocally demonstrated by others. Rinderpest antisera, however, has been shown to inhibit the hemagglutinating activity of measles virus (Waterson et al 1963).

Further, the receptor sites for measles hemagglutinin on monkey erythrocytes can be blocked by prior treatment with rinderpest antigens (Anon. 1966). Ferrets inoculated with the lapinised or virulent strain of rinderpest did not develop clinical disease, but when challenged with distemper virus, they were totally protected for at least 11 months (Goret et al 1957, 1960). Distemper is 100 percent fatal in ferrets. Monkeys and children convalescing from measles have been shown to have neutralizing antibody against rinderpest (Plowright 1962, DeLay et al 1965). Rinderpest virus probably does not cause clinical disease in humans; however, nearly all human adults have been shown to have serum-neutralizing antibody, most likely from exposure to measles either by immunization or infection (Plowright & Ferris, 1959; Warren 1960). Serum from rinderpest-immunized cattle contained higher neutralizing antibody to measles than to rinderpest (Plowright 1962). This strong immunological cross-reactivity between measles and rinderpest implies that the nucleotide sequence of the F and HA genes of the two viruses will be quite homologous.

As demonstrated for other members of the Paramyxoviridae, it is likely that both the HA and F polypeptides are essential as protectively immunizing antigens (Norrby & Penttinen 1978). Therefore, the scientists at WSU propose to engineer a recombinant that will express both the HA and F genes of rinderpest virus and to accomplish this by using the cDNA* of the HA and F genes of the measles virus to clone the equivalent genes in rinderpest virus. (The measles genes are gifts of Dr. Robert Lazzarini of the NIH).

4) Vaccinia and Vaccine Technology

Modern vaccination began in 1796 when Edward J ner noticed that milkmaids who had been infected with cowpox did not subsequently develop smallpox. He began to vaccinate people against smallpox by scratching them with scabs from cowpox lesions. The exact origin of vaccinia virus is unknown; the virus used for smallpox vaccination does not exist in nature and is distinct from the cowpox virus and the variola virus of smallpox. Vaccinia virus may have originated from horsepox. What is clear, however, is that the vaccinia virus vaccination procedure protected hundreds of millions of people worldwide from smallpox with minimal side effects and with no adverse environmental impacts, despite release of the vaccinia virus on thousands of occasions under poor hygienic conditions.

The principal advantage of live vaccines over inactivated/subunit/synthetic vaccines is that they multiply in the host thereby providing more antigen. But they also have the following drawbacks: 1) interference with export/import of animals and disease control measures (a drawback shared with inactivated vaccines); 2) the potential that the attenuated strains might revert to virulence in either the vertebrate host or any insect vector; 3) the possibility that new virus strains may arise by recombination of vaccine virus with other strains in the animal population; 4) the possibility that pregnant animals or their offspring may be adversely affected by the vaccine strain; and 5) the possibility that the vaccine virus might regain virulence.

Many of these disadvantages could be overcome by subunit or synthetic peptide vaccines, which also is advantageous because the immune system of the animal is challeneged with only one antigen, thereby omitting other components of the virion that might have an adverse effect on the immune response (say, by generation of suppressor T cells*) or the host (teratongenic effects* on the embryo). The major problem with subunit and synthetic peptide vaccines (and inactivated vaccines), however, is that the antigenic mass cannot be greater than the amount of protein injected - there is no amplification of the antigen, as occurs, for example, in an attenuated live virus vaccine.

As an alternative to these vaccination methods, the vaccinia vector system offers more than a compromise - it offers the best of all worlds. First, it permits the expression of a single gene coding for an immunogenic protein (as in a subunit vaccine); second, there is amplification through replication of the vaccinia DNA (as in a live virus vaccine); and third, several genes representing the various serotypes of an infectious agent can be expressed (as in a polyvalent vaccine). Furthermore, rapid diagnostic kits can be developed to distinguish vaccinated animals from those exposed to the whole virus by either infection or vaccination - for example, the kits which were developed for vesicular stomatitis and foot-and-mouth disease.

The nature of the host's immune response to an injected antigen is critical in vaccination. Inactivated vaccines commonly employ an adjuvant of some type to boost the immune response. Live vaccines cannot use adjuvants because they inactivate the immunizing agent; on the other hand, live agents generally do not need additional help to induce immunity.

In a separate study, WSU demonstrated the adjuvant effect of recombinant bovine interferon gamma (BoIFN-y) when administered with G. glycoprotein of vesicular stomatitis virus in cattle. They are now evaluating the adjuvant effects of BoIFN-y and bovine interleukin-2 (BoIL-2) genes in vaccinia virus recombinants by expressing them along with other heterologous genes.

Incorporation of the BoIFN-y and BoIL-2 genes is a novel idea, and the adjuvant effects of these genes in vaccinia recombinants are presently under evaluation. If an adjuvant response is seen, the BoIFN-y or BoIL-2 gene can be incorporated into vaccinia vectors along with the rinderpest genes to enhance the immune response of cattle to the rinderpest virus. This area of vaccinia research is unique to the laboratory at WSU. This will be a significant step forward in the art of vaccination because it will allow finer management of the immune response than has been possible in the past. For one virus, it may be necessary to stimulate humoral responses*; for another, cell mediated immunity; for a third, natural killer cells*. The importance of being able to manipulate the host's immune response cannot be understated in our future efforts to construct polyvalent vaccinia vectors for viral, bacterial, fungal and parasitic diseases, particularly for those agents that normally provoke a weak immune response.

5) Vaccinia virus

Vaccinia virus, a member of the poxvirus family, is a large, double-stranded DNA virus that packages a complete transcription system and replicates in the cytoplasm of infected cells (Moss 1974). Advantages of vaccinia are the large genome (Geshlin & Berns 1981), evidence that vaccinia virus genes contain unique transcriptional regulatory signals recognized by the viral RNA polymerase (Puckett & Moss 1983, Venkatesan et al 1981, 1982a, Weir & Moss 1983), the existence of convenient marker rescue techniques (Venkatesan et al 1981, Weir et al 1982, Nakano et al 1982), and the ability of homologous sequences in plasmid and virus DNA to recombine within infected cells (Weir et al 1982).

Inoculation with vaccinia virus provides a high degree of immunity against variola virus, the etiological agent of smallpox, because of the live nature of the vaccine and the close genetic relationship of the two viruses. Recently, it was proposed that vaccinia virus recombinants could be similarly used to express genes of unrelated pathogens (Weir et al 1982, Mackett et al 1982, Panicali & Paoletti

1982). This involved construction of a plasmid containing a chimeric gene, consisting of a vaccinia virus promoter fused to a foreign protein-coding genome. The plasmid was used to transfect cells already infected with vaccinia virus so that homologous recombination could occur between the sequences flanking the chimeric gene and the same sequences within the vaccinia virus genome. Recombinants were selected either by their expression of the inserted foreign gene or by insertional inactivation of the vaccinia virus thymidine kinase (TK) gene. A related approach of insertional inactivation of the TK gene has been used to produce and select herpesvirus recombinants (Mocarski et al 1980). Furthermore, plasmid vectors that facilitate the insertion and expression of foreign genes in vaccinia virus, as well as the selection of recombinants, have been constructed, and the prokaryotic chloramphenicol acetyltransferase gene (previously used with simian virus 40 vectors) has been adopted to characterize and optimize the system (Mackett et al 1984).

An additional selection marker that WSU uses in their laboratory for rapid screening of recombinants of interest is the co-expression of the Escherichia coli B-galactosidase (Lac Z) gene along with the foriegn gene of interest. Expression of the Lac Z gene leads to the development of blue plaques in the presence of XGal (5-bromo-4-chloro-3-indolyl-B-D-galoctopyranoside). Thus one is able to distinguish TK" recombinants from spontaneous TK" mutants (Chakrabarti et al 1985).

Initial experiments indicate that vaccinia virus vectors retain infectivity, accommodate at least 25,000 base pairs of exogenous DNA, and synthesize foreign gene products that are properly processed and glycosylated* (Mackett et al 1984). The heterologous genes, which have expressed in this vector system includes; hepatitis B virus surface antigen (Smith et al 1983a, Smith et al 1983b, Paoletti et al 1984), influenza virus hemogglutinin (Panicali et al 1983, Smith et al 1983c), herpesvirus glycoprotein D (Paoletti et al 1984), malaria sporozoite surface antigen (Smith et al 1984), rabies glycoprotein (Kieny et al 1984), vesicular stomatitis virus (VSV) glycoprotein (G) and nucleoprotein (N) genes (Mackett et al 1985, Yilma et al 1985b), the two vesicular stomatitis virus G genes (manuscript in preparation), the BoIFN-y and BoIL-2 genes (manuscript in preparation), and gene segment 2 of bluetongue virus serotype 10 (manuscript in preparation).

Vaccinia virus has a wide host range, including man, cattle, horses, swine, sheep, goats, mice and monkeys. The work WSU proposes here has fundamental significance in developing vaccines for all these species. The broad host range of vaccinia virus has certain advantages. For example, the vaccine vector for rinderpest can be given to cattle, buffalo, and other large ruminants. It also can be given to sheep and goats to prevent petit peste des ruminants. On the other hand, there are some potential problems with the use of vaccinia vectors in humans, such as the possibility of interference

with worldwide smallpox control. These questions were considered in detail at a recent meeting of the NIH and World Health Organization (WHO) (See attached documents - Appendix), in which Dr. Yilma and Dr. Moss participated. As a result of this meeting, a special advisory group recommended an accelerated program of research on the use of recombinant vaccines employing viral vectors in humans and animals. A similar meeting was called by the USDA Animal and Plant Health Inspection Service (USDA/APHIS) to address the safety of vaccinia virus recombinants on January 7, 1986, and Dr. Yilma and Dr. Moss were the two invited speakers. The conclusions of that meeting were that there was no reason to suspect unforeseen safety problems with vaccinia vector vaccines and that ssuch vaccines could be licensed and approved through existing USDA/APHIS procedures.

The scientists at WSU have answered one safety question with respect to the spread of the virus from the site of intradermal inoculation. They have used the vaccinia virus-hepatitis B recombinant and the V50 recombinant expressing the G gene of vesicular stomatitis virus serotype New Jersey (VSV-NJg) in cattle to demonstrate that the virus does not spread from the site of intradermal inoculation, presumably due to the inactivation of the TK gene by the inserted foreign DNA. Therefore, WSU does not anticipate reversion to the virulent vaccinia virus in cattle, or spread of the recombinant from one animal to another. Furthermore, for the development of the vaccinia vector vaccines, WSU will use the attenuated New York City Board of Health strain of vaccinia virus previously used by Wyeth Laboratories to prepare the U.S. smallpox vaccine. If necessary, WSU has the potential to attenuate the virus further by insertional inactivation of other nonessential regions of the vaccinia virus genome besides the TK gene.

WSU expects to field test its vaccinia vector recombinant vaccine for vesicular stomatitis virus serotype New Jersey/Indiana (VSV-NJ/IN) in Mexico during 1986-88 as part of a study funded by USDA/APHIS to compare inactivated whole virus, G subunit, and synthetic peptide vaccines for vesicular stomatitis (see attached letters - Appendix 3). This will be the first large-scale field study of the vaccinia vector system.

In the WHO eradication campaign, smallpox vaccine was produced by extensively scarifying the shaved skin of a calf and seeding the wounds with vaccinia virus. The scabs from the calf were collected after seven days, ground in saline, filtered, and the filtrate used for vaccination of humans after testing for pyrogenicity. One calf would yield over 100,000 doses of vaccine at a cost of less than three U.S. cents/dose. Once lyophilized* smallpox vaccine is very stable for several years. The vaccine was administered to humans by scarifying the skin using a bifurcated needle or an airgun injector. Similar production and delivery procedures could be used for the rinderpest recombinant, or the virus could be grown in tissue

culture where facilities are available. Nothing needs to be invented or re-invented for developing countries to produce large quantities of rinderpest vaccine cheaply. Once the recombinant is available, nomadic hersmen and other livestock owners could independently conduct both the vaccice production and vaccination phases of the program. Alternatively, the governments of developing countries could centrally produce the vaccine for distribution, as was done to eradicate smallpox. In either case, it is not necessary to import manufactured vaccine or syringes and needles.

Inoculation of cattle has precedents in the culture of African nomadic herdsmen. For example, for over a thousand years, the Somali nomads in the Ogaden region of East Africa have vaccinated their cattle against contagious bovine pleuropneumonia, which is caused by Mycoplasma mycoides. This disease causes the accumulation of fibrin in the pulmonary interlobular septa and pleura and copious accumulation of serous fluid in the lungs. To produce the vaccine, the herdsmen take the lung of an animal that has died of the disease and dry it in the sun. They then inoculate other cattle by making an incision in the skin over the nose and inserting a piece of the dried lung. The area swells temporarily, and the animal develops a mineralized keloid that resembles an antler. Animals so treated are protected for life. Since the rinderpest vaccine can be easily made from the dried cutaneous scabs of animals previously vaccinated with the vaccinia vector, the vaccine can be administered merely by scratching it into the skin, without the need for a syringe. The herdsmen could thus readily adopt the practice of independently inoculating their herds against rinderpest.

Vaccinia is particularly well suited to the development of a rinderpest vaccine that provides long-term immunity for several reasons:

1) There is only one serotype of rinderpest as far as immunity is concerned. There are different strains of rinderpest manifested by different degrees of pathogenicity in the field. However, from the immune point of view a vaccine against one serotype will immunize against all serotypes, including petit peste des ruminants virus in sheep and goats. It is not necessary to make a polyvalent vaccinia vector (as one must, for example, for the five U.S. serotypes of bluetongue virus).

2) Rinderpest is a systemic disease, and immunity has been directly correlated to any detectable level of humoral antibody response (D.E. DeTray, personal communication). Vaccinia vectors are very effective in controlling systemic diseases, expecially those with a hematogenous phase. For example, WSU has recently demonstrated the effectiveness of a single vaccination with V50 (vaccinia recombinant expressing VSV-G gene) against a systemic, rabies-like disease in mice (Yilma et al 1985a, 1985b; Mackett et al 1985).

3) If a cell-mediated immune response were essential for immunity to rinderpest, which does not appear to be the case, this could also be achieved with vaccinia since it was recently demonstrated that heterologous antigens of influenza virus expressed in vaccinia induced a high-level cellular immune response in mice (Bennink et al 1984).

4) The use of current tissue culture vaccine has demonstrated clearly that rinderpest can be controlled by a vaccine. The vaccinia vaccine will have all the immunogenic properties of the tissue culture vaccine, but will have none of its logistical disadvantages.

Finally, it is very important that rinderpest vaccination be sustained for several years, even in the absence of clinical disease, in order to eradicate the virus. It is difficult to persuade farmers in any part of the world to continue vaccination under these circumstances. Vaccinia technology is useful in overcoming thisreluctance. It is possible and feasible to incorporate into vaccinia vector immunogenic genes for a number of diseases. As new immunogenic genes are incorporated into a vector, it is not necessary to discard other ones. Theoretically, some 12 to 20 different immunogenic genes can be incorporated into a single vaccinia genome without inactivating the virus (or several different vaccinia vectors could be given as a cocktail at one time).

2.0 Research Strategy - Technical

2.1 Hypothesis

Vaccination of cattle with a vaccinia virus recombinant that expresses the immunogenic genes of rinderpest, namely the HA and F genes, will provide protection from rinderpest for the economic life of the animal and can be practically achieved under conditions prevailing in developing countries.

Construction of the vaccinia vector vaccine for rinderpest will involve the following: 1) insertion of the immunogenic genes of the rinderpest virus into the appropriate vectors; 2) homologous recombination of these vectors with the Wyeth strain of vaccinia virus in CV-cells; 3) evaluation of the protective immune responses to these recombinant vaccines in cattle; and 4) development of more effective promoters and improved techniques to enhance expression of immunogenic genes in vaccinia virus recombinant vectors.

2.2 Networking and Training

Dr. Yilma through his research with vaccinia virus has established a broad international network within the scientific community. Additionally, WSU's extensive international programs have broadened this network into the developing countries. The African collaborators in the project will become a part of the network. The network will continue to be expanded and serve as a resource during the course of this project. Two African veterinary postdoctoral candidates will be trained during the three year life of this project in Dr. Yilma's laboratory, learning molecular biology and vaccinia technology. (It is anticipated these postdoctoral candidates will be from Kenya.) They will also participate in the laboratory testing of the vaccine. It is envisioned that these scientists will return to Kenya and apply the techniques they have learned to develop future vaccines for important diseases in Africa.

2.3 Overview of the Research Strategy

Presented here and in the following sections on project objectives is a general description of the research to be conducted. A detailed research plan is contained in Dr. Yilma's proposal on which the following descriptions are based. Dr. Yilma's technical proposal has been reviewed by the National Science Foundation (NSF). A copy of this review is attached as Appendix 8.

To develop a genetically engineered vaccine a number of steps are required. These steps are briefly described below:

1) Identification of the antigen(s) which stimulate the immune system so that the vaccinated animal is protected. For rinderpest, the immunogenic proteins have already been identified as the fusion protein (F) and the hemogluttinin (HA) protein.

2) Developing a system of probes (monoclonal antibodies or DNA probes) to identify the antigens and extract the gene which expresses the antigen.

Rinderpest virus is closely related to, and classified in the same genus as measles virus. These viruses share many common features and the two are antigenically related. This means that antibodies raised against measles cross react with rinderpest. Much work has been done on the measles virus, and the DNA probes and monoclonal antibodies have been developed. Since the two viruses are closely related the tools developed for measles can be used to identify the antigenic genes of rinderpest.

3) Transfer of the gene or genes for the rinderpest antigens to vaccinia virus.

This is accomplished by a two phase process. Initially the rinderpest gene of interest is placed in a plasmid vector which contains some DNA homologous to the DNA of vaccinia (i.e., they have the same sequence). The rinderpest gene is surrounded (flanked) in the plasmid vector by this vaccinia DNA. This plasmid vector may also contain maker sequences which will later be used to verify the insertion of the rinderpest DNA.

The second phase of the insertion of the gene into vaccinia virus is known as homologous recombination. Cells are infected with vaccinia virus and then treated with the plasmid vector containing some vaccinia virus DNA and the rinderpest gene. The similar DNA sequences of the vaccinia virus (contained in the plasmid and the virus) line up and recombination occurs (that is pieces of DNA are exchanged). The rinderpest gene moves from the plasmid vector to the vaccinia virus. The site of insertion of the foreign gene is determined by the flanking vaccinia DNA.

A schematic representation of this process is shown below.

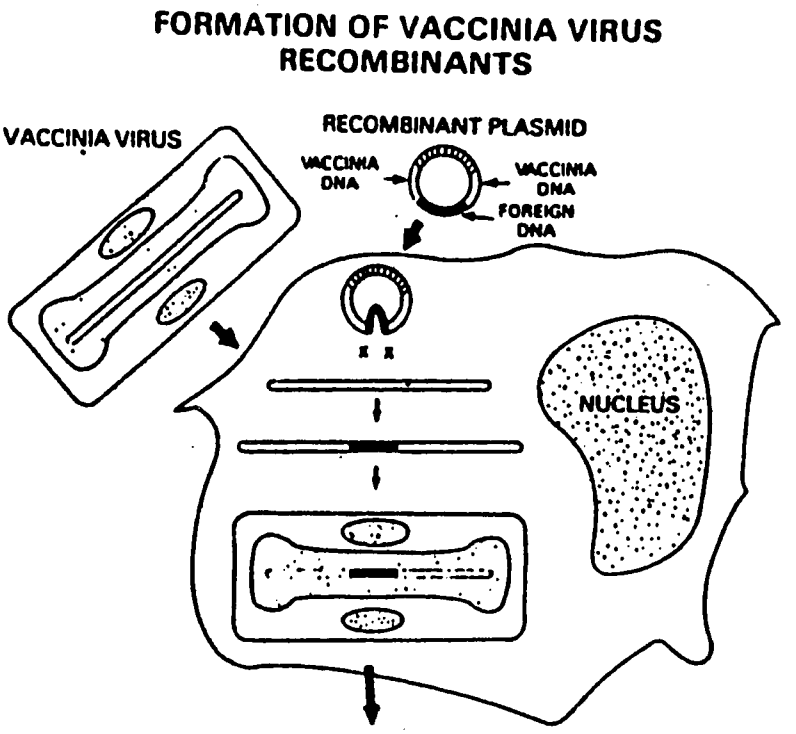

FIG. 2. Formation of vaccinia virus recombinants by homologous recombination.

* From Moleculat Biology of Vaccinia Virus: Strategies for cloning and Expression of Foreign Genes. Bernard Moss in Vaccinia Viruses as Vectors for Vaccine Antigens. 1984 ed. Gerald V. Quinnan, Jr., M.D. Elsevier, NY.

4. Laboratory and field testing of the vaccines.

The efficacy and potency of the vaccine is determined by animal inoculation with the vaccinia vectored vaccine followed by challenge with virulent rinderpest virus.

2.4 Specific Research Strategies

Objective No. 1 - To clone the hemagluttinin and fusion protein immunogenic genes of rinderpest virus into the appropriate plasmid vectors

The messenger RNA for the antigenic genes will be identified using the probes developed from previous work with measles. (These probes will be provided by Dr. Bellini - Center for Disease Control - see

letter Appendix 3). From the messenger RNA, DNA will be prepared and cloned into pBr322 (plasmid vector) by standard methods. Radioactive labeled measles probes will be used to select bacteria which contain the rinderpest genes. The identified clones will be sequenced by California Biotechnology Incorporated. This has been arranged (see attached letter, Appendix 3).

Objective 2. To construct an infectious vaccinia virus recombinant that optimally expresses the two immunogenic genes of rinderpest virus.

The construction of a vaccinia virus recombinant that expresses both the HA and F genes of rinderpest virus will involve several steps. First a vaccinia virus recombinant containing a marker system (LAC Z) will be used. Homologous recombination (between the plasmid vector containing the F gene and the vaccinia virus recombinant containing the marker system) will occur. This will provide a vaccinia virus recombinant which can be selected for and which contains the F gene of rinderpest.

Construction of a recombinant that contains both the F and HA genes will also be accomplished by homologous recombination (between the vaccinia virus which contains the F gene and the plasmid vector which contains the HA genes). The presence or absence of the predicted structures and chimeric genes will be determined by using the marker system and verified by digestion with restriction endonucleases followed by agarose gel electrophoresis.

a. Expression of heterologous genes. Several methods will be used to detect the expression or the production of heterologous (rinderpest) proteins. Preliminary evidence for protein expression will be obtained by incubating cell monolayers containing recombinant vaccinia virus plaques with I-25 labeled specific antibody to HA and F proteins, as routinely performed in WSU laboratory. Alternatively, expression of the proteins in vaccinia infected cells will be determined by immunofluorescence as described in scientific publication (Yilma et al 1985a, Mackett et all 1985).

b. Characterization of proteins: An effective recombinant vaccinia vaccine must produce the rinderpest antigens accurately. This will be determined using the standard immunoprecipitation and gel electrophorisis methods. In WSU scientist's previous reports, they clearly demonstrated expression of immunoprecipitable antigens by vaccinia recombinants.

Objective 3. To measure immunity and protection against viral challenge in cattle vaccinated with this recombinant vaccinia vaccine at intervals up to 12 months after vaccination.

Since this is the ultimate proof, the goal is to demonstrate that the vaccinia virus recombinant vaccine protects cattle against clinical disease after challenge with virulent rinderpest virus.

a. Evaluation of efficacy of the vaccinia virus vector. The efficacy of the vaccine will be tested by experimental challenge with virulent rinderpest virus (Kabete "O" strain) of cattle vaccinated with the recombinant at various periods up to 12 months after vaccination. The results will be compared with those from cattle given the tissue culture vaccine developed by Plowright. Humoral immune responses will be monitored during and after vaccination and challenge. This is a unique experiment which compares the vaccinia recombinant vaccine with Plowright's tissue culture vaccine in the natural host with live virus challenge.

All animal studies involving exposure to the rinderpest virus and assessment of humoral immune response, and challenge studies will be conducted in a P4 facility at the Plum Island Animal Disease Center.

Initially, a pilot study at Plum Island will be conducted to assess the efficacy of vaccine before committing a large number of cattle in Africa to the study.

1. Two groups of six cows will be vaccinated twice with the vaccinia vector on one occasion only. Each group will be challenged with virulent rinderpest virus; one at six months after vaccination, and one at 12 months.

2 Two groups of six cows will be vaccinated twice with the vaccinia vector, on day 0 and day 28. Each group will be challenged with virulent rinderpest virus; one at six months after vaccination, and one at 12 months. The vaccinations will be timed so that there are always two positive control cows vaccinated with the Plowright's tissue culture vaccine and two negative control cows vaccinated with V50 (recombinant expressing the VSV G gene).

b. Evaluation of humoral immunity. Humoral responses will be determined by serum neutralization (SN) as described previously (Plowright 1968). This work can be performed in Kenya in collaboration with Dr. Chema, or in Mali or Nigeria.

Objective 4. To develop more effective promoters and improved techniques for enhanced expression of immunogenic genes in vaccinia virus recombinant vectors.

Several effective vaccinia vector vaccines have been produced by the Washington State University (WSU) and others. The scientists at WSU are confident, therefore, that existing technology is sufficient to produce an effective rinderpest vaccine. But all technology can be improved.

As described earlier, promoters are sequences of DNA which regulate the expression of a gene. A very promising area for improvement in vaccinia is the development of more effective promoters that can lead to a better immune response. In conventional vaccine technology, this can be likened to the continuing search for new and more effective adjuvants. By studying different promoters, we hope to develop vaccinia vectors that will stimulate more long lasting immunity - lifelong for man and animals.

Previous studies have demonstrated that one particular promoter is responsible for a 30-fold more efficient expression of the gene than another promoter. It is believed that an increased level of gene expression or multiple copies of the foreign gene can provide improved protection of the animal.

To produce a live recombinant vaccine it is necessary to integrate stably one or more genes of an infectious pathogen, express these genes efficiently, and maintain infectivity. The time and level of expression of the foreign gene are determined by the choice of the vaccinia viral promoter and the way the chimeric gene is engineered.

For the first vaccinia virus recombinant that expressed the VSV glycoprotein gene (Mackett et al 1985), the WSU scientists used the first vaccinia virus promoter ever defined. Since that time, a number of other vaccinia virus genes have been located. Some of these encode the major structural proteins of the virus and are made in large amounts during a vaccinia virus infection (Wittek at al 1984). It seems likely, therefore, that the promoter regions of these genes would be very efficient. In order to utilize such promoters, the scientists at WSU have sequenced several late genes (Weir & Moss 1984) and are carrying out *in vitro* mutagenesis studies to define the regulatory sequences. These new promoter regions will be used to increase the expression of the rinderpest gene by vaccinia virus recombinants. A second approach that will be taken to exhance expression is the insertion of multiple copies of the chimeric rinderpest gene.

Tentative Schedule

WSU scientists anticipate that they will complete the construction of vaccinia vectors expressing the rinderpest gene (objective 1) during the first year of the grant. During the remaining 24 months of the grant period, they will assess humoral and protective immune responses of cattle to the recombinant (Objectives 2 and 3). Studies with promoters will be conducted throughout the grant period (Objective 4).

3.0 Implementation Plan

Upon project approval, a brief worldwide information cable will be sent to inform missions.

3.1 S&T/AGR Project Management Responsibility

This project will be managed by the S&T/AGR project manager who will deal directly with Washington State University to provide technical and administrative support and oversight. Current AID project staff and precedures are adequate to implement this project. The project manager will spend approximately 60 percent of the time managing the activities to assure that the objectives are achieved, facilitiate acquisition of mission clearances, monitor project operations, approve certain administrative actions within the project, maintain close liaison with the grantee, and communicate regularly with the Principal Investigator, Washington State University.

3.2 WSU Management Responsibility and Qualification

The scope of work of this project is entirely in the area of vaccine development using vaccinia virus as a vector where WSU has particular and unique implementation expertise. It does not require the assignment of new functions or activities nor the expansion of an existing function or activity which would increase capital investment or annual operating costs at WSU. It is administratively part of the Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University. The Dean of the Graduate School and Vice Provost for Research has taken a strong interest in the project and is actively supporting it.

3.2.1 WSU Management Responsibility

The Principal Investigator (PI) has the overall responsibility for implementing the project. The PI draws on advice of world famous scientists, e.g., Dr. Sandra Ristow, Department of Veterinary Microbiology and Pathology, WSU; Dr. Bernard Moss, Chief of the Laboratory of Viral Diseases at the National Institutes of Health and the world's leading expert in the molecular biology of vaccinia; Dr. Jerry Callis, Director of the USDA/ARS Plum Island AnimalDisease Center and Dr. Charles A. Mebus, Supervisory Veterinary Medical Officer, Plum Island; Drs. Beverly Dale, R. Tyler White, and Barbara Cordell from California Biotechnology, Inc. Specifically the PI will have responsibility for implementing the following functions:

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- Design the annual work plans in collaboration with the S&T/AGR project manager and obtain approval prior to implementation. The annual work plans will outline the activities to be implemented during the coming year.
- Insure that appropriate U.S. regulations regarding recombinant DNA research and testing of genetically engineered organisms are followed throughout the project at both U.S. and African locations.
- Insure that all testing involving live animals conforms with U.S. regulations.
- Maintain close working relationships and communicate regularly with the S&T/AGR project manager.
- Develop and implement the required reporting structure to conform with the project reporting requirements.
- Oversee the timely completion of all reports set forth in the reporting requirements described in Section of this project paper.
- Obtain clearance from the S&T/AGR project manager to hire scientists and consultants to implement the activities in this project paper.
- Obtain approval of the requesting missions prior to sending scientists and other professionals to the missions.
- Carry out the annual work plans within the time frame and financial resources allocated.
- Coordinate the work of the WSU headequarters with the work of the collaborating institutions.

3.2.2 WSU Qualification

The proposed three-year project is based on the unique capability of WSU in the progress already made to perfect the vaccinia virus as a vector vaccines. WSU has:

- Developed a vaccine for vesicular stomatitus virus which is now ready for field testing.

- Available probes (DNA) for working on rinderpest which is a critical step in developing a vaccine, as a probe is needed to identify the gene or genes which will be used to make the vaccine.

- A principal investigator, Dr. Yilma who has had experience working in Africa in vaccine development programs and has professional linkages with scientists there.

  Dr. Yilma has established professional relationship with the scientists at USDA/ARS Plum Island and several biotechnology industrial firms.

Adequate animal facilities and research laboratories exist at WSU which are staffed with experienced scientists and technicians who are already working on the development of vaccines and related technologies. WSU has established an ongoing research network with several of the developing countries (e.g., Kenya and Sudan), and many of WSU's scientists have had considerable experience working with tropical diseases in Kenya, Sudan, Senegal, Gambia, Nigeria and elsewhere.

3.2.3 Collaborative Relationship between WSU and AID

WSU has had a long relationship with AID that began in the early 1950s when the University implemented a project in Pakistan. Following that project, WSU designed and/or implemented a number of projects funded by AID, several of which have livestock production and animal health components which are directly relevant to this project; e.g., Small Ruminant Collaborative Research Support Program in Kenya. A list of projects is attached as Appendix 6. WSU faculty members have participated in a variety of AID's planning, committees and related activities, evaluation efforts, and as resource persons for a broad spectrum of activities and interests. Based upon the University's participation with the Agency, the University signed a Memorandum of Understanding in 1983, which was a continuation of five years previous of implementation of a strengthening grant. One of the areas of concentration identified in the Memorandum of Understanding is livestock production and animal health.

WSU has established an effective collaborative relationship with AID that has spanned several decades in which the Agency has utilized the capability of the University and its faculty to address various aspects of their programs. This proposal continues this relationship and will build upon the foundation previously established and proven to benefit both the Agency and the developing countries.

3.3 Advisory Panel of Experts

A panel of experts on vaccine development and molecular biology will be established to advise the Grantee on matters concerning the research and testing of this project. The panel members will include representatives from the following: Small Ruminants - CRSP; International Laboratory for Research on Animal Diseases (ILRAD); International Livestock Center for Africa (ILCA); USDA/ARS; and from the private sector. Specifically, the Panel will assist in the following areas:

1) Review the annual work plans and recommend additions, modification, or deletion of components, as appropriate.

2) Consult with the WSU Principal Investigator on the management implications of the suggested modifications.

3) Participate in the annual reviews and the indepth evaluation of the activities under the Cooperative Agreement.

4.0 Monitoring Plans

Monitoring will be accomplished by the Principal Investigator, the S&T/AGR Project Officer, the collaborators at Plum Island and at the National Institutes of Health, and the scientific staff at California Biotechnology. Annual progress reports and copies of all documents resulting from field and laboratory activities will be submitted to the Project Officer for clearance prior to distribution.

4.1 Annual Work Plans

WSU will develop the annual work plan as a working document to guide the operations and achievements expected from the project. It will be forwarded to S&T/AGR for approval each year. The first plan will be submitted to S&T/AGR no later than 30 days after the cooperative agreement is signed and will cover the period August 1, 1986 to July 31, 1987. Thereafter, the annual work plan will be due 60 days prior to the end of the anniversary date of the cooperative agreement.

S&T/AGR will review the contents of the proposed work plan with the external panel of experts, ask for points of clarification, if required, and grant final approval of the contents as proposed or modified by agreement between WSU and S&T/AGR. This process of review and approval will be completed not later than 30 days after receipt of the original work plan from WSU.

The annual work plan shall include the following:

- A list of activities to be undertaken during the year, including testing at the various locations.
- A statement of how the activities relate to the outputs and research priorities.
- A projected beginning time frame for execution of the activity.
- A projected ending time frame for completion of the activity.
- A projected expenditure of person-months of input for each activity.
- The projected stage of research at the end of the work plan or the projected outputs at the end of the work plan.

4.2 Reporting Requirements

In addition to the Annual Work Plans described above, WSU will submit the following reports within the specified time frame. These reports will provide pertinent data for S&T/AGR to monitor project activities.

4.2.1 Technical and Research Reports

Technical research activities of the project will be summarized in reports and submitted to S&T/AGR and participating agencies, institutions and organizations. These reports will also address regulatory issues related to the project. Normally such reports will be completed 60 days after completion of the activity. Journal articles and other external publications are encouraged. Manuscripts should be submitted to the S&T/AGR Project Manager prior to submission to a publisher as well as a copy of the resulting publication.

4.2.2 Annual Activity Reports

An Annual Activity Report of the WSU international activities will be prepared. Although principally a technical document, it nevertheless must include pertinent statistics on quantitative information regarding the project and its activities described in Section above. A minimum of five copies should be submitted to S&T/AGR Project Manager within 90 days of the end of each project year.

4.2.3 Training Activities

An annual report of the postdoctoral fellows is required indicating their progress in learning how to conduct laboratory work in molecular biology and their participation in the rinderpest research.

4.2.4 Annual Expenditure Reports

WSU will submit annual expenditure reports by project line item. The format will be collaboratively developed by S&T/AGR project manager and the Principal Investigator at WSU.

4.2.5 Trip Reports

Trip reports will be prepared for each TDY or trip to an LDC. Each report will contain, but not be limited to, the following information: logistical information; i.e., type of activity, geographical area of activity, dates of TDY, and team composition; 2) objective of TDY, including scope of work, as appropriate; 3) activities performed while on TDY; 4) summary of any technical reports resulting from TDY; 5) summary of identifiable techniques or

information which could be utilized in this project; and 6) summary of future potential needs of, or opportunities for, assistance to LDCs or missions, including possible networking potential. One copy of this report will be forwarded to S&T/AGR not later than 30 days after the staff member returns to WSU. The trip report generally will not exceed four pages.

4.3 Reviews and Evaluations

4.3.1 Management Reviews

Management reviews will be conducted annually by the S&T/AGR project manager in consultation with WSU, USDA/ARS Scientists at Plum Island, National Institutes of Health, and California Biotechnology, Inc. Such annual reviews will be undertaken after submission of the annual reports as described in Section 4.2 above. The annual reports (activity and impact) will become an integral part of the review process.

4.3.2 In depth Evaluation

In depth comprehensive evaluations will be performed at 1.5 year and 2.5 year by an external panel of experts to review the progress made in achieving the established goal. The panel will be comprised of experts in vaccine development and molecular biology. Recommendations for participants on this panel will be obtained from NSF.

The evaluation factors will include project achievement in developing an improved vaccine for rinderpest. This evaluation will be based on monitoring reports, inspection of physical facilities, WSU progress reports, technical publications, and trip reports. The evaluation will determine if project inputs are being provided as planned, that conditions and covenants of project workscope are being met, and that project outputs are being accomplished as planned. This evaluation will be the basis for recommending changes in the workscope necessary to achieve project purpose and end of project status. The review conducted in the middle of the second year of this project will also address follow-on project development and the future issues involved in bringing the new recombinant rinderpest vaccine to large scale field testing and eventual utilization.

5.0 Participating Institutions

The proposed research involves collaboration among four institutions, i.e., WSU, California Biotechnology, Inc. (CBI), USDA/ARS Plum Island, and at least one in a developing country in Africa, e.g., Kenya. The project will be administered by WSU and Dr. Yilma will be the principal investigator with overall responsibility for all aspects of the proposed work. No problems are anticipated in the interaction of the four institutions, all of which are currently collaborating with Dr. Yilma on other vaccinia vector projects (see documents attached as Appendix 3).

To do small scale animal testing of the vaccine in Africa consent must be obtained from the foreign government involved. WSU has already reached agreement with Dr. Chema of Kenya. During the first year of the grant and depending on the progress of the construction of the vector, the principal investigator, Drs. Callis, DeTray, Henson, and possibly others involved in the project will visit Kenya where the experimental challenges might be carried out. They will inspect the facilities, meet with potential collaborators, and sign agreements with appropriate government officials. Other possible countries who may be interested in collaboration are Niger and Mali. The S&T/AGR Project Manager may also accompany this team. It would be premature to attempt to provide a specific breakdown of the budget required to do the work in Africa until we have determined exactly where and how such trials will take place.

5.1 Washington State University's Contribution

5.1.1 Scientists funded by WSU

WSU will provide the services of the Principal Investigator, Dr. Yilma free of charge and 25 percent of the salary of Dr. Yilma's research technician.

5.1.2 WSU Equipment and Facilities

Washington State University has already made a substantial investment in work on vaccinia virus as a vector for vaccines. The laboratory equipment and facilities are unparalleled in the world for research on vaccinia virus and will be used for the activities under this project. The principal investigator's laboratory is located in the Bustad Veterinary Science Building, a new research/teaching facility of the WSU College of Veterinary Medicine (occupied since November 1978). The investigators have well-equipped laboratories containing equipment for gene cloning, microbiological, chemical, biochemical, physiological, and pathological studies. Equipment includes sampling manifolds, fraction collectors, multiskan MC, DU-50 spectrophotometer

with printer-plotter, scanning densitometer, vacuum oven, MP-4 multipurpose land camera, orbital shaker, shaking water bath, ultra-low freezer, cryological freezers, Geiger counter, microcentrifuges, incubator shaker, circulating water bath, vacuum pump, high pressure liquid and gas chromatographs, and liquid scintillation counters.

In addition to departmental centrifuges, the principal investigator's own laboratory has a tabletop ultracentrifuge, L5-65 floor model ultracentrifuge and vertical, fixed angle, and swinging bucket rotors. The laboratories also have high- and low-speed centrifuges, airfuges, electronic balances, ph meters, fluorometer, thin-layer and column chromatography, gel electrophoresis equipment, cell culture facilities, tissue-processing equipment for light and electron microscopy, Zeiss MOP-3 image analysis system, computer capabilities in statistics, programming and data analysis, and access to automated amino acid analysis.

The College of Veterinary Medicine has a new Hitachi 600/2 TEM and electron microscopy laboratory. Other equipment and facilities include incubators, vertical and horizontal laminar flow hoods, fume hoods, refrigerators, freezers, and central equipment rooms with filtration units for culture media, glassware maintenance, and a sterilization facility.

The principal investigator's laboratory has been inspected by an USDA/APHIS veterinarian and the Washington State Veterinarian and certified for work on VSV in a P-3 facility available for both cattle and small animals. Washington State is an "Agreement State" in terms of federal regulations regarding radioactive materials. WSU, under the provision of the Washington Administrative Code 402-22-090, has a Type A broad-scope radioactive materials license (#WN-C003-1).

5.2 S&T/AGR's Contribution

5.2.1 General - Cost Estimates

The total cost of this three-year vaccinia virus as a vector for vaccines project is $870,000 for S&T/AGR. As indicated above, WSU will be making a tremendous contribution to achieve the goal and purpose of the project. The $870,000 will provide the necessary funding to allow WSU to expand its work in vaccinia virus research to cover rinderpest vaccine. The participating LDC institution will provide partial support for the testing of the rinderpest vaccine.

5.2.2 Line Item Budget

The line item distribution of the funding for the three year budget is shown below:

Table 1
(in thousands)

	Year One	Year Two	Year Three	Grand Total
Training of African Scientists				
Salaries	$ 39	$ 42	$ 45	$126
Benefits (24%)	9	10	11	30
Supplies	27	24	24	75
Equipment	50	-0-	-0-	50
Publications	1	2	2	5
Travel	31	35	38	104
Total Direct Costs	$157	$113	$120	$390
Total Indirect Costs (excl. Equipment	46	47	55	148
Subcontracts	196	55	82	333
Grand Total for CA	$399	$217	$254	$870

Subcontracts

Plum Island				
Direct Costs	$ 21	$ 50	$ -0-	$ 71
Indirect Cost (USDA/ARS 10%)	2	5		7
Indirect Costs (26% of $25000)	6			6
Total Plum Island	29	55	-0-	84
California Biotechnology				
Direct costs	$160	-0-	-0-	$160
Indirect Costs (26% of $25,000)	7			7
Total	167	-0-	-0-	$167
African Institution				
Direct Costs	-0-	-0-	$ 75	$ 75
Indirect Costs (26% of $25,000)	-0-	-0-	6	6
Total	-0-	-0-	$ 81	$ 81

5.3 Collaborating Institutions

5.3.1 Plum Island

The Plum Island Animal Disease Center is the special laboratory of the Federal Government established in 1952 for testing and experimenting with foreign contagious livestock diseases. It has extensive P4 facilities to accommodate large and small experimental animals in isolation and for handling and restraining of cattle. All challenge experiments in cattle will be conducted in a facility for work with Class 4 infectious agents, including rinderpest.

5.3.2 National Institutes of Health

Dr. Moss has seven major molecular laboratories under his direction at NIH. These are fully-equipped for the study of the molecular biology of vaccinia virus. Drs. Cordell, White, and Dale have extensive modern laboratories at California Biotechnology; their equipment and facilities are state-of-the-art.

5.3.3 Collaborating LDC Institution

Ministry of Agricultural and Livestock Development, Department of Veterinary Sciences; Veterinary Research Laboratory.

5.3.4 California Biotechnology, Inc.

This is a major biotechnology research and development company with a scientific staff of approximately eighty. The company has current research programs on subjects including, growth factors, drug delivery systems, neurobiology and vaccines. It has substantial experience in working with vaccinia virus. It has joint development projects with Eli Lilly and Company and Wyeth. In 1985 California Biotechology, Inc.'s revenues increased by 16 percent.

6.0 Economic Considerations

The following types of economic considerations are relevant to this project:

1) Are investments in biotechnology research cost-effective for developing improved technologies for animal tolerance to stress for increasing agricultural productivity; and

2) What are the cost-benefit ratios of comparative techniques.

The usual cost to benefit or cost effectiveness analysis cannot be done ex ante for research program because the products of the program can not be assigned a realistic monetary value. Two factors related to the costs and potential benefits of this research program have been considered. First, the costs of the various research components seem reasonable in that they were developed and cross checked by research administrators who routinely develop similar research budgets and they are similar to costs per scientist year for such research performed in other organizations. Second, returns on investments in agricultural research have been very high in comparison to alternative public sector investments. Several post facto analyses have shown rates of return on public agricultural research investments (since 1910) in the U.S. between 40 and 50 percent. These figures apply to broad based investments in both basic and applied research.

Livestock losses to diseases in Africa is estimated at 30 to 50 percent of production. With these high losses, a very high benefit/cost ratio will be realized for most animal health investments. Present health programs disclose a benefit/cost ratio ranging from 6/1 to 140/1 which indicated that for each dollar spent on disease control and eradication, $6.00 to $140.00 are saved by reduction in animal losses and increased production. A major disease outbreak not only results in financial losses, but has long lasting effects; e.g., it took 20 years for Sudan to recover from an outbreak of rinderpest in 1931. Animal disease outbreaks also have a secondary economical effect since affected meat-producting countries are usually barred from exporting; e.g., Somalis livestock producers lost Saudi Arabia, a major market, after a rinderpest outbreak in their country.

7.0 Future Considerations

7.1 Utilization of Improved Rinderpest Vaccine

At the end of this project an improved rinderpest vaccine will be development. The vaccine will be a recombinant live vaccine constructed using vaccinia virus. Small scale contained field testing will have been successfully conducted.

The next step following the end of this project, is large scale field testing. As discussed above, environmental considerations and the regulations which are in existance at the time will play a major role in the methods of this large scale testing. The issues and funding of large scale field testing will most likely be addressed in a follow-on project.

Additionally, there are AID projects which will contribute to the field testing. Africa Bureau, Niger Mission, currently supports a project with Tufts University and Plum Island to develop a non-thermostable rinderpest vaccine. This project has been developing a field testing strategy which can be used with the vaccinia rinderpest vaccine.

Following large scale field testing the actual utilization of the vaccine begins. Currently the European Economic Council (EEC) is developing a large project which will focus on vaccine delivery. The improved rinderpest vaccine is a likely candidate for this program. EEC will be kept informed of this project's progress and discussions of the delivery aspects of the utilization will be active.

7.2 Future Vaccine Development

Programs to develop vaccines for other important animal diseases will build on this initial effort in rinderpest. The rinderpest work will demonstrate the usefulness of a live vaccinia virus vectored disease. The vaccinia virus is capable of carrying genes from a number of diseases. This would allow a single vaccination to protect animals from multiple diseases. The target diseases for such future vaccine work, as identified by NSF, include anaplasmosis, babesiasis and heartwater.

Vaccine 2
5/14/96:Revised 5/27/86

8.0 Environmental Considerations

In the area of biotechnology regulation is one of the critical issues. It can be divided into two categories:

1) Questions which arise concerning containment

2) Questions which arise concerning release of genetically engineered organisms into the environment

The proposed project deals only with the first of these categories. The rinderpest vaccine will be developed and tested under contained conditions. However the second grouping of questions is important in the development of a useful vaccine, as eventually the vaccine must move out into the field. For this reason the discussions which follow below outline all of the steps required in bringing the vaccine to field. As this project develops all of the necessary agencies will be kept informed of its progress.

The project is designed to develop an improved vaccine for rinderpest, an acute highly contagious viral disease of ruminants. This vaccine will be developed using recombinant DNA technology, specifically using vaccinia virus as a vector. The product that emerges from this project will be an effective live recombinant vaccinia virus for rinderpest which is suitable for use in the developing countries. This vaccine will be tested under laboratory conditions in both the U.S. and Kenya. At the end of this project the rinderpest vaccine will be ready for large scale field testing.

8.1 Areas of Safety

There are 4 major areas of safety concerning this project. These are:

1) Working with rinderpest, Class 4 infectious agent

2) Recombinant DNA laboratory work

3) Testing of a live genetically engineered vaccine

4) Deliberate release of a genetically engineered vaccine in large scale field testing.

8.1.1 Rinderpest - Rinderpest is a class 4 infectious agent requiring defined levels of containment and restrictions or handling. All work with rinderpest virus will be done at Plum Island Animal Disease center where extensive P4 facilities are available to accommodate large and small experimental animals. All challenge experiments will be conducted in a facility for work with class 4 infectious agents.

The regulations for working with an infectious agent such as rinderpest have been established by USDA and Animal and Plant Health Inspection Service. Dr. Yilma, the PI on this project has worked extensively at Plum Island with infectious animal diseases. Dr. Callis, a collaborator on this project, was director of Plum Island for 30 years. It is the responsibility of the PI in conjunction with Dr. Callis to insure that all regulations are followed.

The removal of any part of the rinderpest virus from Plum Island to laboratories in the U.S. must be approved by (APHIS). This is decided on a case by case basis. Typically testing in cell culture and live animals must be performed to insure that the viral component being removed from the island is not infectious in its own right.

The proposed site for animal testing in Africa is in Muguga, Kenya. There is an isolation facility at Muguga that has been used in previous rinderpest work. Prior to conducting research at Muguga, its facility and procedures for testing genetically engineered ogranisms will be reveiwed to ensure that they are adequate based on United States and Kenyan standards. The AID Environmental Coordinator will assist S&T/AGR in developing the Kenyan containment facility certification team and participate in this review process. We will also obtain the written approval of the appropriate Ministries of the Government of Kenya before testing is commenced. If the facility in Kenya is not used, the same kind of review will be conducted regarding any other proposed facility in any other country and approval by appropriate ministeries in that country will be obtained.

8.1.2 Recombinant DNA Laboratory Work - Since the initial recombinant experiments were performed in the 1970's specific guidelines have been developed by NIH. It is the responsibility of the PI to insure that all laboratory procedures follow these regulations.

A general description of the NIH guidelines cited from GAO's report - Biotechnology - Agriculture's Regulatory System Needs Clarification, March 1986:

The 1976 "NIH Guidelines for Research Involving Recombinant DNA Molecules" were the federal government's initial response to the concern about risks associated with recombinant DNA research. The guidelines were developed in response to concern among scientists and the general public about the potential hazards of genetics engineering. They were formalized by an NIH Recombinant DNA Advisory Committee (NIH-RAC) following an international conference held in February 1975 at Asilomar California, and attended by a group of eniment scientists. The guidelines originally were binding

only on research supported by NIH; subsequently, all federal agencies that perform, fund, or regulate recombinant DNA research agreed to adopt the NIH guidelines and to require compliance by federally funded researchers doing work involving recombinant DNA. An agency can encourage the compliance of researchers who receive federal funds by threatening to cut off such funds in the event of noncompliance. The guidelines do not cover privately funded research but contain a section encouraging voluntary compliance by industry.

The guidelines provide an administrative framework that specifies the responsibilities of the scientists, their institutions, and the federal government. For each institution the key organization is the Institutional Biosafety Committee (IBC), comprising experts in relevant scientific disciplines and lay persons representing the public interest. More than 300 IBCs throughout the nation oversee recombinant DNA research to ensure compliance with the guidelines. At the federal level the key parties are the NIH Director, who is the final decision-maker under the guidelines, and the NIH-RAC, a diverse group of experts who advise the NIH Director on the major technical and policy issues involved.

The guidelines also classify experiments into three categories: (1) those requiring special reivew by the NIH-RAC, (2) those requiring containment of potentially harmful organisms and review by the IBC, and (3) those exempt from special NIH-RAC and IBC review. The NIH-RAC reviews experiments in the first category to assess risk and assign an appropriate level of containment. Those in the second category must be conducted in accordance with previously established containment standards that become more rigorous as the level of perceived hazard increases. These standards specify physical types of containment relating to special laboratory features and biological types of containment relating to the deliberate weakening of experimental organisms to reduce their chance of survival outside the laboratory. According to USDA, compliance with these standards is ensured by the IBCs at the local level.

The third category of experiments-those that are exempt from NIH-RAC and IBC review-has grown to the point where it now represents about 80 to 90 percent of all recombinant DNA experiments. Research since 1976 has led to less concern among many scientists about the risks of recombinant DNA research and, as a result, there has been a gradual but substantial relaxation of original containment requirements and oversight mechanisms through a series of formal revisions in the NIH guidelines.

Three limitations of the NIH guidelines, however, have caused more people to be concerned about their adequacy in terms of regulating the biotechnology industry: they (1) apply to nonfederally funded research on a purely voluntary basis, (2) do not apply to organisms created by genetic engineering techniques other than recombinant DNA, and (3) do not adequately address the issue of deliberate release of genetically engineered organisms into the environment. (Orginally, they simply prohibited such releases, but more recently they were changed to require special review under category 1.)

8.1.3 Small Scale Contained Animal Testing - Once the genetically engineered vaccinia virus rinderpest vaccine is developed it will be necessary to conduct tests in live animals. These animals will be inoculated with the new vaccine and then challenged with rinderpest virus. Such tests have two components which are safety issues: 1) the use of the recombinant vaccine and 2) the use of the infectious virus rinderpest. Point 2 has already been discussed in the section on rinderpest. All of these tests will be conducted in facilities which meet the specified regulations - Plum Island and the Muguga facility.

The use of the recombinant vaccine will follow the regulations set forth by APHIS which is currently the agency with jurisdiction over animal diseases. At the writing of this project paper, APHIS is in the process of developing defined procedures. If the proposed procedures are accepted the Institutional Biosafety Committes (IBC)will be responsible for approving the small scale field testing as defined in this project. Additionally international regulations, as they develop, will be taken into consideration.

8.1.4 Deliberate Release of a Genetically Engineered Organism - This is the final step in the testing process of the new vaccine. It requires the administration of the vaccine in an open system-i.e., released into the environment. This is the most controversial step in the process as there is not a data base of risk assessment.

The proposed APHIS regulations indicate this step is the most controlled. A complete environmental assessment fully reviewed by APHIS is required before any testing can be initiated. Again, international regulations, as they develop, will also be considered.

8.2 Responsibility and Approval

8.2.1 Principal Investigator - It is the responsibility of the PI to insure that appropriate regulations regarding facilities and procedures be followed throughout this project.

8.2.2 A.I.D. - It is the responsibility of the project manager in collaboration with environmental officers to monitor the project in regards to all regulations.

8.2.3 Kenyan Government - In all of the above regulatory issues the approval of the Government of Kenya ministries of Health and Agriculture and other appropriate African institutions (e.g., OAS-Organization of African States) will be obtained.

W3407e:Vaccine4:5/28/86

IMPROVED ANIMAL VACCINES

THRU BIOTECHNOLOGY

PROJECT NUMBER 936-4178

APPENDIX I - LOGFRAME

5/29/86

AID 1020-20 (3-79)

PROJECT DESIGN SUMMARY
LOGICAL FRAMEWORK

Project Title & Number: Improved Animal Vaccines thru Biotech - 936-4178

Life of Project:
From FY 1986 to FY 1989
Total U.S. Funding $900,000
Date Prepared: 5/15/86

NARRATIVE SUMMARY	OBJECTIVELY VERIFIABLE INDICATORS	MEANS OF VERIFICATION	IMPORTANT ASSUMPTIONS
Program or Sector Goal: The broader objective to which this project contributes: To increase the stability and efficiency of food production by reducing livestock losses due to rinderpest.	Measures of Goal Achievement: - Overall increased production of food - Reduced incidence of rinderpest.	- National Surveys - AID/W and Mission reports - FAO Production Statistics - Impact Evaluation.	Assumptions for achieving goal targets: - Food production remains a high priority in LDC . - Sufficient interest in LDCs to remove constraints which limit agricultural production systems.
Project Purpose: To develop an improved vaccine for rinderpest which will lead to increased livestock production.	Conditions that will indicate purpose has been achieved: End of project status. - An effective infectious recombinant vaccinia virus vaccine to rinderpest is developed - Completion of preliminary animal tests of the rinderpest vaccine - Rinderpest vaccine is ready for large scale field testing.	- Scientific publications and presentation at scientific meetings - Reports from preliminary animal tests indicate vaccine potency - Preparations for large scale field testing are underway.	Assumptions for achieving purpose: - Within the available funding and time constraints a recombinant vaccinia virus vaccine for rinderpest can be developed - The recombinant vaccinia virus vaccine for rinderpest will be effective and suitable for use in LDCs - LDCs are willing to participate in the field tests of experimental vaccine.

Appendix 1
Page 1 of 2

AID 1020-20 (1-79)

PROJECT DESIGN SUMMARY
LOGICAL FRAMEWORK

Project Title & Number: Improved Animal Vaccines thru Biotech - 936-4178

Life of Project:
From FY 1986 to FY 1989
Total U.S. Funding $900,000
Date Prepared: 5/15/86

NARRATIVE SUMMARY	OBJECTIVELY VERIFIABLE INDICATORS	MEANS OF VERIFICATION	IMPORTANT ASSUMPTIONS
Outputs: An effective vaccine for rinderpest is developed which does not require sophisticated facilities, equipment, and management. An increase in the scientific data available on rinderpest and the use of vaccinia virus as a tool in developing vaccine. Improved ability of LDC scientists and institutions to use molecular biology and apply specifically vaccinia technology t- the study of other important diseases.	**Magnitude of Outputs:** - One recombinant vaccinia virus vaccine is developed containing two of the immunogenic genes of rinderpest - Test data from vaccine trials on cattle in two laboratory settings (Plum Island and Kenya) - Three publications per year - for years 2 and 3 of the project and presentation of the project progress at one scientific conference per year for years 2 and 3 - Development of improved vaccinia virus vectors capable of enhancing the level of gene expression. - Two postdoctoral doctors of veterinary medicine from Africa trained in the use of vaccinia virus as a vector.	- Scientific publications and project reports - On site reviews and evaluations - Development of vaccine data from field trials - Expanded LDC scientists' knowledge and skills in development and use of vaccines - Data from laboratories in LDCs and LDC scientists applying vaccinia technology to develop vaccines for other important diseases - Use of vaccinia virus vectors with improved promoters.	**Assumptions for achieving outputs:** - Research directions pursued by WSU to date will lead to the development of a successful vaccine - Continued cooperation and support by LDC institutions - Laboratory and field trials will be permitted in the United States, LDCs, and IARCs. - Technologies developed are appropriate for use in LDCs - Principal researchers will continue their institutional affiliation with WSU - Vaccinia virus as a tool for vaccine development can still be improved.
Inputs: Scientists working on project: WSU draws together a team which consists of experts in vaccinia virus technology and vaccine program development in Africa. (See Appendix 4)	**Implementation Target (Type and Quantity)** Budget (000) FY 1986 - FY 1990 Salaries $126 Benefits 30 Supplies 67 Equipment 50 Publications 5 Travel 105 Indirect Costs 144 Subcontract 373 TOTAL $900	- Annual workplans in place - Subcontracts are signed on schedule - Equipment is available - Staff of scientists are working - Workplan is implemented on schedule	**Assumptions for providing inputs:** - Funds for the Cooperative Agreement will be obligated in FY 86 - Continuing cooperation and collaboration among the scientists - The various facilities will keep their commitments with regard to this project.

Appendix 1
Page 2 of 2

IMPROVED ANIMAL VACCINES

THRU BIOTECHNOLOGY

PROJECT NUMBER 936-4178

APPENDIX 2 - GLOSSARY

5/29/86

Glossary

erythrocytes - red blood cells

hemagluttinate - the ability to cause red blood cells agglutinate; (i.e. clump togerther due to interactions with the cell surface proteins).

humoral response - the immune response involving the production of specific antibody.

lapinsinized-

lymphocytes - the cells of the immune system which are responsible for such responses as the production of antibodies and the rejection of tissue grafts.

natural killer cells - small lymphocytelike cells that spontaneously and relatively non-specifically, kill a variety of tumor and virus cells in culture. They do not require the target cells to be covered with antibodies in order to kill it. How they distinguish abnormal cells from normal ones is unknown.

nucleocapsid - the protein coat of a virus.

suppressor T cells - a class of T cells (lymphocytes) that are able to suppress the immune response to an antigen.

teratogenic effects - the ability of an agent to cause a cell to lose the

control of differentiation as it divides; this results in the production of a mass of cells which contains all types of cells (a teratoma).

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IMPROVED ANIMAL VACCINES

THRU BIOTECHNOLOGY

PROJECT NUMBER 936-4178

APPENDIX 3 - COMMUNICATIONS WITH COLLABORATORS

5/29/86

California Biotechnology Inc.

April 10, 1986

Dr. Tilahun Yilma
Department of Veterinary Pathology
and Microbiology
Washington State University
Pullman, WA 99164-7040

Dear Dr. Yilma:

This letter will confirm that California Biotechnology Inc. wishes to collaborate with you and your colleagues on experiments designed to generate a recombinant vaccinia vaccine against rinderpest, a devestating viral disease of livestock in Africa and Asia. For our contribution, we will generate full-length cDNA clones of the two rinderpest virus surface genes encoding the hemagglutinin protein and the fusion protein.

We understand that this research will be fully funded by your laboratory. We further understand that California Biotechnology will have no proprietary rights to these cloned genes when expressed in vaccinia.

We feel that this is a very exciting scientific and humanitarian effort and are pleased to be included as a project member. We look forward to beginning the cloning as soon as possible.

Yours sincerely,

John Shine, Ph.D.
Senior Vice President,
Research and Development

JS:ebp

P.O. Box 51650 • Palo Alto, California 94303 • (415) 966-1550 • 2450 Bayshore Frontage Road • Mountain View, California 94043
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Agricultural Research Foundation

P.O. Box 39189
Tel 592071
Nairobi

Ref No. AGREF/CORR/1/VOL.I/109

Date 8th April 1986

Dr. Tilahun Yilma
Associate Professor
Washington State University
Department of Veterinary
Microbiology and Pathology
Pullman
Washington 99164-7040
U. S. A.

Dear Dr. Yilma,

Thank you very much for your phone call of April 5th 1986. After the recent sweep by Rinderpest through Africa, I am extremely excited at the prospect of working with you and Roger on vaccinia vectored Rinderpest vaccine.

As I told you in my earlier letter, we are working on a project draft aimed at developing bio-technology at the University of Nairobi. I have already been in contact with Dr. A. App at Rockefeller on this and he has been encouraging. The Rinderpest work would therefore be right along the lines we have been thinking. African friends at ILRAD and elsewhere have been urging this kind of initiative. We shall all be quite thrilled.

You will find enclosed a budget and my CV's. Please keep me informed of developments.

Best regards to John and Roger.

Yours sincerely,

S. Chema

S. Chema DVM, Ph.D
Director
Agricultural Research Foundation

D.E. DE TRAY, D.V.M.
2441 Capital Ave.
Medford, Oregon 97504

April 9, 1986

Dr. Tilahun Yilma
Dept. of Veterinary Microbiology & Pathology
Washington State University
Pullman, Washington 99164

Dear Dr. Yilma:

I am delighted to learn that you will be developing a new vaccine against rinderpest using a genetic engineering concept. As we both know from our past experiences, the main problems of mass rinderpest vaccination are logistical. Refrigeration is required for the vaccine from the time it leaves the laboratory until it is inoculated. The maintenance of this "chain of cold" is almost impossible in tropical countries. Your new vaccine will eliminate the necessity for refrigeration and will make it possible to eradicate rinderpest.

I would be honored to serve as a consultant during the development and field testing of your new vaccine. I began my career with U.S.D.A. in Mexico working on vaccine production for foot-and-mouth disease and my early work in Africa was with rinderpest vaccine. Thus I am deeply interested in your plan to develop a new rinderpest vaccine.

A copy of my biographical data is enclosed.

Sincerely,

D. E. DeTray

D. E. DeTray, D.V.M.

Encl.

COPY

Washington
State University

Department of Veterinary Microbiology and Pathology, Pullman, Washington 99164-7040
509-335-6030 / FAX 509-335-0997

25 April 1986

Dr. W.J. Bellini
Rabies Unit
Center for Disease Control
Department of Health and Human Services
Lawrenceville Facility
Lawrenceville, GA 30246

Dear Dr. Bellini,

I am writing this letter as we agreed in our telephone conversation of April 25. Thank you for your willingness to assist us in our project of cloning the HA and F genes of rinderpest virus.

We would be grateful if you would make available to us the cDNAs of the HA and F genes of measles virus, as well as monoclonal antibodies directed to the HA and F glycoprotein of measles virus. We will use these in cloning the equivalent genes of rinderpest virus and acknowledge your contribution in any publications that result from this project.

I also would appreciate it if you would send me copies of your publications including those on the cloning of the HA and F genes of measles virus.

If you have any further questions, please call me at (509) 335-6042.

Sincerely,

Tilahun Yilma, D.V.M., Ph.D.
Associate Professor

cc: Dr. Makonnen Fekadu
Dr. Beverly Dale
[redacted]

/kng/0860Y/

JAN 2 8 RECD

United States Department of Agriculture
Agricultural Research Service
North Atlantic Area
Plum Island Animal Disease Center
P.O. Box 848
Greenport, New York 11944

January 17, 1986

Dr. Bernard Moss
Laboratory of Viral D iseases
Building 5, Room 432
NIAID
National Institutes of Health
Bethesda, MD 20205

Dr. T. Yilma
Department of Microbiology
College of Veterinary Medicine
Washington State University
Pullman, Washington 99163

Dear Drs. Yilma and Moss,

This refers to our meeting in Washington on vaccinia as a vector and. in particular to its use for vectoring New Jersey vesicular stomatitis virus (VSV). As stated at the meeting, I have few, if any, concerns relative to safety. It may be that some studies will have to be conducted to develop data on safety where such specific information is not now available. On the other hand, the proposed study in Mexico is of concern to me. We would like to work with you to try to minimize problems.

The vectoring of virus with vaccinia is an intriguing subject. I think it has great possibilities, especially for animal vaccines in the third world. These reasons are well known to all of us. For these reasons, we would like to help avoid any failures which would possibly set the technology back. Therefore, the following is proposed:

1) A batch of VSV, NJ vaccine should be prepared, at Washington State University or N.I.H. Preliminary tests should be conducted on it at WSU in cattle.

2) Following the above, it is proposed that samples of the vaccine be tested at PIADC in a larger group of cattle. They would be held in biocontainment. The method of challenge can be reviewed and agreed upon in advance.

3) We consider it in our joint national interests to assist in further development and evaluation of innovative and important technology. USDA makes this offer to you, as has been done to another group interested in evaluating organically synthesized foot-and-mouth disease polypeptides as FMD vaccines. This suggestion has the approval of APHIS as well as ARS staff.

If you agree with the above, we suggest that we proceed on it at an early date.

Sincerely yours,

J. J. Callis
Director

cc:
J. W. McVicar
C. H. Campbell
C. A. Mebus
R. Breese - Washington State University

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service

National Institutes of Health
Bethesda, Maryland 20205
Building : 5
Room : 436
(301) 496- 9869

COPY

February 3, 1986

Dr. J. J. Callis, Director
North Atlantic Area
Plum Island Animal Disease Center
Agricultural Research Service
United States Department of Agriculture
P. O. Box 848
Greenport, New York 11944

Dear Jerry:

I enjoyed the opportunity to speak with you in Washington and appreciate your continued interest in the use of vaccinia virus vectors for veterinary vaccines. In particular, your offer of providing the facilities of Plum Island for cattle testing is very generous. First, however, our plan is to reconstruct the recombinant in order to put it into the Wyeth NYCBH strain and also increase the level of expression of VSV G. When these modifications have been made and preliminary data obtained, we can discuss your offer in more detail. Yilma and I greatly value your experience and advice on these matters.

Sincerely yours,

Bernard Moss

cc: T. Yilma

IMPROVED ANIMAL VACCINES

THRU BIOTECHNOLOGY

PROJECT NUMBER 936-4178

APPENDIX 4 - PROJECT PERSONNEL

[illegible]/29/86

PERSONNEL

Key Personnel

A. Washington State University

The Washington State University Vaccinia Research Group is headed by Dr. Tilahun Yilma, whose area of expertise is vaccinia vector construction and molecular biology. The group includes Dr. Sandra Ristow, who is in charge of protein biochemistry. Kjerstie Guenther is the group's administrative assistant.

The principal investigator, Dr. Tilahun Yilma was a USAID-sponsored scholar to the University of California to study veterinary medicine between 1965-1970 in preparation for his participation in the JP 15 rinderpest eradication program in East Africa. After earning his DVM degree, Dr. Yilma was the Head of the Veterinary Services and of the JP 15 program for eastern Ethiopia. In the pre-JP 15 campaign, Dr. Yilma participated in the vaccination of over 40 million cattle in East Africa. In 1972, Dr. Yilma was again sponsored by the USAID for graduate training in microbiology at the University of California at Davis in preparation for joining the faculty of medicine and veterinary medicine in Ethiopia. Unfortunately, with the change of government in Ethiopia, Dr. Yilma was unable to return and instead joined the scientific staff at the Plum Island Animal Disease Center of the USDA in 1977. Dr. Yilma worked for over 2 years on the pathogenesis and molecular biology of exotic vesicular disease viruses and also received a "Certificate of Training" for both courses on exotic animal diseases offered at the center.

At present, Dr. Yilma is a virologist and an Associate Professor at WSU. He has been using VSV for several years in the biological assay of IFNs and has developed an effective G subunit vaccine and a rapid diagnostic kit for VS. Furthermore, in collaboration with Drs. Bernard Moss of NIH and John Rose of the Salk Institute, he has developed and tested a vaccinia vector vaccine for VSV. Dr. Yilma constructed the vaccinia vectors expressing the glycoproteins of the two serotypes of VSV and the vector expressing the BoIFN-γ and BoIL-2 genes. In collaboration with Dr. Alice Huang (Director of the Laboratories of Infectious Diseases at the Children's Hospital, Harvard Medical School), Dr. Yilma is also working on synthetic peptide vaccines for VS. Recently, Drs. Huang and Yilma have demonstrated the role of defective interfering particles in the epizootiology of the recent outbreak (1982-83) of VS in the western U.S. [Huang et al in press]. Further, in collaboration with Dr. Dennis Kleid of Genentech, Dr. Yilma has developed a rapid diagnostic kit for foot-and-mouth disease using cloned products of VP1.

During July, 1985, Dr. Yilma was a visiting scientific specialist at Genentech, the world's leading genetic engineering firm, in San Francisco. He also was a Guest Researcher in Dr. Moss's laboratory at NIH from August through October, 1985.

Citizenship welcome reward for WSU professor

By GALE METCALF
Herald staff writer

Today, Tilahun Yilma is in a very real sense a man without a country, yet he is credited with doing very much for America.

"Every time I travel, I really don't have any citizenship, it's been very hard," said the Washington State University professor of veterinary medicine who is credited with discovering a vaccine to cure a deadly cattle disease.

"Now I don't have any citizenship," he added.

But on Monday, Yilma, 42, will become an American.

It will happen in the Tri-Cities at Richland's Federal Building, when the native of Ethiopia and 71 other residents of 27 Eastern Washington communities become naturalized citizens in ceremonies presided over by U.S. Magistrate Lenny Suko of Yakima. Benton-Franklin Superior Court Judge Al Yencopal is to give a keynote address.

"I'm delighted," Yilma said during a telephone interview from his WSU office. "I've never voted in my life and for the first time I will be able to do that."

It culminates a long journey for the award-winning doctor of veterinary medicine who is unable to return to his native country because of political conditions there. Emperor Haile Selassie was deposed in 1974 and replaced by a Socialist state. Yilma's cousin, a general, headed security for Selassie and was killed in the revolution.

"I couldn't return because of the changa in government," said Yilma whose mother, two sisters, and a brother still live in Ethiopia. He maintains contact with them, he said.

"I've lived totally independent after the eighth grade," Yilma said.

His eighth grade science and math test scores were so high they placed him in the top 50 science students in Ethiopia, enabling him to enter a high school there run by Oklahoma State University. He stayed four years, and then entered OSU in his country.

Yilma started out to be a physician.

"When I was five years old there was a hospital close to my house," he said. "This hospital was run by Mennonites from Lancaster, Pennsylvania and Maryland."

There he attended Sunday school and came under the influence of a physician named Robert Eshelman, Yilma said.

"Every morning at 7:30 I walked with him on clinical rounds," Yilma said.

Yilma may have wanted to be a doctor, but the U.S. State Department, which agreed to sponsor him at the University of California, noted there was a critical need for veterinarians in Africa.

He attended the Berkeley campus, and then was accepted for veterinary school at the University of California at Davis where he received his veterinary degree.

"I was sent back (to Ethiopia) and I participated in a cattle disease eradication program in East Africa," Yilma said.

Called JP-15 (for joint program), it was the largest program of its kind in history, he said, and was under international sponsorship through U.S. Aid to International Development.

In the three years Yilma participated in the program, 40 million cattle were vaccinated throughout East Africa.

Yilma returned to Cal-Davis to acquire a doctorate degree in microbiology, to be used at a veterinary school being planned for Ethiopia. Then Selassie was dethroned.

He completed his degree at Davis and because of his experience the U.S. Agriculture Department asked him to come to Plum Island (at the tip of the north fork of Long Island) where exotic diseases are studied.

Yilma still desired to be a physician, and a chance meeting with a cardiologist who was a professor at New York's Cornell Medical School led Yilma to try for a third doctorate degree — a doctor of medicine. Cornell immediately accepted him.

But earning three doctorates means a lot of years of hard work.

"I sort of got tired of school in the middle of it," Yilma said.

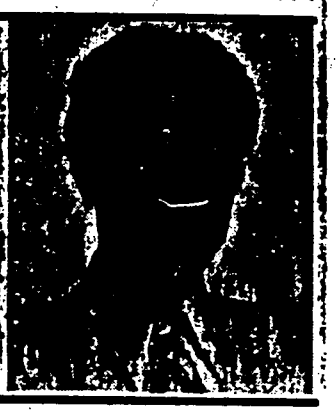
Meanwhile, Leo Bustad, dean of WSU's veterinary school and former professor at Davis, was very much interested in Yilma. In February 1980 Yilma came to WSU.

An outbreak of vescular stomatitis, similar to hoof-and-mouth disease, among cattle in 13 western states gave Yilma a chance to use his talents.

He developed a genetically engineered vaccination that successfully prevented the disease.

At the ceremony today, staff sergeants David W. Paine and Robert Tauff of the U.S. Army Recruiting Station are to serve as color guard.

Some 250 women of the Columbia River Chapter of the National Society of the Daughters of the American Revolution, the Kennewick Chapter of the National Society of the Daughters of the American Revolution, and the Providence Chapter of the National Society of Colonial Dames XVII Century are assisting in the reception following the ceremony that runs 1:30 p.m. to 3:30 p.m.



"I'm delighted. I've never voted in my life and for the first time I will be able to do that."

Tilahun Yilma
WSU professor

Tri-City Herald 4/13/83

Dr. Yilma's participation in the rinderpest eradication program in Africa, his research experience in exotic animal diseases at the Plum Island Disease Center, his experience in the vaccinia expression vector system, and his relationship with outstanding collaborators among federal, university, and industry scientists, make him uniquely qualified to conduct and lead this project to a successful completion.

Dr. Sandra Ristow, Assistant Scientist in the Department of Veterinary Microbiology and Pathology, WSU, is a biochemist with expertise in protein chemistry and considerable experience in monoclonal antibody production and assessment of immune function. She will immunobiologically characterize proteins expressed by the vaccinia virus recombinant expressing the two rinderpest genes.

We also plan to engage one postdoctoral and one post-DVM student to assist in the construction of vaccinia vectors expressing the the rinderpest gene and in the promoter studies.

B. National Institutes of Health

Dr. Bernard Moss, a collaborative investigator on this project, is the Chief of the Laboratory of Viral Diseases at the National Institutes of Health and the world's leading expert in the molecular biology of vaccinia. The technology for the use of vaccinia viruses as expression vectors for foreign genes was developed in his laboratory. His laboratory will be in charge of the development of better expression vectors that will be used for the expression of immunogenic genes of rinderpest.

C. USDA-ARS Plum Island Animal Disease Center

Dr. Jerry Callis is the Director of the USDA-ARS Plum Island Animal Disease Center and has extensive experience in exotic animal viral diseases. His scientific staff will perform parts of the project that involve virus work and the evaluation of immunity to the vaccinia virus recombinant in cattle under the overall direction of the principal investigator.

Dr. Charles A. Mebus is the Supervisory Veterinary Medical Officer at the Plum Island Animal Disease Center. He will supervise the clinical pilot study and and perform the pathological evaluation of cattle at Plum Island.

D. California Biotechnology, Inc.

At California Biotechnology, Inc., Drs. Beverly Dale, R. Tyler White, and Barbara Cordell will clone the HA and F rinderpest genes.

Dr. Beverly Dale is a staff scientist and project leader of animal vaccines at California Biotechnology, Inc. She has extensive experience in cloning retroviral genes from genomic DNA. More recently, she was involved in generating cDNA clones and in the construction of vaccinia virus recombinants. Recently Dr. Dale and her colleagues cloned the hemagglutinin

and the neuraminidase genes of both serotypes of equine influenza viruses and the gene for the surface glycoprotein of bovine virus diarrhea and then expressed these genes in infectious vaccinia virus recombinants. Dr. Dale will lead the group at CBI in cloning the complete coding sequences of the F and HA genes of rinderpest virus.

Dr. R. Tyler White is a staff scientist at California Biotechnology, Inc., with extensive experience in the organization and regulation of eukaryotic gene families, expression of cloned genes and cDNA sequences in heterologous systems, and molecular basis of organismal evolution. As a member of the pulmonary group, he was the molecular biologist responsible for cloning the human pulmonary surfactant apoprotein (PSAP) gene and also directed the introduction of the human PSAP gene and cDNA into mammalian tissue culture cells for the production of high levels of PSAP. As a member of the veterinary vaccine group, he cloned a portion of the bovine viral diarrhea (BVD) virus genome corresponding to one of the major antigenic envelope glycoproteins and expressed this cDNA in several bacterial expression systems.

As part of the study of the evolution of ruminant physiology, Dr. White cloned the cDNA and gene for the lysozyme expressed as a digestive enzyme in cow stomach. As a graduate student with Dr. Paul Berg in the Department of Biochemistry at Stanford University, he constructed mutants of Simian Virus 40 to probe the transcription and processing of late lytic viral RNAs, and characterized the aberrant RNA species expressed by these mutants. Dr. White will be involved in the cloning of the complete coding sequences of the F and HA genes of rinderpest virus.

Dr. Barbara Cordell is a Senior Scientist and Assistant Director of Research at California Biotechnology, Inc. Dr. Cordell earned her Ph.D. degree at Indiana University in 1972; from 1973-1977, she was a Postdoctoral Research Fellow in the laboratory of Dr. J. Michael Bishop in the Department of Microbiology at the University of California at San Francisco. She was Assistant Research Biochemist with Howard M. Goodman at the Department of Biochemistry and Biophysics at U.C. San Francisco for 2 years, and then worked as an Associate Investigator at the Howard Hughes Medical Institute in the Department of Biochemistry and Biophysics at U.C. San Francisco from 1980-1981. From 1981-1983, Dr. Cordell was an Assistant Molecular Biologist in the Department of Molecular Biology at Massachusetts General Hospital, as well as being an Assistant Professor in the Department of Genetics at Harvard Medical School. For the 2 years prior to assuming her present position, she was program leader of animal vaccines, and as such, was involved in generating cDNA libraries from total influenza.

The percentage of time that key personnel will devote to the proposed project is as follows:

%	Institution	Name
20	WSU	T. Yilma, Principal Investigator
5	WSU	S. Ristow, Collaborative Investigator
5	NIH	B. Moss, Collaborative Investigator
5	USDA-ARS	J. Callis, Collaborative Investigator
5	USDA-ARS	C. Mebus, Collaborative Investigator
5	CBI	B. Dale, Collaborative Investigator
5	CBI	R.T. White, Collaborative Investigator
5	CBI	B. Cordell, Collaborative Investigator

We have attached the curricula vitae for all key personnel.

Consultants

An experienced group of consultants has been assembled to assist with the international work.

Dr. Donald E. DeTray, now semiretired, spent 17 years working in a USDA program to control and eradicate animal diseases exotic to the U.S. and to prevent the entrance of foreign animal diseases into the U.S. In 1947, Dr. DeTray worked in Mexico on food-and-mouth disease as a member of a team that developed the vaccine used in the Mexican campaign, returning to Mexico in 1953 to help produce vaccine when foot-and-mouth disease reappeared. From 1954 to 1961, Dr. DeTray led a team of researchers working on African swine fever in Kenya. From 1966 until 1973 Dr. DeTray was the USAID representative for the African-wide campaign against rinderpest, the most extensive animal disease program ever undertaken. Since retiring in 1973, Dr. DeTray has returned to Africa three times as a consultant. He also has served as a consultant to USDA, APHIS, Veterinary Services, Emergency Programs and in this capacity has lectured on rinderpest at various regional meetings and at a number of veterinary colleges. Dr. DeTray's curriculum vitae is attached.

Dr. J. Henson is Director of International Programs at WSU. He has experience in field work in animal diseases in Gambia, Sudan, and Kenya, where he was formerly head of the International Laboratory for Research on Animal Diseases. He is currently involved in the WSU portion of the USAID Small Ruminant CRSP in Kenya. Dr. Henson's expertise will be used to set up and coordinate field studies and experimental infections in Africa.

Dr. Samson Chema is the director of the veterinary services of the Ministry of Agriculture and Livestock Development of the Kenyan government. Dr. Chema is an expert in a number of animal diseases of major economic importance exotic to the United States, including rinderpest. His office and the Department of Veterinary Services and the Veterinary Research Laboratory in Kabete, Kenya, will conduct the field evaluation of the recombinant vaccine for rinderpest once the efficacy of the vaccine is established at the Plum Island Animal Disease Center. Dr. Chema's curriculum vitae is attached.

Dr. Roger G. Breeze is Professor and Chairman of the Department of Veterinary Microbiology and Pathology at WSU. He will be involved in the pathology and experimental and field animal challenge studies. Dr. Breeze's curriculum vitae is attached.

Dr. Jack E. Moulton, a Professor of Pathology at the University of California, Davis, School of Veterinary Medicine, also has agreed to serve as a consultant. Dr. Moulton has experience in working with exotic diseases in tropical countries, including African swine fever, theileriosis, and trypanosomiasis. His curriculum vitae is attached.

We have identified other potential collaborators in Africa including Dr. Farouk Hamdi (USDA-APHIS) in Mali, formerly of Plum Island, and Dr. K. Adesegun Majiyagbe, who did his Ph.D. work on African swine fever at Plum Island and is now working on rinderpest in Vom, Nigeria.

Curriculum Vitae
Tilahun Yilma

Born: [redacted]

Nationality: U.S. Citizen

Business Address: Department of Veterinary Microbiology and Pathology
College of Veterinary Medicine
Washington State University
Pullman, WA 99164

Telephone Number: Office (509) 335-6042
Laboratory (509) 335-6063 or 335-6028

Personal Address: [redacted]

Education:

Institution	Degree	Year	Field of Study
University of California, Davis	B.S.	1968	Vet. Science
University of California, Davis	D.V.M.	1970	Vet. Medicine
University of California, Davis	Ph.D.	1977	Microbiology
Cornell Univ. Medical College New York, NY	--	1979-1980	Medicine

Selected Additional Technical Training

July 1970 Michigan State University, East Lansing, Michigan. Attended a one-week training course in "Methods of Communication" sponsored by the State Department and received a "Certificate of Training."

May 1977 United States Department of Agriculture, the Plum Island Disease Center, New York. Attended a training course on the recognition and diagnosis of foreign animal diseases and received a "Certificate of Training."

April 1979 United States Department of Agriculture, the Plum Island Disease Center, New York. Attended a training course on the recognition and diagnosis of African swine fever and received a "Certificate of Training."

Research and Professional Experience:

1970-1971 Head, Veterinary Service and Joint International Program (J.P.-15) to Eradicate Rinderpest, for Eastern Ethiopia, Ministry of Agriculture of Ethiopia, Harar, Ethiopia.

1971-1972 Lecturer, Food and Agricultural Organization of the United Nations, School for Animal Health Assistants, Debre Zeit, Ethiopia.

1973-1977	Graduate Student in Microbiology, Department of Veterinary Microbiology, University of California, Davis, California.
1977-1978	Assistant Professor, Department of Veterinary Microbiology, University of California, Davis. Duties performed at USDA, the Plum Island Animal Disease Center, Greenport, New York.
1978-1979	Research Associate, New York State College of Veterinary Medicine, Department of Microbiology, Cornell University. Duties performed at USDA, the Plum Island Animal Disease Center, Greenport, New York.
1979-1980	Medical Student, Cornell University Medical College, New York, New York.
1980-1985	Assistant Professor, Department of Veterinary Microbiology/Pathology, Washington State University, Pullman, Washington.
1985-Present	Associate Professor, Department of Veterinary Microbiology/Pathology, Washington State University, Pullman, Washington.

Scholarships Received

- Aid for International Development to Study Veterinary Medicine (1965-1970)
- George H. Hart Freshman Veterinary Student Award for 1966 to the outstanding first-year student (based on preveterinary scholastic achievement)
- Redwood Empire Association Award presented to the outstanding student in large animal medicine from the graduating class of 1970
- Educational tour of Kenya in 1972 (sponsored by F.A.O. of the U.N.)

Honorary and Professional Society Memberships

- California Veterinary Medical Association (former member)
- American Society for Microbiology
- Honor Society of Phi Kappa Phi
- Invitation to Phi Zeta
- American Association for the Advancement of Science
- Association of American Veterinary Colleges
- American Society for Virology

Special Invited Presentations and Honors (1984-86)

- Sixth International Congress of Virology, Sendai, Japan: "Infectious Vaccinia Virus Recombinants That Express Vesicular Stomatitis Virus Genes," 1984.
- Pirbright Animal Disease and Wellcome Research Laboratories, London: "Subunit, Synthetic, and Vector Vaccines for Vesicular Stomatitis," May 14, 1984.
- The Royal Society of Medicine, London: "Modern Vaccines for Vesicular Stomatitis," May 16, 1984.

- Institute of Biological Chemistry, Washington State University, Pullman: "Subunit, Synthetic, and Vector Vaccines for Vesicular Stomatitis," May 29, 1984.
- The Third International Congress on the Immunobiology of Proteins and Peptides, Tahoe, California: "Subunit and Synthetic Vaccines for Vesicular Stomatitis," June 25, 1984.
- International Conference of Vesicular Stomatitis, Mexico City, (Co-sponsored by U.S. State Department and the Mexican Government): "Subunit, Synthetic, and Vaccinia Vector Vaccines for Vesicular Stomatitis," September 26, 1984.
- Workshop on Genetically and Antigenically Modified Vaccinia Viruses for Use as Vaccines, Bethesda, Maryland (Co-sponsored by the U.S. Public Health Service, the World Health Organization, and the National Institute for Biological Standards and Control, London), November 14, 1984.
- Genentech, Inc., San Francisco (A leading genetic engineering firm): "Recombinant Bovine Interferons," three invited seminars during 1984-1985.
- University of Washington Medical School, Division of Respiratory Diseases, Seattle: Seminar, "Modern Vaccines, Including Infectious Vaccinia Virus Recombinants," May 22, 1985.
- The 1985 Maurice R. Hilleman Lecturer, Montana State University, Department of Microbiology, June 14, 1985.
- United States Department of Agriculture, Animal and Plant Inspection Services, Washington, D.C.: "The Use of Live Genetically Altered Vaccine Viruses: Their Potential and Risks," January 7, 1986.
- California Biotechnology, Mountain View, California: "Infectious Vaccinia Virus Recombinants," January 28, 1986.
- Immunomed, Tampa, Florida: "Infectious Vaccinia Virus Recombinants," February 13, 1986.
- Washington Council for Technology Advancement, Report to Washington State Government on the Washington Technology Center; Presentation to State Legislators, Olympia, Washington: Guest Speaker, "Vaccinia Vector System--The Vaccine Technology of the Future," March 3, 1986.
- United States Department of Agriculture, Animal and Plant Inspection Services, Ames, Iowa: Symposium Speaker, "Subunit, Synthetic Peptide, and Vaccinia Vector Vaccines for Vesicular Stomatitis," April 2, 1986.
- Washington Technology Center, Report to the Board of Directors, Gonzaga University, Spokane, Washington: Progress Report , April 3, 1986.
- Recipient of Beecham Award for Research Excellence, College of Veterinary Medicine, Washington State University, 1986.
- California Biotechnology, Mountain View, California: Seminar, "Infectious Vaccinia Virus Recombinants to Eradicate Rinderpest," April 8, 1986.

Invited Research Collaborations

- Visiting Consultant Scientist. Genentech, Inc., San Francisco (A leading genetic engineering firm), July 8-26, 1985.
- Guest Researcher. Laboratories of Viral Diseases, National Institute of Allergy and Infectious Diseases. I constructed several vaccinia virus recombinants expressing interferons and various types of viral immunogenic proteins in Dr. Bernard Moss's Laboratory, August 1-October 11, 1985.

Special Appointments

- Member of Technical Advisory Committee for the Science and Education Research Grants for Service on the Biotechnology-Animal Molecular Biology Review Panel, 1985
- Served as a fact finder for Albert Yates, Executive Vice President and Provost of Washington State University, 1982.
- Leader of the panel to assess the use of live genetically altered vaccines for the United States Department of Agriculture.
- Chairman, Washington State University Graduate School's Distinguished Lecture Series in Molecular Biology. A number of internationally recognized scientists from the U.S. and Europe have participated in this program since October, 1983.
- Member of the committee to recruit the Dean of the Graduate School and Vice Provost for Research, Washington State University, 1986.

Consultant to

- Genentech, Inc., San Francisco (A leading genetic engineering firm).
- AMGen, Inc., Thousand Oaks, Calif. (A genetic engineering firm).
- Bioassay Systems Corporation, Woburn, Mass.
- California Biotechnology Inc., Mountain View.

Teaching Awards

- Outstanding Instructor Award, given by the freshman class of 1971. Food and Agriculture Organization of the United Nations School for Animal Health Assistants, Debre Zeit, Ethiopia
- Super Professor, second-year veterinary students, Washington State University, 1980
- Super Professor, second-year veterinary students, Washington State University, 1984

Publications (last 5 years):

Yilma T, Breese SS Jr: Morphogenesis of the assembly and release of bovine enterovirus. J Gen Virol 49:225-230, 1980

Yilma T: Morphogenesis of vesiculation in foot-and-mouth disease. Am J Vet Res 41:1537-1542, 1980.

Blackwell HJ, Yilma T: Localization of infectious foot-and-mouth disease virus in alveolar cells of the bovine mammary gland. Am J Vet Res 42:770-773, 1980.

Yilma T: High levels of antiviral activity of equine interferons on bovine, ovine and human cells. Abstr Annu Meet Am Soc Microbiol 82:252 (T19), 1982.

Yilma T, McGuire TC, Perryman LE: Preliminary characterization of equine interferons and their antiviral activities on bovine, ovine, and human cells. J Interferon Res 2:363-370, 1982.

Yilma T, Perryman LE, McGuire TC: Deficiency of interferon-γ, but not interferon-ß in Arabian foals with severe combined immunodeficiency. J Immunol 129:931-933, 1982.

Yilma T: Ovine choroid plexus cells are uniquely sensitive to the antiviral effects of interferons alpha, beta, and gamma of humans and domestic animals. Abstr Third Annu Int Congr Interferon Res, 1982.

Yilma T: Deficiency of interferon gamma in Arabian foals with severe combined immunodeficiency. Abstr Third Annu Int Congr Interferon Res, 1982.

Yilma T, Adams SD: Endogenous interferon in sera of normal and caprine arthritis-encephalitis virus infected goats. Abstr Annu Meet Am Soc Microbiol 83:310 (R88), 1982.

Yilma T: Sensitivity of ovine choroid plexus cells to human and other animal interferons. J Gen Virol 64:2013-2016, 1983.

Yilma T, Breeze RG, Leib SR: Greater sensitivity of caprine synovial membrane cells to human interferon-alpha than human or bovine cells. Am J Vet Res 45:2094-2095, 1984.

Yilma T, Sass TM, Davis WC, Ristow SS, Gorham JR, Leib SR, Weidner J, Breeze RG: A subunit and synthetic vaccines for vesicular stomatitis. Abstr Fifth Annu Western Conf Food Anim Vet Med, 1984.

Yilma T: Immunological responses of mice and cattle to the glycoprotein and synthetic peptides of vesicular stomatitis virus. Proc Third Int Congr Immunobiol Proteins Peptides, 1984.

Yilma T, Mackett M, Breeze RG, Rose JK, Moss B: Immune response of cattle and mice vaccinated with vaccinia virus recombinant expressing the glycoprotein of vesicular stomatitis virus. Abstr Sixth Int Congr Virol, 1984.

Davis WC, Yilma T, Perryman LE, McGuire TC: Perspectives on the application of monoclonal antibody and transfection technology in veterinary microbiology. Prog Vet Microbiol Immunol 1:1-24, 1985.

Mackett M, Yilma T, Rose JK, Moss B: Vaccinia virus recombinants: Expression of VSV genes and protective immunization of mice and cattle. Science 227:433-435, 1985.

Yilma T: Use of ovine and caprine cells to measure antiviral effects of interferon. Methods Enzymol 119:551-558, 1986.

Yilma T: Induction of equine interferons. Methods Enzymol 119:130-136, 1985.

Yilma T, Breeze RG, Ristow S, Gorham J, Leib SR: Immune responses of cattle and mice to the G glycoprotein of vesicular stomatitis virus. Adv Exp Med Biol 150:101-115, 1985.

Yilma T, Breeze RG, Ristow, S, Gorham J, Leib RS: The immune response of cattle and mice to the G glycoprotein of vesicular stomatitis virus. Proc Int Conf Vesicular Stomatitis (in press).

Pollard A, Magnuson NS, Yilma T, Reeves R, and Magnuson JA: Suppression of the bovine mitogenic response by infectious bovine rhinotracheitis (IBR) virus. Fed Proc 44: 1985

Yilma T, Mackett M, Rose JK, Moss B: Vaccinia virus recombinants expressing vesicular stomatitis genes immunize mice and cattle. In: Quinnan GV ed. Vaccinia Viruses as Vectors for Vaccine Antigens. New York: Elsevier-North Holland, pp 187-200, 1985.

Yilma T, Breeze RG: New technology for prevention and control of infectious bovine respiratory diseases. Vet Clin North Am 1(2):419-439, 1985.

Yilma T, Owens S, Adams DS: Preliminary characterization of a serum viral inhibitor in goats. Am J Vet Res 46:2360-2362, 1985.

Jones L, Ristow S, Yilma T, Moss B: Accidental vaccination of a human with a vaccinia virus recombinant expressing the nucleoprotein gene of vesicular stomatitis virus. Nature 319:543, 1986.

Wu T-Y, Yilma T, Huang SA: Correlation between virulence of vesicular stomatitis virus isolates and continued high titers during undiluted passage. Proc Int Conf Vesicular Stomatitis (in press).

Huang A, Wu T-Y, Yilma T, Lanman G: Resistance to interference by defective particles exhibited by virulent isolates of vesicular stomatitis virus. Microbial Pathogenesis (in press).

Sass T, Shen D, Woodard L, Yilma T: An enzyme-linked immunoassay for the detection of bovine antibodies to vesicular stomatitis. Am J Vet Res (in press).

Yilma T, Adams SD: High level of interferon-gamma in synovial fluids of goats persistently infected with caprine arthritis-encephalitis. J Interferon Res (accepted with revision).

Yilma T, Breeze RG, Ristow, S, Leib RS: Glycoprotein of vesicular stomatitis virus protectively immunizes cattle and mice (submitted).

Englen MD, Ristow S, Yilma T: A serum-neutralizing, non-precipitating, partially protective monoclonal IgA antibody against VSV-NJ (submitted).

Yilma T, Ristow S, Moss B, Jones L: A novel approach for the production of monoclonal antibodies using infectious vaccinia virus recombinants (submitted).

CURRICULUM VITAE

Sandra S. Ristow
Assistant Scientist
Born: [redacted]

Education:

Wisconsin State University, Eau Claire, B.S. 1963, Chemistry.
University of Minnesota, Minneapolis, MN, Ph.D. 1972, Biochemistry
University of Minnesota Medical School, Minneapolis, MN, Post-doctoral, 1973, Tumor Immunology

Professional Experience:

Assistant Scientist, Washington State University, Department of Veterinary Microbiology and Pathology, December 1985 to the present.

Microbiologist, USDA-ARS, Washington State University, December 9, 1984 to December 9, 1985.

Research Associate, Washington State University, October 1, 1983 to December 1984, Department of Veterinary Microbiology and Pathology.

Adjunct Assistant Professor, University of Idaho, January 1, 1980 to October 1, 1984, Department of Bacteriology and Biochemistry.

Research Associate, Washington State University, November, 1979 to March, 1980, Department of Veterinary Microbiology and Pathology.

Instructor in Surgical Sciences and Biochemistry, University of Minnesota, July 1, 1976 to June 30, 1978.

Research Fellow, Department of Surgery, University of Minnesota, July 1, 1972 to June 30, 1976.

Graduate Student, Department of Biochemistry, University of Minnesota, 1968-1972.

Associate Scientist, Department of Neurology, University of Minnesota, 1965-1968.

Scientist I, Archer-Daniels Midland Corporation, Minneapolis, Minnesota, 1963-1965.

Honors:

Undergraduate NSF Fellowship
B.S. Summa cum laude
Elected to Iota Sigma Pi, Phi Lambda Upsilon and Sigma Xi
Received Cyrus Barnum Award for the outstanding teaching of medical students

Societies:

Iota Sigma Pi, Sigma Xi, American Association for the Advancement of Science, Tissue Culture Association, American Association of Immunologists.

Publications:

Ristow SS, Wetlaufer DB: Formation of three dimensional structure in lysozyme: Evidence for non-random route. Fed Proc 30:1287, 1971.

Ristow SS: Nucleation in lysozyme folding. Ph.D. Thesis, University of Minnesota, 1972.

Ristow SS, Wetlaufer DB: Evidence for nucleation in the folding of reduced hen egg lysozyme. Biochem Biophys Res Commun 50:544-550, 1973.

Burk MW, Ristow SS, McKhann CF: The role of antigen load in tumor specific immunity. Surgical Forum XXV:107-108, 1974.

Burk MW, Yu S, Ristow SS, McKhann CF, Holmes-Gray B: Refractoriness of lymph node cells from tumor bearing animals. Fed Proc 33:69, 1974.

McKhann CF, Ristow SS, Burk MW: Biological activities of cell surface components: Immunosuppressive effects of tumor antigen. In: The Cell Surface-Immunological and Clinical Approaches. Advances in Experimental Medicine and Biology, Vol 51 BD Kahan and RA Reisfeld, eds., Plenum Press, pp 201-210, 1974

Ristow SS, Cleveland PH, McKhann CF: Detection of tumor specific antigen in serum by means of a humoral cytotoxicity assay. XIth International Cancer Congress, Florence 1:288, 1974.

Burk MW, Yu S, Ristow SS, McKhann CF: Refractoriness of lymph node cells from tumor bearing animals. XIth International Cancer Congress, Florence 1:281, 1974.

McKhann CF, Ristow SS, Burk MW: Lymphocyte responsiveness in cancer and transplantation. Transplant Proc 7:287-290, 1975.

Burk MW, Yu S, Ristow SS, McKhann CF: Refractoriness of lymph node cells from tumor bearing animals. Int J Cancer 15:99-108, 1976.

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Ristow SS, Cleveland PH, Pier TH, McKhann CF: Purification of tumor associated antigens from a methylcholanthrene induced murine sarcoma as monitored by humoral cytotoxicity. Prep Biochem 5:479-495, 1975.

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Ristow SS, Starkey JR, Osborne C, Hass GM: Protease inhibitors inhibit the action of natural killer (NK) cells. Fed Proc 40(6):1601, 1981.

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Starkey JR, Prieur DJ, Ristow SS: Natural killer cell activity in fawn-hooded rats. Experientia 39:308, 1983.

Starkey JR, Ristow SS, Talmadge JE, McDonald TL: Immunologic enhancement of experimental metastasis in the rat. Cancer Immunol Immunother 17:42-50, 1984.

Davis WC, Seifert S, Ristow SS: Establishment of continuous porcine lymphocyte cell lines by transfection. Fed Proc 43:1605 (1984).

Yilma, T, Sass TM, Davis WC, Ristow SS, Gorham JR, Leib SR, Weidner J, Breeze RG: A subunit and synthetic vaccine for vesicular stomatitis. Abstracts of the Fifth Annual Western Conference for Food Animal Veterinary Medicine, 1984.

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Ristow SS, Starkey JR, Stanford DR, Brooks CG: Cell surface thiols, but not intracellular glutathione, are essential for cytolysis by cloned murine natural killer cell line. Immunol Invest 14:401-414, 1985.

Yilma T, Breeze RG, Ristow S, Gorham J, Leib SR: Immune responses of cattle and mice to the G glycoprotein of vesicular stomatitis virus. Adv Exp Med Biol 150:101-115, 1985.

Jones L, Ristow S, Yilma T, Moss B: Accidental vaccination of a human with a vaccinia virus recombinant expressing the nucleoprotein gene of vesicular stomatitis virus. Nature 319:543, 1986.

Review of Literature:

Wetlaufer DB, Ristow SS: Acquisition of three dimensional structure in proteins. Ann Rev Biochem 42:135-138, 1973.

Ristow SS, McKhann CF: Tumor associated antigens. In: Mechanisms of Tumor Immunity, J Wiley and Sons, Inc., Green I, Cohen S, McCluskey RT (eds.), pp 109-145, 1977.

Magee W, Ristow SS: Targeting to lymphoid cells of the immune network. Pharmac Therap 21:295-323.

Submitted for Publication:

Yilma T, Breeze RG, Ristow, S, Leib RS: Glycoprotein of vesicular stomatitis virus protectively immunizes cattle and mice (submitted).

Englen MD, Ristow S, Yilma T: A serum-neutralizing, non-precipitating, partially protective monoclonal IgA antibody against VSV-NJ (submitted).

Yilma T, Ristow S, Moss B, Jones L: A novel approach for the production of monoclonal antibodies using infectious vaccinia virus recombinants (submitted).

Curriculum Vitae
Bernard Moss

Born: [redacted]

Citizenship: United States

Marital Status: Married 1961, three children

Education:

Institution	Degree	Year	Field of Study
New York University	B.A.	1957	Biology, with honors
New York University School of Medicine	M.D.	1961	Medicine
Massachusetts Institute of Technology	Ph.D.	1966	Biochemistry

Brief Chronology of Research and Professional Experience:

1961-1962 Intern Children's Hospital Medical Center, Boston; Fellow, Harvard Medical School

1966-1970 Investigator, Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases, National Institutes of Health

1973-1974 Acting Chief, Laboratory of Biology of Viruses, NIAID, NIH

1971-1984 Head, Macromolecular Biology Section, Laboratory of Biology of Viruses, NIAID, NIH

1984-present Chief, Laboratory of Viral Diseases, NIAID, NIH

Military Service:

1966-1968 USPHS (Surgeon)

1974-1976 USPHS (Sr. Surgeon)

1976-present USPHS (Medical Director)

Professional Societies:

- American Association for the Advancement of Science
- American Society for Microbiology
- American Society of Biological Chemists
- American Society for Virology

Honors, Awards, Fellowships:

- Phi Beta Kappa, 1957
- Sigma Xi, 1957
- Alpha Omega Alpha, 1961
- National Foundation Research Fellowship
- PHS Postdoctoral Fellowship, 1963-1966
- PHS Commendation Medal, 1979

- Foundation for Microbiology Lecturer, 1980-1981
- Becton-Dickinson Traveling Lecture Award, 1984
- PHS Meritorious Service Award, 1984
- Schultz Memorial Lecturer, 1985
- Stanhope Bayne-Jones Lecturer, 1985
- One of Science Digest's 100 Most Innovative Scientists of 1985

Editorial Board Memberships:
- Journal of Virology, 1972-present
- Antimicrobial Agents and Chemotherapy, 1973-present
- Intervirology, 1974-present
- Journal of Biological Chemistry, 1982-present
- Virology, Associate Editor, 1976-present
- Advances in Virus Research, Advisory Board member, 1984

Committee Memberships:
- Virology Task Force, NIAID, 1976-1977
- Molecular Biology Study Section, special member, NIH, 1977
- NIH Working Group on Virus Recombinant DNA Guidelines, 1978
- WHO Study Group on Orthopoxviruses, 1979
- NIH Ectromelia Advisory Committee, 1980
- ACS, Nucleic Acid and Protein Synthesis Advisory Committee, 1983-present
- WHO, Temporary Advisor, 1983, 1985
- The Foundation for Advanced Education in the Sciences, Board of Directors, 1985

Conference Chairmanships:
- mRNA Structure and Synthesis Session, Gordon Conference on Animal Cells and Viruses, 1975
- Symposium on mRNA Methylation at American Society for Biological Chemistry, 1976
- Poxvirus Workshop, Fourth International Congress for Virology, 1977
- mRNA and rRNA Session of International COnference on Transmethylation, 1978
- Translation Session of Poxvirus-Iridovirus Workshop, Cold Spring Harbor, 1979
- Symposium on Transcription of DNA Virus Genomes at American Society for Microbiology, 1980
- Poxvirus Iridovirus Workshop, Cold Spring Harbor, 1980
- Poxvirus Workshop, Fifth International Congress for Virology, 1981
- Gordon Conference on Animal Cells and Viruses, 1982-1983
- Cytoplasmic DNA Virus Workshop, International Congress for Virology, 1984

Research Interests
Regulation of viral and eukaryotic gene expression; synthesis and modification of viral and cellular messenger RNA; virus replication, antiviral agents, vaccines.

Publications (last 5 years):

Cooper JA, Wittek R, Moss B: Hybridization selection and cell-free translation of mRNA's encoded within the inverted terminal repetition of the vaccinia virus genome. J Virol 37:284-294, 1981.

Belle Isle H, Venkatesan S, Moss B: Cell-free translation of early and late mRNAs selected by hybridization to cloned DNA fragments derived from the left 14 million to 72 million daltons of the vaccinia virus genome. Virology 112:306-317, 1981.

Moss B, Winters E, Cooper N: Instability and reiteration of DNA sequences within the vaccinia virus genome. Proc Natl Acad Sci USA 78:1614-1618, 1981.

Venkatesan S, Moss B: In vitro transcription of the inverted terminal repetition of the vaccinia virus genome: Correspondence of initiation and cap sites.

Wittek R, Cooper JA, Moss B: Transcriptional and translational mapping of a 6.6 kilobase-pair DNA fragment containing the junction of the terminal repetition and unique sequence at the left end of the vaccinia virus genome. J Virol 39:722-732, 1981.

Cooper JA, Wittek R, Moss B: Extension of the transcriptional and translational map of the left end of the vaccinia virus genome to 21 kilobase pairs. J Virol 39:733-745, 1981.

Moss B: 5' end labeling of RNA with capping and methylating enzymes. In: Chirikjian JG, Papas TS eds. Gene Amplification and Analysis. New York: Elsevier/North Holland, pp 254-266, 1981.

Venkatesan S, Baroudy BM, Moss B: Distinctive nucleotide sequences adjacent to multiple initiation and termination sites of an early vaccinia virus gene. Cell 25:805-813, 1981.

Moss B, Winters E, Cooper JA: Deletion of a 9,000 base pair segment of the vaccinia virus genome that encodes nonessential polypeptides. J Virol 40:387-395, 1981.

Langberg SR, Moss B: Post-transcriptional modification of mRNA. Purification and characterization of cap I and cap II RNA (nucleoside$^{2'}$-)-methyltransferase from HeLa cells. J Biol Chem 256:10054-10060, 1981.

Keith JM, Venkatesan S, Gershowitz A, Moss B: Purification and characterization of the messenger ribonucleic acid capping enzyme GTP: RNA guanylyltransferase from wheat germ. Biochemistry 21:327-333, 1982.

Weir JP, Bajsar G, Moss B: Mapping of the vaccinia virus thymidine kinase gene by marker rescue and by cell-free translation of selected mRNA. Proc Natl Acad Sci USA 79:1210-1214.

Baroudy BM, Venkatesan S, Moss B: Incompletely base-paired flip-flop terminal loops link the two DNA strands of the vaccinia virus genome into one uninterrupted polynucleotide chain. Cell 28:315-324, 1982.

Venkatesan S, Moss B: Eukaryotic mRNA capping enzyme-guanylate covalent intermediate. Proc Natl Acad Sci USA 79:340-344, 1982.

Wittek R, Moss B: Colinearity of RNAs with the vaccinia virus genome: Anomalies with two complementary early and late RNAs result from a small deletion or rearrangement within the inverted terminal repetition. J Virol 42:447-455, 1982.

Moss B, Cooper N: Genetic evidence for vaccinia virus-encoded DNA polymerase: Isolation of phosphonoacetate-resistant enzyme from the cytoplasm of cells infected with mutant virus. J Virol 43:673-378, 1982.

Baroudy BM, Moss B: Sequence homologies of diverse length tandem repetitions near ends of vaccinia virus genome suggest unequal crossing over. Nucleic Acids Res 10:5673-5679, 1982.

Venkatesan S, Gershowitz A, Moss B: Complete nucleotide sequences of two adjacent early vaccinia virus genes located within the inverted terminal repetition. J Virol 44:637-646, 1982.

Mackett M, Smith GL, Moss B: Vaccinia virus: a selectable eukaryotic cloning and expression vector. Proc Natl Acad Sci USA 79:7415-7419, 1982.

Bajsar G, Wittek R, Weir JP, Moss B: Vaccinia virus thymidine kinase and neighboring genes: mRNAs and polypeptides of wild-type virus and putative nonsense mutants. J Virol 45:62-72, 1983.

Baroudy BM, Venkatesan S, Moss B: Structure and replication of vaccinia virus telomeres. Cold Spring Harbor Symp Quant Biol 47:723-739, 1983.

Smith GL, Mackett M, Moss B: Infectious vaccinia virus recombinants that express hepatitis B virus surface antigen. Nature 302:490-495, 1983.

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Buller RML, Smith GL, Moss B, Cremer K, Notkins AL: Infectious vaccinia virus TK⁻ recombinants that express foreign genes are less virulent than wild-type virus in mice. In: Lerner RA, Chanock RM, Brown F eds. Vaccines 85. Molecular and Chemical Basis of Resistance to Parasitic, Bacterial and Viral Diseases. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., pp. 163-168, 1985.

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Twardzik DR, Brown JP, Ranchalis JE, Todaro GJ, Moss B: Vaccinia virus-infected cells release a novel polypeptide functionally related to transforming and epidermal growth factors. Proc Natl Acad Sci USA 82:5300-5304, 1985.

Buller RML, Moss B: Genetic basis for vaccinia virus virulence. In: Quinnan GV ed. Vaccinia Viruses as Vectors for Vaccine Antigens. New York: Elsevier-North Holland, pp 37-46, 1985.

Winters E, Baroudy B, Moss B: Molecular cloning of the terminal hairpin or vaccinia virus DNA as an imperfect palindrome in an Escherichia coli plasmid. Gene 37:221-228, 1985.

Rohrmann G, Moss B: Transcription of vaccinia virus early genes by a template-dependent soluble extract of purified virions. J Virol 56:349-355, 1985.

Weir J, Moss B: Use of a bacterial expression vector to identify the gene encoding a major core protein of vaccinia virus. J Virol 56:534-540, 1985.

Moss B: Vaccinia virus expression vector: A new tool for immunologists. Immunol Today 6:243-245, 1985.

Buller RML, Smith GL, Cremer K, Notkins AL, Moss B: Decreased virulence of recombinant vaccinia virus expression vectors is associated with a thymidine kinase-negative phenotype. Nature 317:813-815, 1985.

Rosel J, Moss B: Transcriptional and translational mapping and nucleotide sequence analysis of a vaccinia virus gene encoding the precursor of the major core polypeptides 4b. J Virol 56:830-838, 1985.

Ensinger M, Weir J, Moss B: Fine structure marker rescue of temperature-sensitive mutations of vaccinia virus within a central conserved region of the genome. J Virol 56:1027-1029, 1985.

Chakrabarti S, Brechling K, Moss B: Vaccinia virus expression vector: Coexpression of ß-galactosidase provides visual screening of recombinant virus plaques. Mol Cell Biol 5:3403-3409, 1985.

Moss B: Use of vaccinia virus vectors for the development of live vaccines. In: Kamely D, Fields B, Martin M eds. Banbury Report 22: Genetically Altered Viruses and the Environment. Cold Spring Harbor NY: Cold Spring Harbor Laboratory, pp 291-300, 1985.

Yilma T, Ristow S, Moss B, Jones L: A novel approach for the production of monoclonal antibodies using infectious vaccinia virus recombinants (submitted).

Curriculum Vitae

PERSONAL INFORMATION:

Name: Jerry J. Callis [redacted]

Born: [redacted]

Present Address: [redacted]

Education:

Auburn University - 1947 - D.V.M.

Purdue University - 1949 - M.S.

Purdue University - 1979 - D. Sc. (Honorary)

Southampton College- 1980 - D. Sc. (Honorary)

WORK EXPERIENCE: Entire work experience has been with USDA.

1949-1951 - Work Study Program on FMD and Vaccine Production
State Veterinary Research Institute
Ministry of Agriculture
Amsterdam, Holland

1952 - First six months
Veterinarian - Vesicular Diseases Project
Beltsville, MD

1952-present - Plum Island Animal Disease Center
Greenport, NY

1952-1956 - In-charge, Research

1956-1962 - Assistant Director

1963-1986 - Director

March 10, 1986-Present - Senior Scientific Advisor for A.R.S.
Tel. No. 516-323-2500

Responsibilities: As Director of PIADC, responsible for providing scientific and administrative direction for the USDA program of diagnosis and research on approximately 40 animal diseases which do not exist in the United States. The staff of 300 includes 40 scientists in five different scientific disciplines. Others include safety technicians, biological laboratory technicians, animal caretakers, guards, engineers and maintenance and operating staff. Responsibilities also include technical direction of scientists stationed in Kenya working on East Coast Fever. Have also been responsible, in the past, for cooperative research in Scotland, England, Spain, Holland, Yugoslavia, Turkey, India, Egypt, Brazil, Colombia, Argentina and Denmark.

J.J. Callis

PUBLICATIONS (last 5 years)

Transmission of diseases by bull semen and fertilized bovine ova. J.J. Callis, A.H. Dardiri and D.E. Herrick, 1980.

Foot-and-mouth disease. G.E. Cottral and J.J. Callis. Chapter in Bovine Medicine and Surgery. Second Ed. Santa Barbara. American Vet. Pub. Inc., p. 153-175, 1980.

Foot-and-Mouth Disease. J.J. Callis, G.E. Cottral and P.D. McKercher. Viral Zoonose 2:168-176, 1981.

Frontiers of science to tomorrow's food (Animal reproduction, genetic engineering, biological control of pests, fish farming). R.D. Jackson, R.A. Bellows, J.J. Callis, C.W. Carlson, R.C. Valentine, C. Ignoffo, M.D. Ruff, H.D. Danforth and R.T. Lovell. The Yearbook of Agriculture - USDA, p. 116-130, 1981.

Foot-and-mouth disease (Livestock, etiology, epidemiology). J.J. Callis and P.D. McKercher. In. Diseases of swine, p. 278-287, 1981.

Surveillance and Control of Virus Diseases: Americas. J.J. Callis, G.C. Poppensiek and D.H. Ferris. In. Virus Diseases of Food Animals. Vol. I: International Perspectives. Academic Press, p. 45-68, 1981.

Agricultural Productivity: Key to the Future. J.J. Callis. Proceedings and Minutes. Thirtieth Annual Meeting of the Agricultural Research Institute. October 8, 198 Agricultural Research Institute, Washington, D.C. p. 97-116, 1981.

Genetically-Engineered Foot-and-Mouth Disease Vaccine, AVMA, July 1982, Salt Lake City, Utah.

Genetic Engineering: Animal Health and Production. J.J. Callis. Vol. 17, 1983.

Residual viruses in fresh and cured meat. P.D. McKercher and J.J. Callis. Official proceedings: Annual Meeting. Livestock Conservation Institute, p. 143-, 1983.

Achievements in genetic engineering and their influence on the control and prevention of animal disease vaccines. H.L. Bachrach, J.J. Callis, F. Brown and K. Strohmaier. Office International des Epizooties, 2(3):629-653, 1983.

The thermal death time curve for foot-and-mouth disease virus contained in primarily infected milk. J.S. Walker, P.W. de Leeuw, J.J. Callis and J.G. van Bekkum. Journal of Biological Standardization, 12(2):185-189, 1984.

Foreign Animal Diseases of concern to U.S. Stockmen. J.J. Callis. In. Beef Cattle Science Handbook 20(62):497, 1984.

Foot-and-mouth disease. J.J. Callis. In. Foreign Animal Disease, p. 166-177, 1984.

Genetic Engineering: Animal Health Production. J.J. Callis. Proceedings 17th Conference FMD Commission. Revue Office International Des Epizooties, 3:269-270, 1984

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Artificial insemination, fertilized ova, and disease transmission. J.J. Callis. In. Beef Cattle Science Handbook 20(63):498, 1984.

Genetic Engineering: Animal Health Production. J.J. Callis. Rev. Office International Office Des Epizooties 3:259-270, 1984.

Session of the Research Group of the Standing Technical Committee of the European Comm. for the Control of Foot-and-Mouth Disease. P.D. Mckercher, J.H. Blackwell, R. Murphy, J.J. Callis, G.F. Panina, A. Civardi, M. Bugnetti, F. Simone. Report of A, FAO, Brescia, Italy, pp. 26-28, 1984.

Residual viruses in animal products and by-products. P.D. McKercher and J.J. Callis. Beef and Cattle Science Handbook, eds, F.H. Baker and M.E. Miller, Westview press, Boulder, Co., pp. 499-, 1984.

Distribucion mundial e importancia de la fiebre aftosa. J.J. Callis and P.D. McKercher. Bovis 3:13-, 1985.

Bluetongue in the United States, in Bluetongue and Related Orbiviruses. J.J. Callis. Alan R. Liss, Inc., New York, 37-42, 1985.

Dose-response evaluation of a genetically engineered foot-and-mouth disease virus polypeptide immunogen in cattle. P.D. McKercher, D.M. Moore, D.O. Morgan, B.H. Robertson, J.J. Callis, D.G. Kleid, S.J. Shire, D.G. Yansura, D. Dowbenko and B. Small. Am. J. Vet. Res. 46(3):587-590, 1985.

Resumen historico de la efectividad y de la peligrosidad de la vacuna inactivada. P.D. McKercher and J.J. Callis. Pruebas de potencia de una vacuna. Nueva generacion de vacunas de FA. Bovis, 3, pp. 65-70, 1985.

Survival of swine vesicular disease virus in "Prosciutto di Parma" (Parma Ham). P.D. McKerhcer, J.H. Blackwell, R. Murphy, J.J. Callis, G.F. Panina, A. Civardi, M. Bugnetti, F. De Simone and F. Scatozza. Can. Inst. Food Sci. Technol. 18(2): 163-167, 1985.

CHARLES A. MEBUS
Supervisory Veterinary Medical Officer, GM-701-15
Plum Island Animal Disease Center
Greenport, NY 11944

1) Education Background:

1950-1952	Rutgers University, Pre-veterinary
1952-1956	Cornell University, Veterinary Medicine, DVM, 1956
1960-1962	Kansas State University, Veterinary Pathology, M.S., 1962
1962-1963	Kansas State University, Veterinary Pathology, Ph.D., 1963
1964	Diplomat, American College of Veterinary Pathologists

2) Additional Training:

1956	AMEDS Meat and Dairy Hygiene School, U.S. Army; 8 weeks, food quality and sanitation.
1971	African Swine Fever Short Course, Plum Island Animal Disease Center, Greenport, New York.
1976	Foreign Animal Diseases Training Course, Plum Island Animal Disease Center, Greenport, New York.
1978	Executive Seminar "Science, Technology and Public Policy, Kings Point, New York.
1979	Performance Appraisal and Career Counseling; U.S. Civil Service Commission.
1981	Personnel Management for Supervisors and Managers, Office of Personnel Management.
1982	Improving Managerial Skills of the New or Prospective Manager; American Management Association.
1982	Supervision and the Organization of Work, Office of Personnel Management.
1983	Audio-Teleconference Course in High Performance Liquid Chromatography.

3. Research Experience:

1956-1958	Assistant Chief, Veterinary Section, USAREUR Medical Laboratory, Landstuhl, Germany.
1958-1960	General Practice, Dover, Delaware.
1960-1963	N.D.E.A. Staff Assistant, Kansas State University (50% research).
1963-1965	Associate Professor, Veterinary Pathology, Kansas State University (30% research) (70% teaching).

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1965-1977 Professor, Veterinary Science, University of Nebraska, Lincoln (94% research) (6% teaching) Teaching included the following courses:

Anatomy and Physiology of Domestic Animals
Advanced General Pathology
Systemic Pathology I
Systemic Pathology II

Service work consisted of doing the histopathologic examinations and immunofluorescent examination on tissue sections, for the Veterinary Diagnostic Laboratory in Lincoln, Nebraska.

Areas of research that resulted in publication were:

Transmissible gastroenteritis of swine
Mycoplasma pneumonia of swine
Ascariasis of Swine
Exudative epidermitis of swine
Calf diarrhea
African swine fever

1977 to present Laboratory Chief, Pathobiology, Plum Island Animal Disease Center

1) Honors and Awards:

Member, Phi Zeta
Member, Sigma XI
Nebraska Veterinary Medical Association Special Award, 1976 (for work on neonatal calf diarrhea).

2) Special Invitations: (last 5 years)

*43) African Swine Fever. Swine Disease Course, Ames, Iowa, January 17, 1980.

44) The Rotavirus of calf diarrhea and its impact on human infantile diarrhea. New York Chapter of the American Society of Microbiology, March 25, 1980.

45) Bovine Virus Diarrhea: African Swine Fever. South Carolina Veterinary Association, May 23, 1980.

46) African Swine Fever Seminar, College of Veterinary Medicine, University of Georgia, November 3, 1981.

47) African Swine Fever--American Society of Tropical Medicine and Hygiene, Atlanta, November, 6, 1981.

48) Africon Swine Fever, Annual Meeting, Viral Diseases, American College of Veterinary Pathologists, Miami, December 1981.

*49) Lectured on Diarrhea and Neonatal Diarrhea, Tufts University, January 21, 1981.

*50) African Swine Fever, Comparative Pathology Section, International Academy of Pathology, Chicago, March 1981.

51) African Swine Fever at the APHIS Swine Disease Course, Ames, Iowa, October 27, 1981.

52) Lecture at the Exotic Disease Seminar, College of Veterinary Medicine, University of Minnesota, January 27, 1982.

53) Lecture at the Exotic Disease Seminar, College of Veterinary Medicine, Oklahoma State University, March 11 and 12, 1982.

*54) Lecture on Emergency Disease Seminar, Department of Food and Agriculture, State of California - April 6-9, 1983.

55) Seminar on African Swine Fever, College of Veterinary Medicine, University of California, 1983.

*56) Lecture on Foreign Animal Diseases Seminar for Diagnosticians, Ames, Iowa, May 24-26, 1983.

57) Lecture on Foreign Animal Class, College of Veterinary Medicine, University of Minnesota, March 3, 1984.

*58) Lecture on Purose of an activities at the Plum Island Animal Disease Center, Tufts University, November 1984.

*Expense paid by inviting organization.

3) Membership in Professional Societies:

American Veterinary Medical Association
U.S. Animal Health Association
Conference of Research Workers
American College of Veterinary Pathologists
American Association of Veterinary Diagnosticians

Advisory and Consultant Activities (last 5 years)

1) Participation in National Scientific Meetings, Technical Conferences Workshops, etc.:

Has been invited to present the following papers at international meetings and meetings outside the U.S.:

*20) Guest lecturer, Exotic Disease Course, Grosse Ile, Canada (Animal Diseases Research Institute, Agriculture Canada), September 1981.

*21) Participate in the CEC-FAO ASF Expert Consultation Meeting, Sardinia, Italy, September 23-25, 1981.

*22) Presented one-week course on Exotic Animal Disease, College of Veterinary Medicine in Santo Domingo, Dominican Republic, February 8-12, 1982.

23) African Swine Fever Virus Disease in the Americas: A Chaning Disease, First International Conference on the Impact of Viral Diseases on the Development of Latin American Countries and the Carribean Region, March 22-27, 1982.

*24) Lecture - African Swine Fever, Congress Annual 1983, Ardre des Medicins Veterinarieo du Quebec, September 22-25, 1983.

*25) Participant - CEC, African Swine Fever Meeting, Bash, Switzerland, October 20-21, 1983.

26) Discussant - International Conference on Impact of Diseases on Livestock Production in the Tropics, May 9-13, 1983.

27) Participant - Tufts/Niger Title XII Conference, Tallorres, France, August 18-25, 1984.

*28) Participant - CEC/FAO Export consultation on African Swine Fever, Rome, October 23-26, 1984.

*Expenses paid by inviting organization or third part.

2) <u>Professional Advisory and Consulting Activities</u>: (last 5 years)

h) Member of the Cooperative Research Panel to review the research program at the College of Veterinary Medicine and Poultry Science Department, Auburn University, January 1981.

i) Review manuscripts for Veterinary Infectious Disease Organization, Saskatoon, Canada.

j) Review grant proposals for WHO Diarrheal Diseases Control Program.

k) Review grant proposals for U.S.-Israel Agricultural Research and Development Fund--BARD.

l) Review 1981 Animal Health Special Research Grant proposals, Cooperative Research.

m) Review manuscripts for the Journal of General Virology.

n) Review manuscripts for Veterinary Pathology.

o) Review Spain-U.S. Cooperative ASF Research, Madrid, Spain.

p) Member of the FAO Working Team on ASF.

q) 1979 to present--Adjunct professor of Veterinary Pathology, College of Veterinary Medicine, University of Georgia.

r) 1980 to present--Courtesy Professor, New York State Veterinary College, Cornell University.

s) 1983-present, Graduate Committee, School of Veterinary Medicine, University of California.

t) Consultant for the Animal Resources Review Committee, Animal Resources Branch, DRR, NIH.

3) Special Assignments:

Referee for WHO Diarrheal Disease Control Program Proposals.

Member, Panel on Biotechnology Research in Portugal, National Research Council 1983.

Reviewer, US/Spain Cooperative Research Program, February 13,18, 1983.

4) Contributions to Special Foreign Currency Programs (Public Law 480):

Contractor for Study on the Importance of African Swine Fever in lesser developed countries, Joint Research Committee, Board for International Food and Agriculture Development, Zaire, Zimbabwe, Cameroon, November 1983.

PUBLICATIONS (last 5 years)

79) Schlafer, D.H., and Mebus, C.A.: African swine fever in pregnant sows. Proc. Annu. Meet. U.S. Anim. Health Assoc., pp. 281-285, 1981.

80) Yedloutschnig, R.J., Dardiri, A.H., Mebus, C.A., and Walker, J.S.: Abortion in vaccinated sheep and cattle after challenge with Rift Valley fever virus. Vet. Record 109:383-384, 1981.

81) Gilberg, H.L., Mebus, C.A., and Lewis, R.M.: Experimental vesicula exanthema of swine virus and San Miguel sea lion virus infection in phocid seals. Vet. Pathol. 19(4):406-412, 1982.

82) McVicar, J.W., Mebus, C.A., Brynjolfsson, A., and Walker, J.S.: Inactivation of African swine fever virus in tissues by gamma radiation. Am. J. Vet. Res. 43:318-319, 1982.

83) McVicar, J.W., Mebus, C.A., Brynjolfsson, A., and Walker, J.S.: Inactivation of African swine fever virus in tissues by gamma radiation. Am. J. Vet. Res. 43:318-319, 1982.

84) Wyatt, R.G., Kapikian, A.Z., and Mebus, C.A.: Induction of cross-reactive serum neutralizing antibodies to human rotavirus in calves after in vitro administration of bovine rotavirus. J. Clin. Microbiol. 18(3):505-508, 1983.

85) Mebus, C.A., and McKercher, P.D.: Foot-and-mouth disease vaccination A review. Bovine Pract 17:134-136, 1983.

86) Sanchez-Vizcaino, J., Mebus, C.A., McVicar, J.W., and Valero, F.: Studies on humoral and cell-mediated immunity in pigs and piglets infected with different field isolates of African swine fever. Agriculture: African swine fever. EVR 8466 European Communities Commission, Luxembourg, pp. 195-205, 1983.

87) Mebus, C.A., McVicar, J.W., and Dardiri, A.H.: Comparison of the pathology of high and low virulence African swine fever virus injections. Agriculture: African swine fever EVR 8466 European Communities Commission, Luxembourg. CEC/FAO Research Seminar, Sassari, Sardinia (September 23-25, 1981), pp. 183-194, 1983.

88) Wardley, R.C., Andrade, Cde, M., Black, D.N., de Castro Portugal, F.L., Enjuanes, L., Hess, W.R., Mebus, C.A., Ordas, A., Rutili, D., Sanchez-Vizcaino, J., Vigario, J.D., and Wilkinson, P.J.: African swine fever virus. Arch Virol. 76:73-90, 1983.

89) Schlafer, D.H., Mebus, C.A., and McVicar, J.W.: African swine fever in neonatal pigs: Passively acquired protection from colostrum or serum of recovered pigs. Am. J. Vet. Res. 45:1367-1372, 1984.

90) Schlafer, D.H., and Mebus, C.A.: Abortion in sows experimentally injected with African swine fever virus: Clinical features. Am. J. Vet. Res. 45:1353-1360, 1984.

91) Schlafer, D.H., McVicar, J.W., and Mebus, C.A.: African swine fever convalescent sows: Subsequent pregnancy and the effect of colostral antibody on challenge inoculation of their pigs. Am. J. Vet. Res. 45:1361-1366, 1984.

Other Scientific Publications (last 5 years)

17) Mebus, C.A.: Calf Coronavirus Diarrhea: Current Veterinary Therapy, Food Anim. Pract., pp. 600-601, 1981.

18) Mebus, C.A.: Rotavirus infections in ruminants: Current Veterinary Therapy: Food Anim. Med., 1980, pp. 597-599.

19) Mebus, C.A.: Rotavirus diarrhea. Comp. Pathol. Bull., 11(3):2, 1971.

20) Mebus, C.A.: Diseases of calves (E. coli). Cienc. Vet. (Mexico), 16:518-520, 1971.

21) Mebus, C.A.: Rotavirus diarrhea. Revista Colombians de Ciercias Pecuarias.

22) Mebus, C.A.: Comparison of diagnostic techniques for research in neonatal diarrhea. Current topics in Vet. Med. and Anim. Sci., Martinus Nijhoff. The Hague, 13:62-69, 1981.

23) Mebus, C.A.: History of the Nebraska calf diarrhea virus in virus infections of the gastrointestinal track. Tyrell and Kapikian, Editor. Infect. Dis. and Antimicrobiol. Agents, 3:291-294, 1984.

24) Mebus, C.A.: Bovine coronavirus in viral infections of vertebrates (in press).

25) Mebus, C.A.: Bovine and ovine rotavirus in viral infections of vertebrates (in press).

26) Garnett, W.H., and Mebus, C.A.: Species specimen listing, collection and shipping procedures for suspected foreign animal diseases. Reference Manual, Foreign Animal Disease Course.

27) Mebus, C.A.: Bovine rotavirus. Current Vet. Therapy (in press).

28) Mebus, C.A.: Calf coroona virus diarrhea. Current Vet. Therapy (in press).

CURRICULUM VITAE

Beverly Dale

Date and Place of Birth [redacted]

Business Address

California Biotechnology, Inc
2450 Bayshore Frontage Road
Mountain View, CA 94043
(415) 966-1550

Home Address [redacted]

Education

Ph.D., Microbiology, 1982
University of Texas Health Science Center at Dallas, Dallas, TX

M.S., Biology (Mycology), 1972
University of Akron, Akron, OH

B.S., Microbiology (Honors), 1968
University of Southern Mississippi, Hattiesburg, MS

Additional Academic Training

RNA Tumor Virus Course, Cold Spring Harbor Laboratory, 1979.

Postdoctoral studies in Tumor Virology, 1982-1983.
University of Texas Health Science Center at Dallas, Dallas, TX

Publications

Dale, B. and Ozanne, B. (1981) Characterization of mou cellular DNA homologous to Abelson murine leukemia vir Mol. Cell. Biol. 1:731-742.

Grunwald, D.J., Dale, B., Dudley, J., Lamph, W., Sugden, B., Ozanne, B., and Risser, R. (1982) Loss of viral gene

117

expression and retention of tumorigenicity by Abelson lymphoma cells. J. Virol. 43:92-103.

Ozanne, B., Wheeler, T., Zack, J., Smith, G., and Dale, B. (1982) Transforming gene of a human leukaemia cell is unrelated to the expressed tumor virus related gene of the cell. Nature 299:744-747.

Dale, B., Wheeler, T., Zack, J., Smith, R.G., and Ozanne, B. (1982) Unique expression of c-abl(human) sequences in a novel human pre-B lymphoblastic leukemia. 11th Ann. ICN-UCLA Symp. Tumor Viruses and Differentiation.

Dale, B., Brown, R., Miller, J., White, R.T., Air, G.M., and Cordell, B. Nucleotide and deduced amino acid sequence of the influenza neuraminidase genes of two equine serotypes. (Submitted to Virology).

Abstracts

Dale, B., T. Wheeler, and B. Ozanne (1981) Arrangement of Abelson murine leukemia virus related sequences in BALB/c mouse DNA. Abst. Annu. Meet. Am. Soc. Microbiol. T102:254.

Dale, B., T. Wheeler, and B. Ozanne (1981) Mouse nucleic acid sequences homologous to Abelson murine leukemia virus. Abst. RNA Tumor Virus Meet., Cold Spring Harbor, NY, pg. 66.

Ozanne, B., Wheeler, T., Dale, D., Zack, J., and Smith, R.G. (1982) Arrangement and expression of Abelson murine leukemia virus related sequences in a novel pre-B lymphoblastic leukemia. Abst. RNA Tumor Virus Meet., Cold Spring Harbor, NY, pg. 150.

Brown, R., Kloss, J., Damm, D., Cordell, B., and Dale, B. (1986) Expression of functional influenza virus neuraminidase by recombinant vaccinia virus. Abst. ICN-UCI Symposium, Laguna Beach, CA.

Professional Experience

1985-present	Project leader, Animal Vaccines, California Biotechnology, Inc., Mountain View, CA
1984-present	Staff Scientist, California Biotechnology, Inc., Mountain View, CA
1983-1984	Scientist, California Biotechnology, Inc.,

Mountain View, CA

1982-1983	Postdoctoral fellow, Department of Microbiology, University of Texas Health Science Center at Dallas, Dallas, TX
1979-1982	Lecturer, Sexually Transmitted Disease Training Program, Texas, Department of Health and Center for Disease Control, Dallas, TX
1977-1982	Adjunct faculty, Department of Biology, Texas Woman's University (Dallas campus), Dallas, TX
1977-1982	Graduate teaching assistant/research assistant, University of Texas Health Science Center at Dallas, Dallas, TX
1975-1977	Instructor and laboratory coordinator, Department of Biology, Texas Woman's University, Denton, TX
1974-1975	Chief microbiologist, Quality Control, Mary Kay Cosmetics, Dallas, TX
1972-1974	Instructor, Department of Microbiology, University of Southern Mississippi, Hattiesburg, MS
1970-1972	Graduate teaching assistant, Department of Biology, University of Akron, Akron, OH

IN PRESS
J.E. Moulton

1. 1983 Moulton, J. E. (editor) Tumors in Domestic Animals, 3rd edition. University of California Press.

* 2. 1983 Moulton, J. E. and Harvey J. Olander. Tumors of the Lymphoid and Hematopoietic System. In: Tumors in Domestic Animals, 3rd edition. University of California Press.

* 3. 1983 Moulton, J. E. Tumors of the Respiratory System. In: Tumors in Domestic Animals, 3rd edition. University of California Press.

* 4. 1983 Nielson, S. and J. E. Moulton. Tumors of the Urinary System. In: Tumors in Domestic Animals, 3rd edition. University of California Press.

5. 1984 Whitelaw, D.D., Kaaya, G.P., Moulton J.E., Moloo, S.K. and M. Murray. Experimental Studies on Genetic Resistance to African Trypanosomiasis in Small East African Goats. Tropical Animal Health.

6. 1985 Moulton J.E. Relapse Infection after Chemotherapy in Goats Experimentally Infected with Trypanosoma brucei: Pathological Changes in Central Nervous System. Veterinary Pathology.

SUBMITTED
J.E. Moulton

1. 1985 Ekejindu, G.O.C., Moulton, J.E., M. Shifrine. Suppression of Bone Marrow Hematopoietic and Stromal Colony Formation by Splenic Fluids from Deer Mice Infected with Trypanosoma Equiperdum - In Vitro Effects. Tropical Veterinarian. (Submitted).

2. 1985 Moulton, J.E. Structural and Functional Changes in the Lymphoid Organs of Cattle Infected with Trypanosoma congolense. Tropical Veterinarian. (Submitted).

3. 1985 LeGrand, E.K.; Donovan, R.M., Marx, P.A., Moulton, J.E., Cheung, A.T.W., Lewis, A.E., Gardner, M.B. Monocyte Function in Rhesus Monkeys with Simian Acquired Immune Deficiency Syndrome. Inf. and Immun. (Submitted)

4. 1985 Moulton, J.E. and L.K. Phillips. A Technique for Culturing Macrophages from Monkeys. Am. J. Vet. Res. (In Preparation)

5. 1985 Moulton, J.E. Culbertson, R., Rosenblott, L.S. and Goldman, M.: Mammary tumors in a colony of beagle dogs. Am. J. Vet. Res. (Submitted).

6. 1983 Moulton, J. E. Tumors of the Mammary Gland. In: Tumors in Domestic Animals, 3rd edition. pp. 600 est. University of California Press, to be submitted August 1, 1985.

CURRICULUM VITAE

Robert Tyler White

Personal Data

Born: [redacted]

Married to Annette M. Breingan

Address: [redacted]

Telephone: [redacted]
Lab 966-1550

Education

Ph.D. in Biochemistry, 1980
Stanford University School of Medicine
Stanford, California

B.A., magna cum laude, with Honors, in Molecular Biology, 1974
Brown University, Providence, Rhode Island

Research Interests

The organization and regulation of eukaryotic genes and gene families. Expression of cloned genes and cDNA sequences in heterologous systems. Molecular basis of organismal evolution.

Research Experience

September, 1983 to Present:

Staff Scientist at California Biotechnology, Inc., Mountain View, California.

Member of the pulmonary group. Molecular biologist responsible for cloning the human pulmonary surfactant apoprotein (PSAP) gene. Directed the introduction of the human PSAP gene and cDNA into mammalian tissue culture cells for the production of high levels of PSAP.

Member of the veterinary vaccine group. Cloned a portion of the Bovine Viral Diarrhea Virus (BVD) genome corresponding to one of the major antigenic envelope glycoproteins and expressed this cDNA in several bacterial expression systems.

May, 1980 to September, 1983:

Postdoctoral researcher in the laboratory of Dr. Allan C. Wilson, in the Department of Biochemistry, University of California, Berkeley.

As part of a study of the evolution of ruminant physiology, I cloned the cDNA and gene for the lysozyme expressed as a digestive enzyme in cow stomach.

January, 1975 to April, 1980:

Graduate student with Dr. Paul Berg in the Department of Biochemistry at Stanford University.

Constructed mutants of Simian Virus 40 to probe the transcription and processing of late lytic viral RNAS, and characterized the aberrant RNA species expressed by these mutants.

June, 1974 to September, 1974:

Laboratory technician in an epidemiology lab of the Zoonosis Branch at the Center for Disease Control in Atlanta, Georgia.

June 1973 to May 1974:

Conducted an undergraduate Honors project in the laboratory of Dr. Arthur Landy at Brown University. While in Landy's lab, I isolated a DNA fragment containing an E. coli tyrosine tRNA gene by restriction endonuclease digestion and gel electrophoresis techniques.

Principal Publications

Luis P. Villarreal, R. Tyler White and Paul Berg. 1979. Mutational alterations within the Simian Virus 40 leader segment generate altered 16S and 19S mRNAs. **Journal of Virology 29**:209-219.

R. Tyler White, Paul Berg and Luis P. Villarreal. 1982. Simian Virus 40 rabbit B-globin recombinants lacking late mRNA splice sites express cytoplasmic RNAs with altered structures. **Journal of Virology 42**:262-274.

Luis P. Villarreal and R. Tyler White. 1983. A splice junction deletion deficient in the transport of RNA does not polyadenylate nuclear RNA. **Molecular and Cellular Biology 3**:1381-1388.

Brad Benson, Samuel Hawgood, James Schilling, John Clements, Deborah Damm, Barbara Cordell and R. Tyler White. 1985. Structure of canine pulmonary surfactant apoprotein: cDNA and complete amino acid sequence. **Proc. Natl. Acad. Sci. USA 82**:6379-6383.

R. Tyler White, Deborah Damm, Judith Miller, Kaye Spratt, James Schilling, Samuel Hawgood, Brad Benson, and Barbara Cordell. 1985. Isolation and characterization of the human pulmonary surfactant apoprotein gene. **Nature 317**:361-363.

Name: Barbara Cordell
Title: Assistant Director of Research
Birthdate: [redacted]

Education:
Cum Laude Indiana University, Bloomington, Indiana, B.A., 1968

Indiana University, Bloomington, Indiana, Ph.D., 1972.

Fellowships and Academic Honors
NIH Genetics Grant Awardee, 1968-1972
Junior Dernham Awardee (American Cancer Society) 1975-1978
Research Fellowship, University of California, San Francisco, CA., 1973-1977
Senior Dernham Awardee (American Cancer Society) 1975-1978
NSF Grant (3 years) Principal Investigator, 1978-1981
Hoechst Grant Awardee, 1981-1983

Positions Held
Postdoctoral Research Fellow (with J. Michael Bishop), Department of Microbiology, University of California, San Francisco, California, 1973-1977

Assistant Research Biochemist (with Howard M. Goodman), Department of Biochemistry and Biophysics, University of California, San Francisco, CA, 1978-1980

Associate Investigator, Howard Hughes Medical Institute, Deaprtment of Biochemistry and Biophysics, University of California, San Francisco, CA, 1980-1981

Assistant Molecular Biologist, Department of Molecular Biology, Massachusetts General Hospital, 1981-1983.

Assistant Professor, Department of Genetics, Harvard Medical School, Boston, MA, 1981-1983.

Senior Scientist, California Biotechnology Inc., Mountain View, CA, 1983-present

Publications
Twenty one original papers. Nine relevant papers listed.

1. O'Farrell, P.Z., Cordell, B., Valenzuela, P., Rutter, W.J. and Goodman, H.M. Structure and processing of yeast precursor tRNAs containing intervening sequences. Nature 274:438-445 (1978).

2. Cordell, B., Weiss, S., Varmus, H.E. and Bishop, J.M. At least 104 nucleotides are transposed from the 5' termini of smaller viral mRNAs. Cell 15:79-81 (1978).

3. Cordell, B., Swanstrom, R., Goodman, H.M., Bishop, J.M. tRNATrp as primer for RNA-directed DNA polymerase: structural determinants of function. J. Biol. Chem. 254:1866-1874 (1979).

4. Cordell, B., Bell, G., Tischer, E., Ullrich, A., Pictet, R., Rutter, W.J. and Goodman, H.M. isolation and characterization of a cloned rat gene encoding insulin I. Cell 18:533-543 (1979).

5. Bell, G.I., Swain, W.F., Pictet, R., Cordell, B., Goodman, H.M. and Rutter, W.J. Human insulin: nucleotide sequences of cDNA clone encoding preproinsulin. Nature 282:525-527 (1979).

6. Bell, G.I., Pictet, R.L., Rutter, W.M., Cordell, B., Tischer, E. and Goodman, H.M. Sequence of the human insulin gene. Nature 284:26-32 (1980).

7. Cordell, B., Diamond, D., Smith, S., Schone, W. and Goodman, H.M. Disproportionate expression of the two non-allelic insulin genes in a rat pancreatic tumor is due to translational control. Cell 31:531-542 (1982).

8. White, R.T., Damm, D., Miller J., Spratt, K., Schilling, J., Hawgood, S., Benson, B., Cordell, B., Isolation and characterization of the human pulmonary surfactant apoprotein gene. Nature 317:361-363.

9. Benson, B., Hawgood, S., Schilling, J., Clements, J., Damm, D., Cordell, B. and R.T. White. Structure of canine pulmonary sufactant apoprotein: cDNA and complete amino acid sequence. Proc. Natl. Acad. Sci. USA 82:6379-6383.

Reviews

Ten reviews. One relevant review listed below.

1. Graham, F.L., Bacchettii, S., McKinnon, R., Stanners, C.P., Cordell, B. and Goodman, H.M. Introduction of macromolecules into viable mammalian cells. Wistar Symposium (Baserga, R., Croce, C., Rovera, G. eds) Vol. 1, chapter in book, (1979).

DONALD E. DeTRAY

Biographical Data

1. Born: [redacted]

2. Received degree of Doctor of Veterinary Medicine, Ohio State University, 1940.

3. 1940-42 - General practice, Napoleon, Ohio.

4. 1942-43 - American Field Service Ambulance Corps attached to the British 8th Army in North Africa and Middle East.

5. 1943-47 - General practice, Napoleon, Ohio.

6. 1947-48 - Mexican-United States Commission for the control of foot-and-mouth disease. Field work and vaccine production.

7. 1949-51 - Research veterinarian (brucellosis), Bureau of Animal Industry, Beltsville, MD.

8. 1951-53 - USDA research veterinarian dealing largely with rinderpest, a virus disease of cattle and many game animals at Kabete, Kenya, East Africa.

9. 1953 - USDA veterinarian returned to Mexico to help deal with a new outbreak of foot-and-mouth disease.

10. 1954-61 - USDA research veterinarian engaging in and supervising research on African swine fever at Kabete and Muguga, Kenya, East Africa.

11. 1961-63 - Assistant to the Director, Animal Disease and Parasite Research Division, Agricultural Research Service, USDA, Beltsville, MD.

12. 1963-66 - Associate Director as in 11 above.

13. 1966-68 - Regional Livestock Advisor (veterinary) for the Agency for International Development (USAID), Lagos, Nigeria. U.S. representative for the African-wide campaign against rinderpest under the aegis of the Scientific, Technical and Research Commission (STRC) of the Organization of African Unity (OAU).

14. 1968-70 - Same position as in 13 above but located in Nairobi, Kenya.

15. 1970-73 - As above, located in Addis Ababa, Ethiopia (in all, 22 African countries were involved in this highly successful campaign.

Biographical Data Cont.

16. 1973 to present, semiretired:

a. Have returned twice to West Africa for USAID to deal with animal health problems associated with the severe drought in the Sahelian countries and served as a consultant to Kenya in 1977 to assess the potential of the livestock industry.

b. Served as consultant to USDA in presentation of "Rinderpest" at five regional seminars of the USDA Regional Emergency Animal Disease Eradication Organization (READEO).

c. Presented a paper on rinderpest for USDA-APHIS Emergency Programs at the veterinary colleges at Kansas State; Washington State; Texas A&M; Georgia, Auburn, Alabama; Colorado State and Ohio State.

d. Presented papers on African swine fever, contagious bovine pleuropneumonia and rinderpest at Oregon Vet. Med. Assoc. meeting in February 1975.

e. Presented lectures on foreign animal diseases for the faculty and prevetinary students at Oregon State University.

Publications

1. Manthei, C.A. DeTray, D.E., and Goode, E.R. 1950. Brucella Infection in Bulls and the Spread of Brucellosis in Cattle by Artificial Insemination, I. Intrauterine Injection. Proc. Book, AVMA, 87th Ann. Meet., Aug. 1950.

2. Maurer, F.D., DeTray, D.E., Kuttler, K.L., and Webb, A.M. 1953. Rinderpest Adjuvant Vaccine, Patent.

3. DeTray, D.E. 1954. African Swine Fever. Chapter in "Foreign Animal Diseases". pp. 25-32.

4. DeTray, D.E. 1954. Contagious Agalactia. Chapter in "Foreign Animal Diseases". pp. 102-106.

5. DeTray, D.E. 1954. Nairobi Sheep Disease. Chapter in "Foreign Animal Diseases". pp. 118-120.

6. Maurer, F.D., Jones, T.C., Easterday, B., and DeTray, D. 1955. The Pathology of Rinderpest. Proc. Book, AVMA, 92nd Ann. Meet., Aug.

7. Hammond, R.A., and DeTray, D.E. 1955. A Recent Case of African Swine Fever in Kenya, East Africa. J. AVMA, 126:937:389-391.

Publications Cont.

8. DeTray, Donald E., and Scott, Gordon R. 1955. Effect of Hyperimmune Hog Cholera Serum on the Virus of African Swine Fever. J. AVMA, 126:937:313-314.

9. DeTray, D.E. 1957. African Swine Fever - A Review. Bull, Epiz. Dis. Afr., 5:475-478.

10. DeTray, Donald E., and Scott, Gordon R. 1957. Blood changes in Swine with African Swine Fever. Amer. J. Vet. Res., 18:68:484-490.

11. DeTray, Donald E. 1957. African Swine Fever in Wart Hogs. (Phacochoerus aethiopicus). J. AVMA, 130:12:537-540.

12. DeTray, Donald E. 1957. Persistence of Viremia and Immunity in African Swine Fever. Amer. J. Vet. Res., 18:69:811-816.

13. DeTray, Donald E. 1960. African Swine Fever--An Interim Report, Bull. Epiz. Dis. Afr., 8:217-223.

14. DeTray, D.E. 1960. The persistence of African Swine Fever Virus (ASFV) in Carcasses. I.B.A.H./O.I.E. Documents on the persistence of virus of certain animal maladies in the meat of animal products.

15. DeTray, D.E. 1961. African Swine Fever. Council Report, AVMA Council on Public Health and Regulatory Veterinary Medicine. J. AVMA, 139:11:1230,1232.

16. DeTray, D.E., Zaphiro, D., and Hay, D. 1961. The Incidence of African Swine Fever in Wart Hogs in Kenya - A Preliminary Report. J. AVMA, 138:2:78-80.

17. Hess, W.R., and DeTray, D.E. 1960 The Use of Leukocyte Cultures for Diagnosing African Swine Fever (ASF). Bull, Epiz. Dis. Afr., 8:317-320.

18. Scott, G.R., DeTray, D.E., and White, G. 1961. Rinderpest in Pigs of European Origin. Amer. J. Vet. Res., 23:93:452-456.

19. DeTray, Donald E. 1962. Diagnostic capabilities for foreign animal disease at the Plum Island Animal Disease Laboratory. California Veterinarian, Sept.-Oct.

20. DeTray, D.E. 1963. African Swine Fever. Chapter in Advances in Veterinary Science, 8:299-333.

21. DeTray, D.E. 1970. Joint Campaign Against Rinderpest. From the Proceedings of the 74th Annual Meeting of U.S. Animal Health Association 1970.

22. DeTray, D.E. 1971. Animal Diseases of Regional Concern in Africa, Their Prevention and Control. Papers Presented to the Conference on Agricultural Research and Production in Africa - September 1971 Vol. 2 - Supplement 1, August 1973 (Supplement to the Journal of the Association for the Advancement of Agricultural Sciences in Africa) pp. 173-5.

23. DeTray, D.E. 1980. Rinderpest. The Bovine Practitioner, No. 15, Nov. 1980.

CURRICULUM VITAE S.CHEMA

Date of Birth: [redacted]

Place of Birth: Mariakani Kilifi District, Kenya

Marital Status: Married: [redacted]

Children:

Schools:

Mwareni Primary School - To 1949

Kaloleni Intermediate School - To 1951

Alliance High School - To 1955

University Education:

Makerere University - 1956 to 1962

Diploma in Veterinary Science

Ohio State University - 1962 to 1965 and 1966 to 1970

Doctor of Veterinary Medicine

Master of Science

Doctor of Philosophy

Research Postiions Held:

Veterinary Research Officer - Sept. 1965 to Sept.1970

Senior Veterinary Research Officer - 1970 to 1974

Principal Veterinary Research Officer - 1974 to 1977

Chief Veterinary Research Officer - 1977 to date.

Administrative Positions Held:

Officer In Charge, Wellcome Institute for Research on Foot and Mouth Disease - 1970 to 1976

Assistant Director of Veterinary Services (Research) at Veterinary Laboratory, Kabete 1976 to 1977

Deputy Director of Veterinary Services (Research) 1977 to-date

Deputy Director of Livestock Development (Research) 1979 to 1984

Director and Founder, The Agricultural Research Foundation 1985

Host Country Representative, SR-CRSP, 1980 to-date.

Research Papers & Reports

Theses:

1. *S. Chema* (1965).
   "Serological Study of Avian Mycoplasma of the 'H' Serotype. Master of Science Thesis. The Ohio State Univeristy.

2. *S. Chema* (1970).
   "Antigenic and Physicochemical Properties of Picornaviruses: A Comparative Study of Human and Animal Enteroviruses and Rhinoviruses."
   Doctor of Philosophy Dissertation. The Ohio State University.

Publications:

1. *Mugera, G.M. and Chema, S.* (1967).

"Nairobi Sheep Disease: A study of its pathogenicty in sheep, goats and sucling mice."

2. *Hughes, J.H., Chema, S., Lin, N., Conant, R.M., and Hamparian, V.V.* (1974).

"Acid Lability of Rhinoviruses: loss of C and D antigenicty after treatment of pH 3.0."

J. Immun, 112:919

3. *Chema, S.* (1975).

"Vaccination as a method of Foot-and-Mouth Disease control: An appraisal of the success achieved in Kenya, 1968-1973."

Bull. Off, int. Epiz. 83:195-209.

4. *Hughes, J.H., Gnau, J.M., Hility, D.M., Chema, S., Ottolenghi, A.C. and Hamparian, V.V.* (1977).

"Picornaviruses: Rapid Differentiation and Identification by Immune Electromicroscopy and Immunofiffusion."

J. Med. Microbiol. 10:203-212.

5. *Chema, S. and Rweyemamu, M.M.* (1978)

"Selection of SAT 2 Foot-and-Mouth disease vaccine strains for East Africa."

Bull. Off. int. Epiz. 89(11-12): 887-898.

128

6. *Chema, S.* (1980)

"Control of Tickborne Disease under changing Farming Systems."
In "Impact of Animal Disease Research and Control on Livestock Production in Africa." Ed. J.E. Huhn.

7. *Chema. S. and Blocklesby, D.W.* (1981).

"Epidemiology: Appraisal and Future Perspectives." Current Topics in Vet. Med. & Animal Science. 14:100-103.

8. *Ndeti,J.K., Ndiritu, C.G., Chema, S., Schermbrucker, C.G., Pay, T.W.P. and Rweyemamu, M.M.* (1982).

"The Performance of Foot-and-Mouth Disease vaccines in Kenya."
Proc. 16th Foot-and-Mouth Disease Conf. of int. Off. Epiz. 1:51-68.

9. *Ngichabe, C.K., and Chema, S.* (1982).

"Effects of Vaccination on the incidence of Foot-and-Mouth Disease in Kenya."
Proc. 16th Foot-and-Mouth Disease Conf. of int. Off. Epiz. 2:639-654.

10. *Sands, M.W., Fitzhugh, H.A., McDowell, R.E., and Chema, S.* (1982)
"Mixed Crop-Animal Systems on Small Farms in Western Kenya."
In "Small Ruminant Collaborative Research Support Program: Technical Report Series No. 17." Winrock International, Morrilton, Arkansas.

11. *Rossiter, P.B., Jesset, D.M., Wafula, J.S., Karstad, L., Chema, S., Taylor, W.P., Rowe, L.W., Nyange, J.C., Otaru, M., Mumbala, M., and Scott, G.R.* (1983).
"The Re-emergence of Rinderpest as a Threat in East Africa."
Vet. Record 113:459-461.

12. *De Boer, A.J., Fitzhugh, H.A., Hart, R.D., Sands, M.W., Job, M.O. and Chema, S,* (1984).
"Production of Meat and Milk from Goats in Mixed Farming Systems in the High Potential Tropics." In "Livestock Development in Subsaharan Africa". Westview Press. Boulder, Colorado.

13. Chema, S. (1984).
"Low Input Low Output Health Programmes: Generating Data for their Improvement."
Preventive Vet. Med. 2(1-4):481-486.

14. *Chema, S.* (1984)
"Milk Production in Kenya."
In "The Potential for Small Scale Milk Production in Eastern and Southern Africa." IDRC Manuscript Report IDRC - MR98e

Reports

1. *Chema, S.* (1972)

   "Serum antibody levels in cattle regularly vaccinated against Foot-and-Mouth Disease in the Rift Valley Province."

   Departmental Report representing the first definitive study of the serological status of cattle under compulsory vaccination against FMD in Kenya (22 pages).

2. *Chema, S.* (1974)

   "Estimation of Foot-and-Mouth Disease vaccine potency by serological methods - Statistical Evaluation."

   Departmental Report laying down the basis for policy guidelines subsequently used for types A & O vaccine types. (24 pages).

3. *Chema, S.* (1975)

   "Control of Foot-and-Mouth Disease by vaccination."

   Paper given at the Annual Meeting of the Tanzania Society for Animal Production, Arusha, Tanzania. June 1975.

4. *Chema, S.* (1975)

   "Trends in the incidence of Foot-and-Mouth Disease in the main cattle raising areas of Kenya."

   Paper given at the 6th Group FMD Vaccine R & D Meeting of the

Wellcome Foundation, Nairobi, Kenya. November 1975.

5. *Chema, S.* (1975)

"The new SAT 2 outbreaks in Kenya."

Paper given at the 6th Group FMD Vaccine R & D Meeting of the Wellcome Foundation. Nairobi, Kenya. November 1975.

6. *Chema, S.* (1979)

"Current Status of Theileria parva and Theileria lawrencei vaccines."

Departmental Review providing a basis for policy guidelines.

7. *Pay, T.W.F., Chema, S., Ndeti, J.K. and Schermbrucker, C.G.* (1980

"Fifteen Years' Experience in the Production of Foot-and-Mouth disease viruses for use in Africa.

Paper presented at the 2nd International Conference on the Impact of Viral Diseases on the Development of African and Middle Eastern Countries. Nairobi, Kenya. December 1-6th 1980.

8. *Kinyili, J.H., Chema, S. and Ndiritu, C.G.* (1980)

"Trends in the Incidence of Foot-and-Mouth Disease in the main cattle raising area of Kenya."

Paper presented at the 2nd International Conference on the Impact of Viral Diseases on the Development of Africa and Middle Eastern Countries. Nairobi, Kenya. December 1-6th 1980.

9. *Chema, S.* (1981)

"Current Tsetse and Trypanosomiasis distribution in Kenya with particular reference to Development Plans.

10. *Chema, S.* (1983)

"Review of Dairy Development in Kenya."

Paper presented at the International Development Research Centre Workshop on Potential for Small Scale Milk Production in Eastern and Southern Africa. Nairobi, Kenya. November 19-20th 1983.

11. *Chema, S. and James, A.D.* (1984)

"An Approach to the Information Needs of a Ministry of Livestock Development."

Paper on Data Management Systems based on microcomputers - presented at the conference on Advancing Agriculture in Africa. Arusha, Tanzania. February 12-18th 1984.

12. *Chema, S.* (1984)

"Evaluation of New Acaricides in Kenya and Views on Selection for Acaricide Resistance in Past and Future."

Paper presented at FAO Expert Consultation on Research on Tick-borne Disease and their Vectors. Rome, November 28-December 2 1983.

13. *Chema, S.* (1984)

"Public Sector Responsibilities in Tick Control Programmes."

Paper presented at FAO Seminar on African Centres for Tick and Tickborne Disease Control, Ouagadougou, Burkina Faso. November 25-29, 1984.

14. *F. Ruvuna, F. Rurangirwa, Kiio, T. McGuire, T. Cartwright, S. Chema, M.A. Okeyo* (1985)
"Dual-purpose goat potential in tsetse areas of Kenya; growth and survival evaluation at Ukunda Research Station".
Proc. 4th SR-CRSP Workshop (in press)

15. *F. Rurangirwa, T. McGuire, S. Chema, A. Kibor, D. Mwamachi* (1985).
"Dose response and shelf life studies on freeze-dried F-38 vaccine".

Proc. 4th SR-CRSP Workshop (in press)

16. *F. Rurangirwa, T. McGuire, S. Chema, A. Kibor, J. Bari* (1985).
"Diagnosis of contagious caprine pleuropneumonia (CCPP) caused by F-38 strain of mycoplasma (Mycoplasma Capripneumonia)

Proc. 4th SR-CRSP Workshop (in press)

17. *F. Rurangirwa, T. McGuire, S. Chema, A. Kibor* (1985).
"Attempts to immunize against CCPP with protein bands.

Proc. 4th SR-CRSP Workshop (in press)

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18. *S. Chema, A.D. James and G. Moll* (1985)

Veterinary Involvement in Integrated Livestock Projects.

Paper presented at the 4th International Symposium on Veterinary Epidemiology and Economics. Singapore Nov. 18-22.

19. *S. Chema* (1986)

Constraints to Livestock Development and Effective Animal Health Control in Kenya.

Paper presented at the Workshop on Constraints and Strategies for Livestock Development and Improvement of Animal Health. West-Berlin 23-28 January 1986.

20. *S. Chema* 1986

Strategies for Improvement of Animal Health Care for Integrated Livestock Production in Small-Scale Farming Systems.

Paper presented at the Workshop on Constraints and Strategies for Livestock Development and Improvement of Animal Health. West-Berlin 23-28 January 1986.

Membership of Societies

1. Society of Phi Zeta - An Honorary Veterinary Society (USA)
2. Kenya Veterinary Association
3. Animal Production Society of Kenya
4. The Camel Forum

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Biographical Sketch: Roger G. Breeze

Employment:

Professor and Chair, Department of Microbiology and Pathology, WSU, 1984-present

Professor (1982-present) and Associate Professor (1977-82) Department of Microbiology and Pathology, WSU

Associate Dean for Research, College of Veterinary Medicine, WSU, 1982-84

Lecturer, Department of Veterinary Pathology, University of Glasgow, 1968-1977

Societies:

American Thoracic Society, AAAS, International Academy of Pathology, Royal College of Veterinary Surgeons, Royal Society for Medicine.

Selected Publications:

Total refereed papers 1974-date about 140. Selected titles of last few years given below.

In 1973, I received the PhD degree in Pathology from the University of Glasgow for my investigations of fog fever (acute bovine pulmonary emphysema, ABPE) in cattle, during which two new bovine respiratory diseases, hypersensitivity pneumonitis and fibrosing alveoliits were first discovered. I moved to WSU in 1977 to collaborate with Dr. J. R. Carlson on studies into the etiology of ABPE. We have conducted integrated clinical, physiologic, pathologic, ultrastructural, biochemical and metabolic studies of naturally occurring and experimentally induced toxic lung injuries of animals, using such chemical agents as 3-methylindole, paraquat, bleomycin, perilla ketone and 4-ipomeanol in a wide range of species (cattle, horse, goats, rats, mice, primates). In 1980, our lung disease research program was expanded to include infectious causes of pneumonia in domestic animals. Using respiratory syncytial virus/*Pasteurella* interactions as a model, we are studying lung defense mechanisms involving alveolar macrophages and how viral/stress-induced immunosuppression can be controlled with such immunomodulators as interferons and interleukins. We are also investigating the chemical mediation of infectious and non-infectious lung injury. Vaccination against respiratory disease is ineffective in many animal species. To develop vaccine technology to address this and other problems, I became associated with Dr. T. Yilma in a research program to develop subunit, synthetic peptide and infectious vaccinia virus recombinant vaccines for vesicular stomatitis. Using this experience, we intend to develop vaccines for other important viral diseases of livestock, including those involving the respiratory system. There was no respiratory disease research program in the College of Veterinary Medicine when I came to WSU. Now there is a broad research effort dealing with chemical and infectious lung injury, lung defense mechanisms, lung inflammation and vaccination at the applied and basic levels.

PUBLICATIONS:

Hammond, A.C., J.R. Carlson, and R.G. Breeze. (1980) Indole toxicity in cattle. Vet. Rec., 107, 344.

Hammond, A.C., J.R. Carlson, and R.G. Breeze. (1980) Prevention of tryptophan-induced acute bovine pulmonary edema and emphysema (fog fever). Vet. Rec., 107, 322.

Breeze, R.G. and Carlson, J.R. (1980) Acute Bovine Pulmonary Edema and Emphysema. In "Current Veterinary Therapy: Food Animal Practice," Ed. J.L. Howard, W.B. Saunders, p. 832.

Breeze, R.G., Lauerman, L.H., Schmitz, J.A., and Magonigle, R.A. (1980) Evaluation of a long-acting oxytetracycline for treatment of Pasteurella pneumonia in calves. Bovine Practitioner, 15, 96.

Breeze, R.G., Carlson, J.R., Potchoiba, M.J., Nocerini, M.R., and Hammond, A.C. (1981) Sodium bicarbonate does not prevent acute bovine pulmonary edema and emphysema. Bov. Pract., 16, 409.

Breeze, R.G., and Gay, C.C. (1981) Plasma levels of a long-acting oxytetracycline in cattle. Bov. Pract., 16, 22.

Breeze, R.B., and Carlson, J.R. (1982) Chemical induced lung injury in domestic animals. Adv. Vet. Sci. Comp. Med., 26, 201.

Trigo, F.J., Morrison, W.B., and Breeze, RG. (1982) An ultra-structural study of canine mesothelioma. J. Comp. Pathol., 91, 531.

Laegreid, W.W., Breeze, R.G., and Counts, D.F. (1982) Isolation and some properties of equine alpha-1-antitrypsin. Int. J. Biochem., 14, 327.

Potchoiba, M.J., Carlson, J.R., and Breeze, R.G. (1982) Metabolism and pneumotoxicity of 3-methylindole, 3-methyloxindole and indole-3-carbinol in goats. Am. J. Vet. Res., 43, 1418.

Hammond, A.C., Carlson, J.R., and Breeze, R.G. (1982) Effect of monensin pretreatment on tryptophan-induced acute bovine pulmonary edema and emphysema. Am. J. Vet. Res., 43, 753.

Breeze, R.G., Magonigle, R.A., McManus, R.F., Grimson, R.E., Stilborn, R.P., and Howlett, D.D. (1982) Control of shipping fever in feedlot cattle with a long-acting oxytetracycline injectable. Bovine Practice, 3, 32.

Carlson, J.R., and Breeze, R.G. (1983) Cause and prevention of acute pulmonary edema and emphysema in cattle. In "Handbook of Natural Toxins," Ed. Keeler, R.F., and Tu, A., Marcel Dekker, pp 85-116.

Carlson, J.R., Hammond, A.C., Breeze, R.G., Potchoiba, M.J. and Heinemann, W.W. (1983) Effect of monensin on bovine ruminal 3-methylindole production after abrupt change to lush pasture Am. J. Vet. Res., 44, 118.

Nocerini, M.R, Carlson, J.R., and Breeze, R.G. (1983) Effect of glutathione status on covalent binding and pneumotoxicity of 3-methylindole in goats. Life Sci., 32, 449.

Leung, C.T., Carlson, J.R., and Breeze, R.G. (1983) Effects of mixed function oxidase inducers and inhibitors on cytochrome P-450 content, gel electrophoresis profiles and 3-methylindole-induced lung toxicity in goats. Can. J. Physiol. Pharmacol., 61, 395.

Turk, M.A.M., Breeze, R.G., and Gallina, A.M. (1983) Pathologic changes in 3-methylindole induced equine bronchiolitis. Am. J. Pathol, 110, 209.

Bayly, W.M., Grant, B.D., Breeze, R.G., and Kramer, J.W. (1983) The effects of maximal exercise on acid-base balance and arterial blood-gas tension in Thoroughbred horses. In, Equine Exercise Physiology, Eds. Snow, Persson and Rose, Blackwell, Oxford, p. 400.

Breeze, R.G., and Turk, M.A.M. (1984) Cellular structure, function and organization in the lower respiratory tract. Environ. Hlth. Persp., 55, 3-24.

Becker, G.M., Breeze, R.G., and Carlson, J.R. (1984) Autoradiographic evidence of 3-methylindole covalent binding to pulmonary epithelial cells in the goat. Toxicology, 31, 109-121.

Potchoiba, M.J., Carlson, J.R., and Breeze, R.G. (1984) Effect of energy or protein supplements containing monensin on ruminal 3-methylindole formation in pastured cattle. Am. J. Vet. Res., 45, 1389-1392.

Breeze, R.G., Laegreid, W.W., Bayly, W.M., and Wilson, B.J. (1984) Perilla ketone toxicity: a chemical model for the study of equine restrictive lung disease. Eq. Vet. J., 16, 180-184.

Breeze, R.G., Brown, C.M., and Turk, M.A.M. (1984) 3-Methylindole as a model of equine obstructive lung disease. Eq. Vet. J., 16, 108.

Breeze, R.G., Laegreid, W.W., and Olcott, B.M. (1984) Role of metabolism in immediate effects and pneumotoxicity of 3-methylindole in goats. Brit. J. Pharmacol., 82, 809-815.

Trigo, F.J., Breeze, R.G., Gallina, A.M., and Evermann, J. (1984) Pathogenesis of bovine respiratory syncytial virus infection in lambs. Am. J. Vet. Res., 45, 1663-1670.

Trigo, F.J., Breeze, R.G., Gallina, A.M., Evermann, J. and Liggitt, H.D. (1984) Interaction of bovine respiratory syncytial virus and Pasteurella hemolytica in the ovine lung. Am. J. Vet. Res., 45, 1671-1678.

Honeyfield, D.C., Carlson, J.R., Nocerini, M.R., and Breeze, R.G. (1984) Duration of inhibition of 3-Methylindole production by monensin. J. Anim. Sci., 60, 226-231.

Nocerini, M.R., Honeyfield, D.C., Carlson, J.R., and Breeze, R.G. (1984) Reduction of 3-methylindole production and prevention of acute bovine pulmonary edema and emphysema with lasalocid. J. Anim. Sci., 60, 232-238.

Carlson, J.R., and Breeze, R.G. (1984) Ruminal metabolism of plant toxins with emphasis on indolic compounds. J. Anim. Sci., 58, 1040.

Trigo, E., Liggitt, H.D., Breeze,R.G., Leid, R.W. and Silflow, R. (1984) Bovine pulmonary alveolar macrophages: ante-mortem recovery and in vitro evaluation of bacterial phagocytosis and killing. Am. J. Vet. Res., 45, 1842-1847.

Trigo, E., Liggitt, H.D., Evermann, J.F., Breeze, R.G., Huston, L.Y., and Silflow, R. (1984) Effect of in vitro inoculation of bovine respiratory syncytial virus on bovine pulmonary alveolar macrophage functions. Am. J. Vet. Res., in press.

Becker, G.M., Nocerini, M.R., Carlson, J.R. and Breeze, R.G. Alkylation of bronchiolar epithelial cells by 3-methylindole metabolites in the horse. Toxicology Letters, in press.

Laegreid, W.W., Breeze, R.G., and Gregory, E.M. (1985) Mechanism of endotoxin protection against 3-methylindole pneumotoxicity. Life Sci., in press.

Yilma, T., Breeze, R.G., and Leib, S.R. 1984. Greater sensitivity of caprine synovial membrane cells to human interferon-alpha than human or bovine cells. Am. J. Vet. Res., 45, 1809.

Yilma, T., Breeze, R.G., Ristow, S. and Leib, S.R. (1985) The immune response of cattle and mice to the G glycoprotein of vesicular stomatitis virus. Adv. Exp. Bio. Med., in press.

CURRICULUM VITAE

NAME: Jack Edward Moulton

ADDRESS: Department of Pathology
School of Veterinary Medicine
University of California
Davis, California 95616

TELEPHONE: (916) 752-7234

TITLE: Professor of Pathology

DATE OF BIRTH: [redacted]

PLACE OF BIRTH: [redacted]

Academic Training

University of Washington (Pre-veterinary Medicine), 1942-43
Washington State University (Veterinary Medicine), 1943-44
(Military Service 1944-46)
Washington State University (Veterinary Medicine), 1946-49, B.S. 1947, DVM 1949
University of Minnesota (Veterinary Medicine-Pathology), 1949-52, Ph.D. 1953

Honors and Awards:

Phi Zeta and Sigma Psi (National honor societies in Veterinary Medicine)

Professional Specialty:

Veterinary Pathology

Employment:

University of Minnesota, Instructor--1949-52
University of California, Davis, Professor - 1952-present

Professional Societies

American Veterinary Medical Association
Research Workers in Animal Diseases
Society of Experimental Biology and Medicine
New York Academy of Science
Northern California Association of Parasitologists
Veterinary Cancer Society

Consultancies: Universities Associated for Research and Education in Pathology (UAREP), 1977 food additive study.
UAREP, 1980, food additive study
FAO, 1979, Colombia, South America, African swine fever
FAO, 1979, Dominican Republic, African swine fever
FAO, 1981, Zambia, African swine fever
Council for International Exchange of Scholars, African Fulbright awards, 1982-1985

Foreign Experience (6 years):

1958-59 Cambridge University, sabbatical leave. Research on scropie of sheep.

1966-67 East African Veterinary Research Organization, Muguga, Kenya Sabbatical leave - research on ASF, CBPP

1969-71 University of Nairobi, (Director, University of California Education Abroad Program) - teaching and research (E. Coast Fever, Jaagsiekte of sheep)

1972-73 University of Nairobi, teaching and trypanosomiasis research

1981-82 International Laboratory for Research on Animal Diseases, Narrobi, Kenya - research on African trypanosomiasis and East Coast fever.

Scientific Experience:

I have been a member of the Department of Veterinary Pathology of the University of California since 1952. During this period I have participated in research projects in all phase of morphological pathology and have specialized in several areas of experimental pathology. In morphological pathology most of my research has been on the pathogenesis of infectious diseases and on animal neoplasia. I have written a book (3rd edition in preparation) on the latter subject. I have taught general and special pathology, autopsy and surgical pathology, and give graduate courses in comparative oncology and tropical diseases of animals. During the last 10 years I have worked on exotic diseases of tropical countries, including African swine fever, theileriosis, and trypanosomiasis, and have had 5 years working in Kenya. Since 1974 my total research effort has been in the field of trypanosomiasis. I have actively participated in the training of veterinary graduate students and have served on many graduate committees. My department has trained over 150 Ph.D. candidates during my tenure.

During 1981-82 I received a Fulbright Research Award for a sabbatical leave at the International Laboratory for Research on Animal Diseases (ILRAD) in Kenya. After returning to the U.S. I was asked to serve on the national committee for selecting Fulbright awards for Africa south of the Sahara. In 1986 I received another Fulbright Award, this time for teaching at the Veterinary Faculty, University of Zimbabwe. I leave in May, 1986.

PUBLICATIONS
(since 1978)

* 95. 1978 Moulton, J. E. and D. R. Stevens. Animal Model: Trypanosomiasis in deer mice. American Journal of Pathology 91(3): 693-696.

* 96. 1978 Moulton, J. E. Preparation of primary cultures of tick cells. American Journal of Veterinary Research 39: 1558-1564.

* 97. 1979 Moulton, J. E. and J. L. Coleman. A soluble immunosuppressor substance of spleen of deer mice infected with Trypanosoma brucei. American Journal of Veterinary Research 40: 1131-1133.

* 98. 1980 Moulton, J. E. Experimental Trypanosoma brucei infection in deer mice: Splenic changes. Veterinary Pathology 17: 218-225.

* 99. 1981 Moulton, J. E., C. von Tscharner and R. Schneider. Classification of lung carcinomas in the dog and cat. Veterinary Pathology 18:513-528.

* 100. 1982 Oyejide, A., E. B. Wheeldon, J. E. Moulton and J. A. Wolcott. The effect of Trypanosoma equiperdum on the lesions of experimental influenza virus pneumonia. Journal of Comparative Pathology 92:169-175.

* 101. 1983 Ikede, B. O., A. Oyejide, J. E. Moulton and J.C. Schmidt. Splenic cytology during chronic Trypanosoma equiperdum infection in deer mice: A semi-quantitative electron microscopic study. Zentralblatt fuer Veterinaermedizin Reihe A. 30:290-295.

102. 1984 Oyejide, A. and J.E. Moulton. Splenic cell immunosuppression in experimental trypanosomiasis. Immunobiology, Vol. 167, No. 1/2/3, p 221.

103. 1984 Oyejide, A, J.E. Moulton and J.A. Wolcott. Depression of serum and pulmonary neutralizing antibodies to influenza virus in Trypabosoma brucei infected mice. Transactions of the Royal Society of Tropical Medicine and Hygiene

104. 1984 Moulton, J. E., G. Buscher, D. Bovell and S. Doxsey. Blast Transformation of Adherent Macrophages infected in vitro with sporozoites of Theileria parva. American Journal of Veterinary Research. 45:678-684

105. 1985 Whitelaw, D.D., J.E. Moulton, W.I. Morrison and M. Murray. Central nervous system involvement in goats undergoing primary infections with Trypanosoma brucei and relapse infections after chemotherapy. Parasitology 90:255-268.

106. 1985 Ekejindu, G.O.C., Shifrine, M., Wilson, R.D. and Moulton, J. Chronic and acute trypanosomiasis in mice: A study by in vitro cloning of lymphohaematopoietic progenators. Trop. Med. Parasit. 36:46-52.

IMPROVED ANIMAL VACCINES

THRU BIOTECHNOLOGY

PROJECT NUMBER 936-4178

APPENDIX 5 - REGULATORY CONSIDERATIONS

5/29/86

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Nature Vol. 312:299, 1984

Vaccinia virus

WHO to back vector vaccines

Washington

A SPECIAL advisory group to the World Health Organization (WHO) has approved an accelerated programme of research on recombinant vaccines using viral vectors. Such vaccines, now under development, might be used in mass immunization programmes where alternatives do not exist or are prohibitively expensive. Though yet to be approved by WHO's regular scientific advisory group, the decision puts to rest speculation that the reintroduction of vaccinia immunization programmes might be thought unnecessarily dangerous.

Vaccinia (cowpox virus) has long been a favourite organism for virologists, being easy to culture and safe to handle. With the elimination of smallpox, its use in vaccination was discontinued, because of severe reactions, sometimes fatal, that occur at the rate of 1 or 2 per million vaccinations. Furthermore, vaccinia can be transmitted by skin contact between people and from animals, albeit rarely.

Despite some reservations expressed at a workship held last week jointly by WHO, the US Public Health Service and the British National Institute of Biological Standards and Control, the advisory group decided that the potential benefits of research into recombinant vaccines outweigh the possible risk. Protection against a number of diseases has already been demonstrated in animals using vaccinia as a vector for antigens, including hepatitis B, herpes simplex virus type 1 and rabies (see for example *Nature* 311, 67; 1984). Although the safety of these vaccines has not been systematically studied, it appears that deletion of the thymidine kinase gene of vaccinia — which is replaced by the foreign gene — may make the virus even less easily transmitted than the normal type.

In its advice to WHO, the advisory group stresses the importance of developing alternative vectors likely to be of use against diseases of the respiratory tract and enteric diseases, including bacterial, protozoal and helminthic diseases. Because much early experience will be with animal vaccines, WHO is urged to establish links with the United Nations Food and Agriculture Organization to monitor veterinary results. And to avoid countries using different strains of vaccines haphazardly, WHO is invited to set up a collaborative centre for dissemination of information and storage of reference samples, probably to be located at the National Institute for Allergy and Infectious diseases at Bethesda, Maryland. **Tim Beardsley**

United States Department of Agriculture

Animal and Plant Health Inspection Service

Federal Bldg.
Hyattsville, MD
20782

Dr. Tilahum Yilma
Department of Veterinary
Microbiology and Pathology
Washington State University
Pullman, WA 99164

DEC 2 6 1985

Dear Dr. Yilma:

In response to your request, a meeting to discuss the use of live genetically altered vaccine viruses is scheduled for 9 a.m. on January 7, 1986, in Room 1143, South Building, 14th and Independence Avenue, Southwest, Washington, D.C.

Veterinary Services is currently reviewing data submitted in support of license applications for live recombinant derived virus-vectored vaccines. Although current regulations are considered to be adequate for licensing these products, we want to assure that these products will not pose any unacceptable risk to animals, man, or the environment.

Assessing hypothetical risks associated with the use of live recombinant-derived vaccines using a live vector should be included as one of the items on the agenda. For the purpose of these discussions, we suggest using vaccinia-vectored vaccines as a model. The following are conjectoral events that need to be considered:

1. Determine the probability of proliferation and probable effects of the organism in the host.

2. Spread of the organism from vaccinated to nonvaccinated animals of the target species.

3. Spread from vaccinated target species to other animals, and potential spread to man.

4. Potential establishment of the organisms within the environment, including physical dispersal and genetic changes.

5. Plans for release of the organism by vaccination, including conditions for conducting such studies, starting with limited numbers and advancing to large field trials.

Dr. T. Yilma

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It is expected that, following this meeting, Veterinary Services will develop preliminary guidelines for authorization of field trials and for licensing these veterinary biological products.

Sincerely,

E. L. Mackey, for
S. T. Wilson, Jr.
Director
National Program Planning Staffs
Veterinary Services

IMPROVED ANIMAL VACCINES

THRU BIOTECHNOLOGY

PROJECT NUMBER 936-4178

APPENDIX 6 - International Contracts with AID

5/29/86

INFORMATION ABOUT CURRENT OR PREVIOUS INTERNATIONAL CONTRACTS

	Name of Project	Country	Time Period
1.	West Pakistan Agricultural University Development	Pakistan	1954 - 1972
2.	University of Jordan College of Agriculture Development AID-287-UNN-81-T	Jordan	1975 - 1979
3.	Eastern Islands Agricultural Education Project AID/497-0293	Indonesia	1979 - 1984
4.	Lesotho Farming Systems Research Project AID/AFR-C-1517	Lesotho	1979 - 1986
5.	Western Sudan Agricultural Research Project AID/AFR-C-1539	Sudan	1979 - 1985
6.	Dry Beans/Cowpeas Collaborative Research Support Program AID/DSAN/XII-G-0261	Guatemala Tanzania	1980 - 1987 1980 - 1987
7.	Small Ruminant Collaborative Research Support Program AID/DSAN/XII-G-0049	Kenya	1978 - 1987
8.	Jordan Valley Agricultural Services Project AID/NEB-0241-C-00-2031	Jordan	1982 - 1986
9.	Yemen Agricultural Development Support Program Library Development AID/NE-C-1698 Main Contract WSU/YE/CORE-1698-01 (WSUSC#)	Yemen	1984 - 1986
10.	Pakistan Irrigation Systems Management Project 391-01167-C-00-5044-00	Pakistan	1985 - 1989
11.	Technical Assistance to Zimbabwe Tax Commission CO-690-0206-13-C-00-5022	Zimbabwe	1985 - 1986

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APPENDIX 7 - ENVIRONMENTAL THRESHOLD DETERMINATION AND INITIAL ENVIRONMENTAL EXAMINATION

5/29/86

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MEMORANDUM TO: S&T/FA, Dr. Anson R. Bertrand

FROM: S&T/AGR, Tejpal Gill

SUBJECT: Environmental Threshold Determination

REF: Improved Animal Vaccines
Through Biotechnology - 936-4178

I have concluded that this project involves controlled experimentation exclusively for the purpose of research and field evaluation, in the form of testing on live animals in containment facilities both of which are carefully monitored. The categorical exclusion from environmental review set forth in Section 216.2(c)(2)(ii) of AID's Environmental Procedures is applicable, therefore to this project. Nevertheless, S&T/AGR has conducted an Initial Environmental Examination of this project.

On the basis of the Environmental Threshold Determination, I recommend that you make the following determination:

_X_ 1. The proposed agency action is not a major Federal action which will have a significant effect on the human environment.

____ 2. The proposed agency action is a major Federal action which will have a significant effect on the human environment, and:

____ a. An Environmental Assessment is required; or

____ b. An Environmental Impact Statement is required.

The cost of and schedule for this requirement is fully described in the referenced document.

_____ 3. Our environmental examination is not complete. We will submit the analysis no later than _____ with our recommendation for an environmental threshold decision.

Approved: _____

Disapproved _____

Date: _____

Clearance:Environmental Officer CWCollier 5/28/86
Carroll Collier

KAJones: Revised 5/28/86

Improved Animal Vaccines Through Biotechnology

INITIAL ENVIRONMENTAL EXAMINATION

PROJECT LOCATION: WORLDWIDE

PROJECT TITLE: Improved Vaccines Through Biotechnology

PROJECT NUMBER: 936 4178

LIFE OF PROJECT: Three Years

IEE PREPARED BY: Kerri-Ann Jones/in collaboration with Steve Tisa

DATE: May 28, 1986

ACTION RECOMMENDED: NEGATIVE DETERMINATION

CONCURRENCE: _____

DATE: _____

Improved Animal Vaccines through Biotechnology

ENVIRONMENTAL ANALYSIS

I. Project Description - (The following is a brief project description. An expanded discussion can be found in Attachment 1)

This project is designed to develop an improved vaccine for rinderpest, an acute highly contagious viral disease of ruminants. This vaccine will be developed using recombinant DNA technology, specifically using vaccinia virus as a vector. The product that emerges from this project will be an effective live recombinant vaccinia virus vaccine for rinderpest which is suitable for use in the developing countries. This vaccine will be tested under laboratory conditions in both the U.S. and Kenya. At the end of this project the rinderpest vaccine will be ready for large scale field testing.

There are three major areas of safety concern in this project. These are:

1) Working with rinderpest, Class 4 infectious agent - The USDA special facility at Plum Island Animal Disease Center, specifically designed for handling such diseases, will be the site of all work with the whole virus.

2) Recombinant DNA laboratory work - The laboratory procedures for laboratory work of this nature have been defined by the National Institutes of Health.

3) Testing of a live genetically engineered vaccine - Regulations of the Animal Plant Health Inspection Service (APHIS) and other appropriate U.S. agencies, international regulations as they develop, and regulations of the government of Kenya (appropriate ministeries) will be adhered to in all instances.

This project only proposes to do limited testing under isolated laboratory conditions. Therefore, it is subject to a categorical exclusion from environmental review under Environmental Procedures 22, CFR Section 216.2 (C), (2), (ii). Nevertheless, we have reviewed the project to determine whether there is a significant effect foreseeable on the human environment. The definitions used for "effects", "significantly" and "human environment" are those cited in the regulations issued by the Council on Environmental Quality under the National Environmental Policy Act. A discussion of these components of this project and our determination can be found in Attachment 2.

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Washington State University (WSU), as the principal institute, will assure that:

1) No research involving gene alterations and/or manipulation of already available altered genes will be undertaken until the experimental protocols and laboratory facilities have been reviewed by appropriate Federal regulatory agencies and/or safety review boards;

2) Appropriate U.S. Government and international quarantine regulations involving experimentation with and movement of infectious diseases will be rigorously adhered to and the project leaders will stay up to date with the appropriate agencies and regulations (e.g., APHIS and Environmental Protection Agency (EPA)) as they develop; and

As research progresses, it is expected that a live recombinant virus vaccine for rinderpest will be developed. Testing of this vaccine under laboratory conditions will follow APHIS regulations and be conducted at Plum Island. In Africa, the government of Kenya's regulations will be followed in the tests conducted at the isolation laboratory in Muguga. Facilities and procedures at Muguga will be reviewed to ensure they are adequate based on U.S. and Kenyan standards. The approval of the Ministries of Health and Agriculture of the Government of Kenya will be obtained before testing commences.

Large scale field testing is not a component of this project. However, all the necessary agencies will be kept informed of the project's progress in order that the procedures for large scale field testing can move as smoothly as possible.

II. Recommendations

From the discussion above and the analysis contained on the following "Impact Identification and Evaluation Form," it is determined that the initial implementation of this project will not have a significant direct affect on the environment. A negative determination is recommended.

Improved Animal Vaccines Through Biotechnology

ENVIRONMENTAL ANALYSIS

IMPACT IDENTIFICATION AND EVALUATION FORM

Impact Areas and Sub-Areas	Impact Identification *
A. LAND USE	
1. Changing the character of the land through:	
a. Increasing the population	N
b. Extracting natural resources	N
c. Land clearing	N
d. Changing soil character	N
2. Altering natural defenses	N
3. Foreclosing important use	N
4. Jeopardizing man or his work	N
5. Other factors:	
Reducing pesticide pollution and contamination	N
B. WATER QUALITY	
1. Physical state of water	N
2. Chemical and biological states	N
3. Ecological balance	N
4. Other factors	N
C. ATMOSPHERIC	
1. Air additives	N
2. Air pollution	N
3. Noise pollution	N
4. Other factors	N
D. NATURAL RESOURCES	
1. Diversion, altered use of water	N
2. Irreversible, inefficient commitments	N
3. Other factors	N

E. CULTURAL	
1. Altering physical symbols	N
2. Dilution of cultural traditions	N
3. Other factors	N
F. SOCIO-ECONOMIC	
1. Changes in economic/employment patterns	N +
2. Changes in population	N
3. Changes in cultural patterns	L +
4. Other factors	L +
G. HEALTH	
1. Changing a natural environment	N
2. Eliminating an ecosystem element	N
3. Other factors	
Reducing pesticide poisoning	N
Reducing nitrates in water	N
H. GENERAL	
1. International impacts	L +
2. Controversial impact	U
3. Larger program impacts	M/H
4. Other factors	N
I. OTHER POSSIBLE IMPACTS (not listed above)	
1. Introduction of new plant species	U
2. Agricultural chemicals	L +
3. Other factors	M - (Regulatory Climate)

* The following symbols are used for impact identification

N = No environmental impact
L = Little environmental impact
M = Moderate environmental impact
H = High environmental impact
U = Unknown environmental impact

\+ = Beneficial impact
\- = Negative impact

Kerri-Ann Jones:5/15/86

Attachment 1

Expanded Environmental Discussion

In the area of biotechnology regulation is one of the critical issues. It can be divided into two categories:

1) questions which arise concerning containment

2) questions which arise concerning release of genetically engineered organisms into the environment

The proposed project deals only with the first of these categories. The rinderpest vaccine will be developed and tested under contained conditions. However the second grouping of questions is important in the development of a useful vaccine, as eventually the vaccine must move out into the field. For this reason the discussions which follow below outline all of the steps required in bringing the vaccine to field. As this project develops all of the necessary agencies will be kept informed of its progress.

The project is designed to develop an improved vaccine for rinderpest, an acute highly contagious viral disease of ruminants. This vaccine will be developed using recombinant DNA technology, specifically using vaccinia virus as a vector. The product that emerges from this project will be an effective live recombinant vaccinia virus for rinderpest which is suitable for use in the developing countries. This vaccine will be tested under laboratory conditions in both the U.S. and Kenya. At the end of this project the rinderpest vaccine will be ready for large scale field testing.

There are 4 major areas of safety concering this project. These are:

1) Working with rinderpest, Class 4 infectious agent

2) Recombinant DNA laboratory work

3) Testing of a live genetically engineered vaccine

4) Deliberate release of a genetically engineered vaccine in large scale field testing.

1) Rinderpest - Rinderpest is a class 4 infectious agent requiring defined levels of containment and restrictions or handling. All work with rinderpest virus will be done at Plum Island Animal Disease center where extensive P4 facilities are available to accommodate large and small experimental animals. All challenge experiments will be conducted in a facility for work with class 4 infectious agents.

The regulations for working with an infectious agent such as rinderpest have been established by USDA and Animal and Plant Health Inspection Service. Dr. Yilma, the PI on this project has worked extensively at Plum Island with infectious animal diseases. Dr. Callis, a collaborator on this project, was director of Plum Island for 30 years. It is the responsibility of the PI in conjunction with Dr. Callis to insure that all regulations are followed.

The removal of any part of the rinderpest virus from Plum Island to laboratories in the U.S. must be approved by (APHIS). This is decided on a case by case basis. Typically testing in cell culture and live animals must be performed to insure that the viral component being removed from the island is not infectious in its own right.

The proposed site for animal testing in Africa is in Muguga, Kenya. There is an isolation facility at Muguga that has been used in previous rinderpest work. Prior to conducting research at Muguga, its facility and procedures for testing genetically engineered ogranisms will be reveiwed to ensure that they are adequate based on United States and Kenyan standards. The A.I.D. environmental coordinator will assist S&T/AGR in developing the Kenyan containment facility review team and participate in this review process. We will also obtain written approval of the appropriate ministries of the Government of Kenya before testing is commenced. If the facility in Kenya is not used, the same kind of review will be conducted regarding any other proposed facility in any other country and approval by appropriate ministeries in that country will be obtained.

2) Recombinant DNA Laboratory Work - Since the initial recombinant experiments were performed in the 1970's specific guidelines have been developed by NIH. It is the responsibility of the PI to insure that all laboratory procedures follow these regulations.

A general description of the NIH guidelines cited from GAO's report - Biotechnology - Agriculture's Regulatory System Needs Clarification, March 1986:

> The 1976 "NIH Guidelines for Research Involving Recombinant DNA Molecules" were the federal government's initial response to the concern about risks associated with recombinant DNA research. The guidelines were developed in response to concern among scientists and the general public about the potential hazards of genetics engineering. They were formalized by an NIH Recombinant DNA Advisory Committee (NIH-RAC) following an international conference held in February 1975 at Asilomar California, and attended by a group of eniment scientists. The guidelines originally were binding only on research supported by NIH; subsequently, all federal agencies that perform, fund, or regulate recombinant DNA research agreed to adopt the NIH guidelines and to require compliance by federally funded researchers doing work involving recombinant DNA. An agency can encourage the compliance of researchers who receive federal funds by threatening to cut off such funds in the event of noncompliance. The guidelines do not cover privately funded research but contain a section encouraging voluntary compliance by industry.
>
> The guidelines provide an administrative framework that specifies the responsibilities of the scientists, their institutions, and the federal government. For each institution the key organization is the Institutional Biosafety Committee (IBC), comprising experts in relevant scientific disciplines and lay persons representing the public interest. More than 300 IBCs throughout the nation oversee recombinant DNA research

The guidelines also classify experiments into three categories: (1) those requiring special reivew by the NIH-RAC, (2) those requiring containment of potentially harmful organisms and review by the IBC, and (3) those exempt from special NIH-RAC and IBC review. The NIH-RAC reviews experiments in the first category to assess risk and assign an appropriate level of containment. Those in the second category must be conducted in accordance with previously established containment standards that become more rigorous as the level of perceived hazard increases. These standards specify physical types of containment relating to special laboratory features and biological types of containment relating to the deliberate weakening of experimental organisms to reduce their chance of survival outside the laboratory. According to USDA, compliance with these standards is ensured by the IBCs at the local level.

The third category of experiments-those that are exempt from NIH-RAC and IBC review-has grown to the point where it now represents about 80 to 90 percent of all recombinant DNA experiments. Research since 1976 has led to less concern among many scientists about the risks of recombinant DNA research and, as a result, there has been a gradual but substantial relaxation of original containment requirements and oversight mechanisms through a series of formal revisions in the NIH guidelines.

Three limitations of the NIH guidelines, however, have caused more people to be concerned about their adequacy in terms of regulating the biotechnology industry: they (1) apply to nonfederally funded research on a purely voluntary basis, (2) do not apply to organisms created by genetic engineering techniques other than recombinant DNA, and (3) do not adequately address the issue of deliberate release of genetically engineered organisms into the environment. (Orginally, they simply prohibited such releases, but more recently they were changed to require special review under category 1.)

3) Small Scale Contained Animal Testing - Once the genetically engineered vaccinia virus rinderpest vaccine is developed it will be necessary to conduct tests in live animals. These animals will be inoculated with the new vaccine and then challenged with rinderpest virus. Such tests have two components which are safety issues: 1) the use of the recombinant vaccine and 2) the use of the infectious virus rinderpest. Point 2 has already been discussed in the section on rinderpest. All of these tests will be conducted in facilities which meet the specified regulations - Plum Island and the Muguga facility.

The use of the recombinant vaccine will follow the regulations set forth by APHIS which is currently the agency with jurisdiction over animal diseases. At the writing of this project paper, APHIS is in the process of developing defined procedures. If the proposed procedures are accepted the Institutional Biosafety Committes (IBC)will be responsible for approving the small scale field testing as defined in this project.

4) Deliberate Release of a Genetically Engineered Organism - This is the final step in the testing process of the new vaccine. It requires the administration of the vaccine in an open system-i.e., released into the environment. This is the most controversial step in the process as there is not a data base of risk assessment.

The proposed APHIS regulations indicate this step is the most controlled. A complete environmental assessment fully reviewed by APHIS is required before any testing can be initiated.

Kenyan Government - In all of the above regulatory issues the approval of the Government of Kenya ministries of Health and Agriculture and other appropriate African institutions (e.g., OAS-Organization of African States) will be obtained.

Principal Investigator - It is the responsibility of the PI to insure that appropriate regulations regarding facilities and procedures be followed throughout this project.

A.I.D. - It is the responsibility of the project manager in collaboration with environmental officers to monitor the project in regards to all regulations.

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IMPROVED ANIMAL VACCINES

THRU BIOTECHNOLOGY

PROJECT NUMBER 936-4178

APPENDIX 8 - National Science Foundation
Review and Recommendations

5/29/86

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Proposal on "An Infectious Vaccinia Virus Recombinant Vaccine for Rinderpest", T. Yilma Principal Investigator.

I find it hard to imagine a proposal on vaccine development that would have a higher chance of success than this one. Drs. Yilma, Moss, Callis, etc. have the backgrounds that are necessary to insure that an effective recombinant vaccine will be developed and be used in an effective way. There is no reason to think that the techniques that Dr. Moss has used successfully in the past to incorporate genes for other antigens into vaccinia virus will not work in this case. The other scientists involved have excellent training and experience in molecular biology and/or vaccine applications and all should be able to collaborate in producing and testing an effective vaccine.
If all that is proposed can be carried out with the $917,858 requested the AID will be getting a bargain. I recommend funding with the highest enthusiasm.

Philip D. Harriman, Ph.D.
Program Director, Prokaryotic Genetics
National Science Foundation.
May 15, 1986

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