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AN EVALUATION OF THE PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION (PARFR)

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I. INTRODUCTION AND BACKGROUND

Purpose of the Evaluation

This was an in-depth evaluation to assess the accomplishments, future directions and functions of the Program for Applied Research on Fertility Regulation (PARFR), a component of USAID's Population Research Program. The Evaluation Team (the Team) was instructed to consider a variety of different topics. Among the problems and issues to be assessed were the variety of current PARFR projects, the research priorities, the Scientific Advisory Committee, PARFR staff and facilities, the workshop/symposia, training and information dissemination, project planning, funding level, reporting to AID and relationships with other efforts in the field of contraceptive development.

The present evaluation came out of the recommendation of the previous site visit report which was written in 1980. That report suggested that another review of the PARFR program should be made two years after the 1980 site visit and every three years thereafter.

Composition of the Team

Members of the evaluation team were:

1. Michael J.K. Harper, Ph.D., Sc.D. (Chairperson)
   Department of Obstetrics and Gynecology
   The University of Texas Health Science Center at San Antonio
2. D.J. Patanelli, Ph.D.
   Contraceptive Development Branch
   National Institute of Child Health and Human Development (NICHD)
   National Institutes of Health

3. David F. Archer, M.D.

Department of Obstetrics and Gynecology

University of Pittsburgh School of Medicine

Jeffrey M. Spieler, the PARFR Technical Monitor, represented AID, Office of Population, throughout the evaluation.

Approach and Methodology

The visit was made on June 13-17, 1983 and took place at two locations. On June 13 and 14, a site visit was made to three major subcontractees in Birmingham, Alabama: Southern Research Institute, Stolle Research and Development Corporation and the University of Alabama in Birmingham Medical School, Department of Obstetrics and Gynecology.

On June 15-17 the team went to the PARFR offices in Chicago, Illinois to meet with the PARFR staff, and three other subcontractees who were working on different aspects of contraceptive research under the PARFR program and who presented the current status of their research. In addition to these visits, Dr. Patanelli was an observer at the March 28, 1983 SAC meeting and Dr. Archer attended the PARFR Workshop on Long-Acting Contraceptive Delivery Systems in New Orleans, May 31 - June 3, 1983.

The various individuals with whom discussions were held during the evaluation were as follows:

1. Southern Research Institute in Birmingham, Alabama

   Richard L. Dunn, Ph.D.

   Thomas R. Tice, Ph.D.

   Donald R. Cowsar, Ph.D.

2. The University of Alabama in Birmingham

   Charles E. Flowers, Jr., M.D.

   Lee R. Beck, Ph.D.

   Valerie Z. Pope, M.S.

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3. Stolle Research and Development Corporation

Danny H. Lewis, Ph.D.

A complete listing of the projects being undertaken by these various individuals is attached as Appendix I of this report entitled "Current PARFR-supported Research at Southern Research Institute, Stolle Research and Development Corporation and the University of Alabama in Birmingham."

At the PARFR headquarters in Chicago, the Team met with:

1. Lourens J.D. Zaneveld, D.V.M., Ph.D.
   Rush-Presbyterian-St. Luke's Hospital
   Chicago, Illinois
2. Erwin Goldberg, Ph.D.
   Northwestern University
   Evanston, Illinois
3. Robert T. Chatterton, Ph.D.
   Northwestern University Medical School
   Chicago, Illinois

In addition, the following participants from PARFR were present:

1. John J. Sciarra, M.D., Ph.D.
   Program Director
2. Gerald I. Zatuchni, M.D., M.Sc.
   Director of Technical Assistance
3. Alfredo Goldsmith, M.D., M.P.H.
   Head, Research Project Development
4. Diane Krier-Morrow, M.B.A.
   Director of Administration

Project Background

The program for Applied Research on Fertility Regulation (PARFR) is a division of the Department of Obstetrics and Gynecology of Northwestern

University Medical School. PARFR is mainly funded by the United States Agency for International Development (USAID). PARFR provides scientific, technical and financial assistance to United States and foreign institutions for applied research projects aimed at developing safe, effective and acceptable methods of fertility regulation. A primary interest of PARFR is to encourage applied research activity in developing countries either through foreign-based projects or as collaborative efforts between United States and foreign institutions.

Specific research areas which can be supported include laboratory studies, animal studies and human investigations leading to the development of contraceptive methods that:

Interfere with the maturation process of sperm or ovum either at the local or central level;

Interfere with the transport of gametes;

Interfere with the process of fertilization;

Provide for long-acting, sustained release of antifertility agents.

Below are examples of prior and current projects:

Biodegradable contraceptive steroidal-release systems

Intrauterine delivery systems

Intravaginal chemical and barrier methods

Pharmacological methods for male contraception

Neuropeptides for male or female contraception

Pharmacological, mechanical and surgical methods of male and female sterilization

Reversible occlusive devices for male and female sterilization

Both solicited and unsolicited research proposals can be submitted to PARFR either as pilot studies at minimal funding (maximum of $10,000 per

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year); informal proposals or formal proposals (maximum support of $66,000 per year). Pilot studies are intended to provide the investigator with initial funding to accumulate sufficient data for submission of a formal proposal to PARFR. Informal proposals are written with a brief statement of the objectives, protocol, expected end results and budget. When informal proposals meet PARFR objectives, investigators are requested to submit formal proposals.

Proposals are reviewed by PARFR's Scientific Advisory Committee, a committee of 12 experts in the field of reproductive science and clinical fertility regulation. When a proposal is approved, PARFR's funds are provided through a cost-reimbursable subagreement. PARFR staff and the Scientific Advisory Committee continually monitor the progress of all projects, including site visits as indicated.

II. EVALUATION

The objectives of the PARFR program were reviewed by the Team. It was felt that these objectives were perfectly appropriate and that PARFR is accomplishing its objectives of developing contraceptive modalities that will have use in developing countries. The dissemination and review of research findings are being accomplished through PARFR organized workshops and symposia, as well as the distribution of this material through a mailing list of approximately 6,000 names.

Administrative Organization

The first item to be reviewed was the administrative organization of the PARFR program. The Team was told that Dr. Sciarra spent about 10% of his time directing the program and that Dr. Zatuchni was involved 80% and Dr. Goldsmith 90% of the time. Diane Krier-Morrow devoted 100% of effort to the administration of this program. The Project Controller and secretaries all

devoted 100% of effort to the program. A publication coordinator was utilized on a contract basis to deal with specific publications.

The Team was impressed with the staff and facilities of the present PARFR program. In particular, it noted with pleasure that the program now had adequate space to carry out fully the program activities. Dr. Zatuchni felt that the present staff was adequate at this time to carry out all the ongoing activities. However, he did indicate that as more projects progressed to the clinical trial stage, specifically Phase II and later, additional staff would be required. This need would probably occur within the next year. *The Team concurs with the recommendations of the previous site visit team that, when additional professional staff are hired, they should have expertise in the clinical trial area.*

One of the major deficiencies that the program has at this time is the ability to store, collate and retrieve information with regard to ongoing projects, clinical trials, site visits, financial expenditures and program management. The Team recommends very strongly that monies be expended to purchase a mini-computer and to hire (on a part-time contract basis) a programmer to develop the appropriate software necessary for implementation of these recommendations. The Team feels that the present staff are competent, organized and dedicated in carrying out their duties in a very commendable fashion, and that the computer will increase their efficiency.

The Team was gratified to see that, in terms of its role and objectives in the delivery of contraceptive modalities to lesser developed as well as developed countries, there was an excellent interaction between the PARFR administration and the Contraceptive Development Branch of the National Institute of Child Health and Human Development, as well as the World Health Organization, the Population Council and Family Health International. This Interagency dialogue promotes the objectives described above, in addition to

preventing unnecessary overlapping of areas of responsibility and investigation.

PARFR also has an ongoing relationship with private industry, particularly seeking out private sector financial support for projects as they begin to move into the Phase I area of clinical testing. PARFR also is actively soliciting private sector interest for research efforts that appear to be promising while still in the developmental stage.

The Team, based on the recommendations of the prior evaluation, notes that PARFR-supported projects are now being published in scientific peer-reviewed journals, but is unable to determine whether or not the Proceedings of Workshops are indeed being listed in Index Medicus and other reference sources as recommended.

Scientific Advisory Committee (SAC)

The Scientific Advisory Committee may comprise up to 12 members and a Chairman. At this time there are only 10 outside members. The Team reviewed the list of present and past members of the Scientific Advisory Committee. The previous evaluation team had expressed concern about several aspects of the selection of members for the SAC and this remains of concern to the present team. The previous evaluation team had recommended that the term of office of the SAC members should be limited to three years, that no member should serve consecutive terms, and that there should be an interval of at least two years before an individual could be reappointed to the SAC. The present Team are in general greement with these recommendations, but feel that these could be made slightly more flexible. *The Team recommends, therefore, that an individual should be appointed to the SAC for an initial probationary term of one year during which time his performance, interest and expertise can be evaluated. If this individual proves to be satisfactory, he can*

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then be appointed for a further two years. At the end of the initial three-year term, his membership can be extended under special circumstances on a year-to-year basis for an additional two years, up to a maximum of five years. The Team recognizes that there may be a lack of knowledgeable, competent and committed individuals in some areas. Therefore, under special circumstances, such a person can be reappointed to the SAC after an interval of at least two years when he is not a member. *The previous team had recommended that the new SAC members should not receive funds from PARFR at the time of appointment, nor be eligible for contracts while serving on the SAC. The present Team is in general agreement with this recommendation, but feels that under exceptional cicumstances a SAC member should not necessarily be barred from undertaking a* <u>*pilot*</u> *project.* However, if such a pilot project gives rise to a full scale formal research proposal, then that member should resign from the SAC at the time the proposal is submitted to PARFR.

The previous Team had recommended that PARFR broaden the areas of expertise of the individuals on the SAC. It would appear that indeed the PARFR staff have moved in this direction, but the present Team still feels that certain disciplines are lacking — for example, a biostatistician, individual experts in clinical trial methodology and execution (other than the PARFR staff themselves), and a polymer chemist — and recommends that such individuals be added. While the Team does not feel that there is any evidence of conflict of interest, it was noted that at least one institution not only had representatives on the SAC, but also that other individuals from the same institution were recipients of project support from PARFR. Such a practice could give rise to a real or perceived conflict of interest and the Team recommends that PARFR try to eliminate, as much as possible, such potential conflicts. *As a minimal requirement, the Team recommends that individuals*

from an institution absent themselves from discussions concerning projects originating from their own institution, even though they are not principal investigators.

The previous evaluation team had recommended that the Chairperson of the SAC should not be an administrative member of PARFR. The present Team considered this question quite carefully, but were of the opinion that Dr. Sciarra was indeed an excellent chairman of the SAC and his leadership was commended. *However, the Team recommends that Dr. Sciarra should not vote on the actual projects, although he can continue as Chairman of the SAC.* Another area of concern was the practice of PARFR staff members, after they had described the various projects to be considered by the SAC, indicating their recommendations as to approval or disapproval before the members of the SAC had discussed them. *The Team therefore recommends that, in the future, administrative staff of PARFR present the individual projects, placing them in perspective with regard to their total program and possibly also pointing out the strengths and weaknesses in the protocols, but reserving any indication of their own recommendations as to funding until after the SAC members have had a full discussion on the individual project.*

After a lengthy discussion the Team is of the opinion that the SAC could more fully be involved in certain areas of strategic planning of the PARFR program. *It appeared that it was not the usual practice for the SAC to be involved in discussions of strategy, and the Team recommends that, in the future, a portion of the SAC meeting be devoted to a brain-storming session at which the SAC members would be encouraged to discuss new lines of research, to re-evaluate priorities of ongoing lines of research and to suggest potential new areas and investigators that might be of interest to PARFR and AID.*

PARFR uses special consultants for site visits and other technical matters. In some cases, it appears that principal investigators, particularly in the case of multi-center clinical trials, are often used as these special consultants. *The Team recommends that, in general, the PARFR administrative staff should either go by themselves or accompany the principal investigator on such site visits, but that principal investigators can continue to be used for purely technical visits.* The use of special consultants to review particular projects which fall outside the realm of expertise of the members of the SAC is a useful practice and should be continued.

Proposals

The various mechanisms employed for obtaining proposals (informal proposals, formal proposals and pilot studies) were reviewed and the Team feels that these are perfectly appropriate. There was some concern as to the practicality of obtaining worthwhile unsolicited proposals by advertising. Nonetheless, PARFR should, on at least a yearly basis, advertise in widely distributed international journals, as well as locally distributed national journals, e.g. the Singapore Journal of Obstetrics and Gynecology.

It was noted that the present limitation for funding a formal research proposal is a total of $66,000 per year. Since this includes an overhead component, the actual funds available for research are limited. *In view of recent inflation, the Team recommends that this limit be raised to $100,000 per year. This would more nearly reflect present day realities of the cost of undertaking research, especially in developed countries.*

The Team was told that owing to certain university administrative decisions, it was very difficult for PARFR to advance money to investigators to initiate research work on a contract. *There are many instances where it is essential to purchase expensive animals and/or supplies or hire necessary*

personnel at the beginning of the work. For some investigators without other funds this can prove impossible. Therefore, the Team recommends tht PARFR negotiate an agreement with the University's Accounting Department, whereby up to 25% of the total contract amount be advanced for such purchases, when necessary.

Institutional Review Board (IRB)

It was pointed out that, because of the unusual interpretation of liability by the attorneys at Northwestern University, all clinical proposals, even if they had been reviewed and approved by a DHHS approved IRB, still had to be reevaluated and approved by the Northwestern University IRB. This has created hardship in terms of delay in implementation, and has caused considerable additional expense and inconvenience to investigators, especially when consent forms already approved by their own institutional IRBs had to be returned for some minor amendments requested by Northwestern University. *It was pointed out that there is no Federal Regulation which requires double review by two separate IRBs. Therefore the Team strongly recommends that AID consult directly with the administration and attorneys of Northwestern University to see whether this situation might be resolved, so that the approval by a DHHS-approved IRB could be accepted in its entirety without the need for additional review.*

Research

The Team noted that the major research areas of activity supported by PARFR were as follows: female sterilization, male sterilization, intrauterine contraception, systemic contraception, barrier contraception and miscellaneous. During the last two-year funding period, the support for female sterilization, male sterilization, barrier contraception, and miscellaneous had declined while that for systemic contraception and

intrauterine contraception had increased. This reflects the fact that these latter two lines of research are reaching the stage where products are undergoing clinical trials. The Team feels that the allocation of funds to the various areas of research seems to be appropriate. Indeed the Team congratulates the PARFR staff on the excellent progress that certain lines of research have made since the last evaluation. Assessment of these various lines of research suggests that most of them are likely to give rise to projects which will ultimately be suitable for developing country use. In general, the mix of projects with short, medium and long-term focus is reasonable. At this time certain new initiatives are being planned in the field of immuno-reproduction, which will be long-term and these are discussed below.

Research priorities. It was not entirely clear to the Team exactly how the research priorities are set. In some cases, it appears that the PARFR staff decided on priorities and initiated action, and in other cases AID staff members had considerable input as to which lines of research should receive priority. It was, however, clear that the SAC was not really involved in setting research priorities. Obviously, priorities for various lines of research are established and indeed changed from time to time depending on the stage of development of the particular research activity. For example, it now seems that the biodegradable microcapsule systems have — and should have — the highest priority in the program. *However, the Team recommends that PARFR settle in-house, with the help of the SAC if this seems useful, exactly what their priorities are so that this information can be presented to AID with the financial requirements for each individual year's funding.* Thus, if funding is only adequate to support some lines of research, it will be quite obvious which ones would have to be dropped or eliminated. (See comments under heading Financial below.)

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As the PARFR program was originally constituted, the "wide-net approach" of funding any reasonable applied research lead was indeed appropriate. However, at the present stage when several well-defined projects are reaching the clinical trial stage, it seems much more useful to concentrate on pushing these items through the "pipe-line" as fast as possible. New items should be added to the list of ongoing projects only if they are well defined and obviously relevant to PARFR's mission. As mentioned above, the Interagency dialogue in which PARFR is now involved has allowed PARFR to be cognizant of any developments in new areas and progress in other areas in which PARFR is currently involved, and this has clearly avoided duplication in some lines of research. It would seem that at this time, all high priority areas are being pursued with maximal effort. Some of the research lines with lower priorities may not be progressing with the most desirable speed or may be being rushed prematurely. When questioned, PARFR staff indicated that no high priority area of research was being compromised by the fact that additional lines of research were being undertaken. For example, in the biodegradable long-acting systems, the initiation of work on the supplementary systems with estradiol or progesterone was not interfering with the fastest possible progress on the 90-day NET microcapsule system. It was noted that certain lines of research were in their final years of funding, and either were going to be continued with commercial support, or if the results were negative, abandoned entirely. These included, among others, the MCA/FEMCEPT system, the acrosomal enzyme inhibitors and the Shug device. The Team was not apprised exactly how many projects or project lines had been phased out during the past three years, but, in general, it was the impression that most of the projects under investigation at this time seemed appropriate. Where the team had one or two specific concerns about projects, these will be addressed later in this report.

Using the number of informal proposals as one method of assessing how many unsolicited proposals were approved, it would appear that only 13% of informal proposals were approved. This would suggest that relatively few unsolicited proposals were being accepted. The Team did not feel that this was a serious concern since, in fact, there are relatively few new leads available for development. In the same context, it does not seem that any major opportunities have been missed.

During the site visit, certain lines of research were discussed in detail with the Evaluation Team. These are: the biodegradable systems, the Shug device, the acrosomal enzyme inhibitors and the status of research on the sperm enzyme LDH-C_4 and its utility for use as the basis for a contraceptive vaccine. In the section that follows, the specific comments that the site visiting team have about these various areas of activity are set out.

Biodegradable Steroidal Delivery Systems

The various microencapsulation projects that are under development are shown in Appendix II headed "Summary of Microencapsulation Project with PARFR Involvement, June 13, 1983." There are six microencapsulation projects under development. Three of these are with norethisterone (NET). These are a 30-day, a 90-day and a 180-day system. There is a 90-day Norgestrel (NOG) and a 90-day system for delivering estradiol or progesterone. The first four systems will be contraceptive in their own right. The 90-day progesterone system is to be specifically used in postpartum lactating women, while the 90-day estradiol system is being developed so that if bleeding problems arise with the various NET or NOG systems, then addition of the microencapsulated estradiol can be utilized to alleviate or eliminate the bleeding. The estradiol microencapsulated system might also be used as an adjunct to therapy with LHRH agonists for inhibition of ovulation. PARFR is to be commended for

the fact that they have been involved in the development of this system at a very early stage, and are actively collaborating with other agencies. It would seem that these particular microencapsulation systems are probably further developed and more advanced than other biodegradable polymer systems at this time. All the various lines of investigation under development are highly appropriate and should be pursued vigorously. The visit to Southern Research Institute in Birmingham, Alabama confirmed that the technology for delivery of steroids is well advanced, and that it is a very simple matter now to tailor a new microcapsule system to delivery any steroid that may be selected by the program. The Team was informed that although PARFR is not involved, there also is a project underway to microencapsulate testosterone for long-term administration. Such a development could be of great use for male contraceptive systems where replacement androgen therapy will be required. The Team commended PARFR staff and their contractors for the speed with which they have managed to undertake and complete the Phase I trials with the 90-day NET system. Phase II trials are anticipated to commence within the next three months and, given the results of the Phase I trial, there is every reason to believe that these will be satisfactory.

It was previously noted that pharmaceutical companies and private interests were involved at an early stage in the development of these contraceptive systems. The involvement of Stolle Research and Development Corporation seems to have had a very beneficial effect on the progress of this whole area of research. As a result of their involvement, a new two-step process has been developed which greatly improves the efficiency of loading of the microcapsule systems. Exploitation of the polymer technology is being systematically explored. Certain types of polymer fibers have been developed which also release steroids at predetermined rates and can be produced very

cheaply. Use of these fibers is envisioned in being wrapped around the vertical and horizontal arms of an IUD — indeed, even the whole IUD could be constructed out of the fibers — so that estradiol and progesterone can be released into the uterine cavity. In addition, a project is underway to wrap vaginal rings with these fibers as a means of delivering steroids to the vaginal mucosa. These projects are at a somewhat earlier stage of development than the microcapsules, but because of the ease of changing the amount of steroid delivered per unit time by shortening or lengthening the fiber, such technology has a particularly promising future. Because of the cheapness of the manufacture of such fibers, enough fiber to produce large numbers of IUDs or vaginal rings could be spun in a short period of time. The public sector rights in these developments seem to be adequately protected. However, the Team was not given the information to determine exactly how many patents have been applied for as a result of PARFR-supported research.

Acrosomal Enzyme Inhibitors

This work was presented by Dr. Zaneveld and was based on various chemical compounds related to nitrophenol guanidinobenzoate (NPGB). In these new compounds, the nitrophenol grouping had been replaced by various other phenols. Of these compounds, the ones that showed most promise were methylumbelliferone and methylsalicylate. Primate studies with these compounds will be supported by PARFR and will start within six months. However, it was noted that only one compound at one dose would be able to be tested. It is not clear that this study will provide sufficient evidence to permit extension or termination of this area of research. The PARFR decision is that, if the results from this primate study are negative, this line of research will be dropped. Commercial involvement might be forthcoming if the results are positive. In view of the long involvement of PARFR in this

particular program, the Team feels that cessation of funding in this area if the results are negative is probably advisable.

The second area that Dr. Zaneveld presented was the so-called "Shug device," which is an intravasal blocking device. Dr. Zaneveld pointed out that the advantages of this particular device over previous vas plugs were: 1) it was comprised of two plugs instead of one; 2) it was much longer than other types of plugs and, therefore, even though it was not held in place by tissue ingrowth, it was likely to be more effective; and 3) it was maintained at the point of insertion by the fact that the thread joining the two plugs lay outside the vas. Now that a new and modified design has been developed, retrieval of the plug is also easier. Patent applications on this device are pending and it was noted that a commercial company is also involved in its production and development. Dr. Zaneveld informed the team that Phase I trials were planned to start in August 1983 with this device. The Team feels that this is an interesting development that should be pursued and is pleased to see that Phase I trials are being undertaken as rapidly as possible. It is recognized, of course, that intravasal plugs have had a long history of failure and that such failure is always a possibility with this new device.

A final area of research which Dr. Zaneveld mentioned involved the development of quinacrine pellets. He noted that optimal results might be achieved by combination of a slow release (7-day) plus a quick release (24-hour) pellet. This combination of pellets placed in the uterine cavity might produce a better occlusion of the tubal isthmus than pre-existing types of quinacrine pellets or quinacrine suspensions. It is planned to test these new pellets in cynomolgus monkeys in the near future. Pellets of tetracycline are also being developed with a view to similar testing. *The Team is not*

enthusiastic about this approach, given the long history of less than satisfactory occlusion produced by quinacrine when delivered in a variety of different modalities, and recommends that large amounts of money should not be expended on development of this line unless startlingly improved results are achieved.

Anordrin

Dr. Chatterton presented a summary of his recent work with Anordrin, a steroidal compound first developed by Drs. Pincus and Jacques, and more recently, by the Chinese. Dr. Chatterton noted that the most extensive article on this compound was one by Chen and Chen in the Chinese Journal of Obstetrics and Gynecology which he has had translated into English. In Dr. Chatterton's studies, Anordrin is used in a rather different modality than previously. It is being used as an ovulation-suppressing agent, whereas previous studies have concentrated on its use as a luteolytic, post-coital, or vacation-type pill. The most striking finding from his studies is that a single dose of a relatively modest amount of this compound (0.1 or 0.2 mg/kg) given on the first day of the menstrual cycle in cynomolgus monkeys causes inhibition of ovulation and an extension of the follicular phase of the cycle. Large doses of this compound (4 mg/kg early in the cycle) delayed menstruation in these monkeys for up to 4-5 months. With the lower doses, the follicular phase of the cycle was still extended to 40-50 days. The luteal phase of the cycle was claimed to be shorter and pregnanediol levels lower than in normal cycles. The evidence on this point was somewhat equivocal.

If the results reported are indeed correct, this is a most striking finding and seems to contradict previously published data on the effects of Anordrin in humans. One of the disturbing features about the proposed program with this compound is the proposal to expend some $200,000 over the next year

for toxicological and other primate studies without adequate knowledge of the patent situation of this compound. The original patent, if still in force, may not yet have expired. In addition, Dr. Chatterton had not even envisioned doing any efficacy studies in mated monkeys, nor was he set up to do such studies. The Team feels that it is premature to undertake such extensive toxicological studies when it is not even known if this peculiar ovulation-suppressing activity does indeed occur in the human. *The Team recommends that, since Anordrin is widely used in the People's Republic of China, and since PARFR has good relations with the World Health Organization and its clinical network, WHO and/or PARFR should be encouraged to approach Chinese or other non-US clinical centers to determine whether a small Phase I study could be done. Specifically, this study should evaluate whether a small dose of Anordrin given on the first day of menstruation does indeed suppress ovulation and, if so, for how long.* Such studies would, of course, involve all the usual hormone assays. This was a major deficiency of the present monkey studies. Until recently, samples were not being evaluated for changes in luteinizing hormone (LH), follicle stimulating hormone (FSH) and estradiol levels. Such information would be extremely valuable in evaluating the mode of action of Anordrin in inhibiting ovulation. If such a human study can be arranged and if the results are positive, then, of course, a major effort should be undertaken to determine whether or not Anordrin could be developed as an ovulation-inhibiting drug. If the results are negative, the proposed work with Anordrin should be dropped.

Immunoreproduction

Dr. Goldberg then presented a synopsis of his recent work with the sperm enzyme, LDH-C_4. This enzyme appears to be mainly restricted to the tail piece of the sperm as evidenced by immunofluorescent studies. His experiments

indicate that, when peptide fragments of one of the sub-units of this enzyme are used as an antigen, following conjugation with an appropriate protein, such as diphtheria toxoid or tetanus toxoid, antibodies are produced which cross-react with the whole native enzyme. In vitro fertilization tests with such antibodies suggest that fertility can be reduced, but never to zero. It is not at all clear that the particular peptide or peptides under study are the most appropriate or the most useful. This is a very long-term program and there is much basic information still to be gained and, if money is a constraint for the PARFR program, then this particular approach is one that should have lesser priority than, for example, the biodegradable delivery system. If, however, new approaches to contraception are urgently desired, then this area of immunoreproduction, at least, provides a new approach. A contraceptive vaccine would, of course, have a significant impact on contraceptive practices.

The decision whether to continue funding of an essentially long-term project should be made by AID and PARFR after mutual discussions. Major imponderables in this whole area — and these apply to all approaches to development of a contraceptive vaccine — include what antibody levels are required to induce absence of fertility, what immunization schedules will give the best response, what are the most appropriate adjuvants to be used, which is the best protein to use as the conjugate and how long the increased antibody levels can be expected to be maintained. All of these problems require considerable amounts of basic research and are unlikely to be solved in the near future. A commercial company has expressed a modest interest in the LDH-C_4 approach and is providing a small amount of support, presumably with the idea of having first option of licensing any product that should emerge in the future. One of the problems that is faced by Dr. Goldberg is

the lack of a good biologist to assist him in his work. He has funding for a peptide chemist and an immunologist, but the existing biologist who was working with him has left, and this probably has slowed the progress of this particular area of research.

The Team discussed the viability of a new initiative in the area of immuno-reproduction, especially in context of certain Indo-US interest. The Team strongly recommends that advice and consultation be provided by bona fide immunologists prior to the initiation of such a program.

Future Planning and Project Planning

The PARFR staff supplied the Team with a first approximation of a flow chart developed by Dr. Forrest Greenslade. This chart outlined the various steps needed to develop the Poly NET-90-Day System. This chart indicated that it was hoped to have all the necessary data available for an NDA submission in mid-1986 for this system. *The PARFR staff reported that Dr. Greenslade's flow chart was going to be improved and made into a proper PERT chart or critical path map, and that similar charts were going to be developed for each of the other major lines of research. This was felt to be a very commendable and useful exercise. The Team recommends that the PARFR staff accomplish this as soon as possible.* Such charts will be extremely helpful in the forward planning of the different activities to be undertaken and the determination of the times at which such activities should start. *The Team strongly recommends that these charts show the estimated duration of each phase of the developmental process and an estimate of the anticipated target dates for start and completion of the actual activities. The estimated cost for each phase should also be identified, since this will be required for financial projections which are discussed later.* Such charts would enable the PARFR and AID program staff and the SAC to make a better overall assessment of the development of the various lines of research. Such a breakdown into a more

precise time and cost estimates would lend greater credibility to the future budget projections made by PARFR.

Twenty-four different projects were listed as targeted research for the next five-year period. However, the Team was disappointed that the PARFR staff had not attempted to list these in order of priority for development. The Team feels that it would be much more useful, not only for the Team, but also for use by others (AID staff, etc.) if such targeted research areas were listed by priority, so that if some had to be eliminated, then it would be immediately obvious which they should be. Consideration of the importance to the success of the program of determining which projects are to be continued and which are to be phased out and what are the steps to be undertaken in a timely manner leads to the conclusion that the efforts put into the development of the listed targeted research projects was minimal. *The Team recommends that, in the future, all tabulations of projected activities be given careful thought and consideration, and be laid out in much more extensive and informative fashion, being broken down by year and listed by order of priority.*

Workshops

Twelve Workshops have been held since 1973, the most recent one, on Long-Acting Contraceptive Delivery Systems, held in New Orleans from May 31 to June 3, 1983. Dr. David Archer, one of the site visiting team, was present at this meeting. This workshop brought together international investigators working in the area of long-acting delivery systems for steroidal and non-steroidal contraceptive modalities. It was noteworthy that Interagency collaboration was exemplified by the inclusion of individuals who were sponsored by WHO, The Population Council, The National Institute of Child Health and Human Development — Contraceptive Development Branch and AID

sponsored agencies. The exchange of information in this area was extensive and covered both long-acting nonbiodegradable as well as biodegradable delivery systems, intrauterine and vaginal contraceptive delivery systems and an overview of the developments in polymer chemistry that have led to the expansion of the controlled release delivery systems. The meeting not only allowed for the dissemination of information among investigators, but also permitted the personal interaction necessary for the development of collaborative agreements and arrangements between individual investigators. There were 115 attendees representing 16 countries.

The 12 symposia that have been held since 1979 are also known as regional workshops because they were held almost exclusively outside the United States in lesser developed countries. These regional workshops underline and exemplify the accomplishment of one of the objectives of PARFR which is to disseminate developments in contraceptive technology to lesser developed countries. Publication of the workshop and symposia proceedings are handled by PARFR through contractual arrangements with Ms. Kelly Osborn in the Department of Obstetrics and Gynecology at Northwestern University. The interval between the workshop and publication is approximately 6-8 months, which is very commendable. The Team feels that this interval cannot realistically be reduced further at the present time.

The "Research Frontiers in Fertility Regulation" series has been published since 1980, and 3-4 issues are published per year. Overall, the titles represent a reasonable and timely overview of subjects in contraceptive areas of interest. The quality, however, is somewhat variable and the Team has some concern over the level of remuneration for the authors. *The Team recommends that there should be a contract between the author and PARFR for articles to be published in the RFFR series indicating that subquality*

material or material arriving later than the anticipated due date would result in a reduction in the remuneration. The Team feels that it might be reasonable to establish a range of remuneration between $600 to $1000. *The Team recommends: 1) a definite reduction in the level of remuneration and 2) the ability to refuse to publish a manuscript if it is felt to be of poor quality.* This publication is mailed to 6000 individuals. It was not entirely clear as to how the mailing list of 6000 names was generated. The Team is concerned that young investigators who would be interested in working in the area of contraceptive research and development might not be apprised of potential opportunities and funding. *The Team recommends, therefore, that additional efforts be made to identify such individuals and to add their names to the mailing list for PARFR publications.*

Financial

The Team feels that the current level of funding is adequate to maintain the program on the basis of the information that was supplied to them. Whether or not it is adequate to meet major new opportunities is not as clear. The Team was apprised of the fact that, if a major breakthrough were to be presented to PARFR, AID could provide additional funding. Alternatively, since PARFR should have available the priority listing of the various research lines, the decision to drop low priority items in the face of major new breakthroughs can easily be made.

The Team recommends provisions should be made in the budget for the recommendations listed above and for the addition of a mini-computer, as well as payment for the development of the program to be utilized by PARFR in computerizing their data base. It is also the feeling of the Team that the remuneration for the Director of Administration is inadequate and the Team

recommends that every attempt should be made by the AID staff to circumvent the regulatory impasse with Northwestern University, and to pay a salary commensurate with the responsibilities of the job. The Team also understands that some branches of the U.S. Government (e.g. NIH) pay a physician incentive bonus in addition to salary. It seems reasonable to the Team that this bonus should be made available to relevant PARFR staff.

As noted above under Project Planning, it is important that PARFR list their budget by line item and estimate the costs through the next five fiscal years for each line of research that is ongoing, or projected to be brought on line, within this timeframe. Projections for 5, and even 8, years into the future are appropriate to allow the funding agency, as well as future evaluation teams, to assess more realistically the cost estimates for the program. *It should be noted that, even though AID staff in attendance with the Evaluation Team stressed that future funds would be made available, there is no guarantee as to the actual level of funding available for any particular year. This makes forward planning more uncertain, and since many projects are nearing phase I and II trials, the Team recommends that these funds be made available at the budgeted level and in a timely manner for the program.*

It was noted that any shortfall in funds promised, but not paid, for a particular year, had the consequence that the funds actually available for research were significantly diminished since fixed costs such as salaries, fringe benefits, etc. could not be reduced in short order. Coupled with inflation, such reduction leads to much greater diminution in the amount of available research funds than would appear on the surface. The percent of total funds spent in developing countries has been approximately 25% to 35% of the total expenditures. This item has not varied over the last few years, and it is not anticipated that it will be reduced in the foreseeable future.

Reporting of PARFR Activities to AID

The Team recommends that submission of semi-annual progress reports of research activities should be continued. However, the Team recommends that AID and PARFR attempt to reduce the amount of paperwork and redundancy of the material that is submitted in the six-monthly reports, particularly in regard to expenditures, organizational charts, and publications. Thus, an annual report could be a more in-depth assessment of the organization and its capabilities, and the intervening six-monthly report would function only as an interim mechanism for highlighting significant developments in the various projects.

III. CONCLUDING REMARKS

In general, the Team is very impressed with the quantity and quality of work being undertaken by PARFR and its subcontractees. *The Team wishes to congratulate all PARFR staff on the excellent job which they are doing, and has no hesitation in recommending that the program be continued in its present shape and form.* The comments and recommendations that are made in the various sections in the body of this report should be regarded solely as attempting to improve an already excellent program, rather than indicating critical deficiencies. *In view of the excellent progress of the PARFR program, the Team concurs that an interval of 3 years before the next in-depth review seems perfectly appropriate.*

IV. SUMMARY OF RECOMMENDATIONS

1. The Team concurs with the recommendations of the previous site visit team that, when additional professional staff are hired, they should have expertise in the clinical trial area.
2. One of the major deficiencies that the program has at this time is the ability to store, collate and retrieve information with regard to ongoing projects, clinical trials, site visits, financial expenditures and program management. The Team recommends very strongly that monies be expended to purchase a mini-computer and to hire (on a part-time contract basis) a programmer to develop the appropriate software necessary for implementation of these recommendations.
3. The Team recommends that an individual should be appointed to the SAC for an initial probationary term of one year during which time his performance, interest and expertise can be evaluated. If this individual proves to be satisfactory, he can then be appointed for a further two years. At the end of the initial three-year term, his membership can be extended under special circumstances on a year-to-year basis for an additional two years, up to a maximum of five years.
4. The previous team had recommended that the new SAC members should not receive funds from PARFR at the time of appointment, nor be eligible for contracts while serving on the SAC. The present Team are in general agreement with this recommendation, but feels that under exceptional circumstances a SAC member should not necessarily be barred from undertaking a pilot project.
5. The previous Team had recommended that PARFR broaden the areas of expertise of the individuals on the SAC. It would appear that indeed the PARFR staff have moved in this direction, but the present Team still

feels that certain disciplines are lacking — for example, a biostatistician, individual experts in clinical trial methodology and execution (other than the PARFR staff themselves), and a polymer chemist — and recommends that such individuals be added.

6. To avoid perceived conflict of interest, as a minimal requirement, the Team recommends that individuals from an institution absent themselves from discussions concerning projects originating from their own institution, even though they are not principal investigators.
7. The Team recommends that Dr. Sciarra should not vote on the actual projects, although he can continue as Chairman of the SAC.
8. The Team recommends that the administrative staff of PARFR present the individual projects, placing them in perspective with regard to their total program and possibly also pointing out the strengths and weaknesses in the protocols, but reserving any indication of their own recommendations as to funding until after the SAC members have had a full discussion on the individual project.
9. It appears that it is not the usual practice for the SAC to be involved in discussions of strategy, and the Team recommends that, in the future, a portion of the SAC meeting be devoted to a brain-storming session at which the SAC members would be encouraged to discuss new lines of research, to re-evaluate priorities of ongoing lines of research and to suggest potential new areas and investigators that might be of interest to PARFR and AID.
10. The Team recommends that, in general, the PARFR administrative staff should either go by themselves or accompany the principal investigator on site visits, but that principal investigators can continue to be used for purely technical visits.

11. In view of recent inflation, the Team recommends that the limit on the funding for formal proposals be raised to $100,000 per year. This would more nearly reflect present day realities of the cost of undertaking research, especially in developed countries.

12. There are many instances where it is essential to purchase expensive animals and/or supplies or hire necessary personnel at the beginning of the work. For some investigators without other funds this can prove impossible. Therefore, the Team recommends that PARFR negotiate an agreement with the University's Accounting Department, whereby up to 25% of the total contract amount be advanced for such purchases, when necessary.

13. It was pointed out that there is no Federal Regulation which requires double review by two separate IRBs. Therefore the Team strongly recommends that AID consult directly with the administration and attorneys of Northwestern University to see whether the present situation might be resolved, so that the approval by a DHHS-approved IRB could be accepted in its entirety without the need for additional review by the Northwestern University IRB.

14. The Team recommends that PARFR settle in-house, with the help of the SAC if this seems useful, exactly what are their research priorities so that this information can be presented to AID with the financial requirements for each individual year's funding.

15. The Team is not enthusiastic about use of quinacrine for female sterilization, given the long history of less than satisfactory occlusion produced by quinacrine when delivered in a variety of different modalities, and recommends that large amounts of money should not be expended on development of this line unless startlingly improved results are achieved.

16. The Team recommends that, since Anordrin is widely used in the People's Republic of China, and since PARFR has good relations with the World Health Organization and its clinical network, WHO and/or PARFR should be encouraged to approach Chinese or other non-US clinical centers to determine whether a small Phase I study could be done. Specifically, this study should evaluate whether a small dose of Anordrin given on the first day of menstruation does indeed suppress ovulation and, if so, for how long.

17. The Team discussed the viability of a new initiative in the area of immunoreproduction, especially in context of certain Indo-US interest. The Team strongly recommends that advice and consultation be provided by bona fide immunologists prior to the initiation of such a program.

18. The PARFR staff reported that Dr. Greenslade's flow chart was going to be improved and made into a proper PERT chart or critical path map, and that similar charts were going to be developed for each of the other major lines of research. This is felt to be a very commendable and useful exercise. The Team recommends that the PARFR staff accomplish this as soon as possible.

19. The Team strongly recommends that these charts show the estimated duration of each phase of the developmental process and an estimate of the anticipated target dates for start and completion of the actual activities. The estimated cost for each phase should also be identified, since this will be required for financial projections.

20. The Team recommends that, in the future, all tabulations of projected activities be given careful thought and consideration, and be laid out in much more extensive and informative fashion, being broken down by year and listed by order of priority.

21. The Team recommends that there should be a contract between the author and PARFR for articles to be published in the RFFR series indicating that subquality material or material arriving later than the anticipated due date would result in a reduction in the remuneration.

22. The Team recommends:

    1) a definite reduction in the level of remuneration and

    2) the ability to refuse to publish a manuscript if it is felt to be of poor quality.

23. The Team recommends that additional efforts be made to identify young investigators interested in contraceptive research and development and to add their names to the mailing list for PARFR publications.

24. The Team recommends provisions should be made in the budget for the addition of a mini-computer, as well as payment for the development of the program to be utilized by PARFR in computerizing their data base.

25. It is also the feeling of the Team that the remuneration for the Director of Administration is inadequate and the Team recommends that every attempt should be made by the AID staff to circumvent the regulatory impasse with Northwestern University, and to pay her commensurate with her responsibilities. The Team also understands that some branches of the U.S. Government (e.g. NIH) pay a physician incentive bonus in addition to salary. It seems reasonable to the Team that this bonus should be made available to relevant PARFR staff.

26. It should be noted that, even though AID staff in attendance with the Evaluation Team stressed that future funds would be made available, there is no guarantee as to the actual level of funding available for any particular year. This makes forward planning more uncertain, and since many projects are nearing phase I and II trials, the Team recommends that

these funds be made available at the budgeted level and in a timely manner for the program.

27. The Team recommends that submission of semi-annual progress reports of research activities by PARFR to AID should be continued. However, the Team recommends that AID and PARFR attempt to reduce the amount of paperwork and redundancy of the material that is submitted in the six-monthly reports, particularly in regard to expenditures, organizational charts, and publications.

28. The Team wishes to congratulate all PARFR staff on the excellent job which they are doing, and has no hesitation in recommending that the program be continued in its present shape and form.

29. In view of the excellent progress of the PARFR program, the Team concurs that an interval of 3 years before the next in-depth review seems perfectly appropriate.

APPENDIX I

CURRENT PARFR-SUPPORTED RESEARCH

AT

SOUTHERN RESEARCH INSTITUTE
STOLLE RESEARCH AND DEVELOPMENT CORPORATION
UNIVERISITY OF ALABAMA IN BIRMINGHAM

Southern Research Institute
Birmingham, Alabama

Richard L. Dunn, Ph.D.	"Development of a Fibrous IUD Delivery System for Estradiol/Progesterone" (PARFR-324)	9/1/82-8/31/83	$52,065
Richard L. Dunn, Ph.D.	"Development of a Vaginal Ring Delivery System for Norgestimate" (PARFR-325)	9/1/82-8/31/83	50,212
Thomas R. Tice, Ph.D.	"Development of Controlled-Release Progesterone Microcapsule" (PARFR-334SRI)	3/1/83-2/29/84	49,500
	"Preparation of Slow-Release Quinacrine Pellets for Dr. Lourens J.D. Zaneveld's study "Efficacy of Quinacrine and Tetracycline in the Primate" (PARFR-323)		5,000

Stolle Research and Development Corporation
Birmingham, Alabama

Danny H. Lewis, Ph.D.	"Development of an Improved Microencapsulation Process for Norethisterone (Two-Step)" (PARFR-331)	2/1/83-9/30/83	$56,868
Danny H. Lewis, Ph.D.	"Development of an Injectable Long-Acting Estradiol Formulation" (PARFR-332)	3/1/83-2/29/84	70,000
Danny H. Lewis, Ph.D.	"One-Month NET Microcapsules and Six-Months Levonorgestrel Microcapsule Preparation" (PARFR-335)	5/1/83-2/29/84	16,284

University of Alabama in Birmingham
Birmingham, Alabama

Charles E. Flowers, Jr., M.D. and Lee R. Beck, Ph.D.	"Poly (lactide-co-glycolide) Norethisterone Microcapsule Study (Poly NET 90)" (PARFR-300)	8/1/81-7/31/83	$72,984
Lee R. Beck, Ph.D.	"Pharmacokinetic Studies in Baboons Relating to PARFR-334SRI" (PARFR-334UAB)	3/1/83-2/29/84	13,699
Charles E. Flowers, Jr., M.D. and Lee R. Beck, Ph.D.	"Poly-gly NET 180 Microcapsule System" (PARFR-333)	3/1/83-2/29/84	47,200
Walter H. Wilborn, Ph.D. (University of South Alabama - in collaboration with Lee R. Beck, Ph.D.)	"Effects of New Synthetic Progestins on Baboon Endometrium" (PARFR-336)	6/1/83-12/31/83	46,061

APPENDIX II

SUMMARY OF MICROENCAPSULATION PROJECTS WITH PARFR INVOLVEMENT

June 13, 1983

There are seven different microcapsule systems being developed with PARFR support by Dr. Beck and associates at SRI and Stolle R and D. PARFR and Stolle have been funding different aspects of the research and development and have exchanged information for mutual benefit. PARFR's objective is to develop and demonstrate feasibility of microcapsule systems having contraceptive applications, and Stolle's objective is to manufacture microcapsule systems for commercial distribution. PARFR and Stolle's involvement varies on each system. A summary of the seven systems, the current state of development, and involvement by PARFR and Stolle is as follows:

1. <u>6-Month Polylactic Acid NET System.</u>

   Polylactic acid microcapsules for NET delivery have been evaluated in three centers:

   A) Mexico City, Mexico
   B) Durango, Mexico
   C) Bahia, Brazil

   A) 24 patients were injected with 60 to 240 um microcaspules containing 22% NET by weight for intended doses of 200 and 100 mg NET. .The study was conducted in 1979 using microcapsules produced in Mexico City by SRI with PARFR funding. Dr. Ramon Aznar was Principle Investigator.

   B) 19 patients were injected with 90 to 212 um microcapsules containing 24% NET by weight for an intended dose of 200 mg NET. The injections were given from 11/81 through 9/82 using microcapsules made in Durango by SRI with PARFR funding. These microcapsules were made using a scale up process developed by SRI with support from the Stolle Research and Development Corporation. Dr. Roberto Riviera was Principle Investigator for this study.

   C) 18 patients were injected with 90 to 212 um microcapsules containing 24% NET by weight for an intended dose of 150 mg NET. These microcapsules were produced in Durango using the Stolle scale up process mentioned in B). Dr. Elsimar Coutinho served as Principle Investigator on this study.

PARFR Involvement: Develop microcapsule systems at SRI; perform baboon testing at UAB; support clinical studies A through C

Stolle Involvement: Scale up technology for preparing clinical doses; provide equipment for manufacturing microcapsules in Durango, Mexico

2. 3-Month Poly-lactide-co-glycolide NET system.

Phase I. Microcapsules for this study were produced in Dr. Lee Beck's lab at UAB with assistance from SRI, and funding by PARFR. Phase I studies were conducted at 5 centers:

A. UAB (Birmingham, Alabama)
B. San Antonia, Texas
C. Rome, Egypt
D. Cairo, Egypt
E. Durango, Mexico

Centers A-D used 63-90 um microcapsules comprised of 85:15 to 88:12 poly lactic:glycolic acid and 20 to 21% NET by weight, and center E was given 90 to 106 um microcapsules from the same formulation. Two groups of 10 women were treated at each center with intended doses of 75 and 100 mg NET (5 subjects each).

PARFR Involvement: Develop system at SRI; support baboon testing at UAB; support clinical studies A through E

Stolle Involvement: Provide scale up technology, and equipment for making microcapsule doses in Dr. Beck's laboratory.

Phase II. Stolle Research and Development Corporation, in collaboration with Ortho Pharmaceutical Company, has developed an improved process for making steroid releasing microcapsules that results in greater yields and more efficient processing. PARFR gave a contract to Stolle Research and Develpment Corporation to adapt this process for the preparation of the 3-month copolymer formulation for use in Phase II clinical trials. Beginning in July, 1983, Dr. Beck, with the assistance of SRI, will make a master batch of 250 g of the 3-month copolymer formulation utilizing the Stolle microcapsule manufacturing process. The microencapsulation work and the clinical trials are being funded by PARFR.

The microcapsules will be 25% loaded, and will range in diameter between 45 and 90 um, and the polymer ratios will range from 88:12 to 85:15 lactide:glycolide.

The estimated starting date of Phase II studies in 100 patients in the USA is September, 1983. Doses for Phase III studies will be produced by Stolle Research and Development Corporation in a new facility that is currently under construction with an expected completion date of October, 1983.

3. 6-Month Poly-lactide-co-glycolide NET system.

Microcapsules for this study were produced as in #2 above, with the exception that microcapsule diameters ranged from 125 to 212 um and doses were 75 and 150 mg NET. These samples are currently being evaluated at two centers - UAB and San Antonio - with 10 women treated at each center.

PARFR Involvement: Same as #2 above

Stolle Involvement: Same as #2 above

4. 3-Month Progesterone Copolymer System. A progesterone microcapsule system with a duration of action of 3 months is being developed by SRI using the PLGA copolymer at an 88:12 to 85:15 ratio. These microcapsules are being produced by SRI with PARFR funding. Size distribution and loading information can be obtained from SRI. Dr. Beck will test the system in baboons.

PARFR Involvement: Support microencapsulation work at SRI; baboon studies at UAB

Stolle Involvement: Microcapsules being made using the Stolle process

5. 3-Month Estradiol Copolymer System. An estradiol microcapsule system is being produced by Stolle Research and Development Corporation using their process. The anticipated duration of release is 3 months, and microencapsulation work at SRI is now in progress.

PARFR Involvement: Funding microencapsulation work at SRI through Stolle R and D; support for primate studies at UAB

Stolle Involvement: Microcapsules being made using the Stolle process

6. 1-Month NET Copolymer System. A one-month NET system is to be developed at SRI.

PARFR Involvement: Support baboon testing

Stolle Involvement: Support microencapsulation work

7. 3-Month Norgestrel Copolymer System. Poly lactide:co-glycolide microcapsules for the release of levo-norgestrel is being developed at SRI.

PARFR Involvement: Support baboon testing

Stolle Involvement: Support microencapsulation work

The effort to develop controlled release microcapsules for the administration of contraceptive steroids at SRI and the UAB has received funding from two sources: the Program for Applied Research in Fertility Regulation (PARFR) and the Stolle Research and Development Corporation. PARFR funded the effort to develop the prototype polylactic acid 6-month norethindrone system, the copolymer 3- and 6-month NET systems, the 3-month estradiol copolymer system, and the 3-month progesterone system. Stolle funded studies to scale up the process for making microcapsules to develop an improved process for making steroid microcapsule systems, and to develop the one-month norethindrone and 3-month levo-norgestrel systems.

The microcapsules used in all the clinical trials to date were produced using the Stolle scale up process and Stolle equipment. All microcapsules for future clinical studies to be funded by PARFR will be produced by the Stolle improved microencapsulation process.

MICROENCAPSULATION PROJECTS WITH PARFR INVOLVEMENT

June 13, 1983

Microcapsule Characteristics:

System	Polymer	Ratio	Load	um Diameter	mg Dose	# Subjects	DRUG
1A-6 MO	PLA	NA	22%	60-240	200	12	NET
	PLA	NA	22%	60-240	100	12	NET
1B-6 MO	PLA	NA	24%	90-212	200	19	NET
1C-6 MO	PLA	NA	24%	90-212	150	18	NET
2A 3 MO	PLGA	88:15 88:12	21%	63-90	75	5	NET
	PLGA	"	"	"	100	5	NET
2B-3 MO	PLGA	"	"	"	75	5	NET
	PLGA	"	"	"	100	5	NET
2C-3 MO	PLGA	"	"	"	75	5	NET
	PLGA	"	"	"	100	5	NET
2D-3 MO	PLGA	"	"	"	75	5	NET
	PLGA	"	"	"	100	5	NET
2E-3 MO	PLGA	"	"	90-106	75	5	NET
	PLGA	"	"	"	100	5	NET
3A 6 MO	PLGA	88:12 85:15	21%	125-212	75	5	NET
	PLGA	"	"	"	150	5	NET
3B-6 MO	PLGA	"	"	"	75	5	NET
	PLGA	"	"	"	150	5	NET

Total 3-Month NET doses in Phase I	50
Total 6-Month NET doses in Phase I	71
TOTAL NET Doses in Phase I Clinical Trials	131

MICROENCAPSULATION PROJECTS WITH PARFR INVOLVEMENT

UNDER DEVELOPMENT

6/13/83

System	Stage	Drug	Comment
2F-3 MO	Phase II	NET	100 patients, 100 USA centers, starting 9/83
4 -3 MO	Preclinical	P4	Copolymer to be tested in baboons
5 -3 MO	Preclinical	E2	Copolymer to be tested in baboons
6 -1 MO	Preclinical	NET	Copolymer to be tested in baboons
7 -3 MO	Preclinical	NORG	Copolymer to be tested in baboons

fb

TECHNICAL REPORT-I

I. PROJECT REPORT SUMMARY

A. General Information

1. "Poly (lactide-co-glycolide) Norethisterone Microcapsule Study (Poly NET 90)" - PARFR - 300T

2. Jose P. Balmaceda, M.D.
   Assistant Professor
   Department of Obstetrics & Gynecology
   The University of Texas Health Science Center
   at San Antonio, Texas 78284

   Ricardo H. Asch, M.D.
   Jane and Roland Blumberg Professor
   in Obstetrics and Gynecology
   Chief, Division of Human Reproduction
   The University of Texas Health Science Center
   at San Antonio, Texas 78284

3. The University of Texas Health Science Center
   at San Antonio

4. Subagreement Period
   a. May 1, 1982
   b. April 30, 1983

5. NONE

6. September 1, 1982 - February 10, 1983

B. Narrative Summary

During this period of time we have completed the following:
Recruitment of 12 subjects. Two subjects were added to the original ten due to problems with the NET injection that were resolved. We have also forwarded 23 of the 24 endometrial biopsies required by the protocol. The blood sampling schedule has been followed accurately with no patients dropping from the project.

II. SUBSTANTIVE TECHNICAL RESEARCH REPORT

A. State of Objectives

The objective of this protocol is to test the safety and effectiveness of a new 90 day injectable contraceptive system. This phase of the study includes determining the effects of the drug on the cycle hormonal patterns and endometrial histology of the subjects.

B. Progress to Date

The protocol has been completed in 9 to 12 subjects. 23 of 24 endometrial biopsies planned have already been performed. Blood sampling have been completed according to the protocol and there is only three patients remaining to finish it. Changes in methodology are discussed in letter.

C. Unanticipated Results

Difficulties encountered in the injection of the microcapsules in the first two patients of our group. Determinated a chance in the modality of preparation of the NET suspension.
(See details - D)

D. Research Design

The original form of preparation of the NET microcapsules for injection did not consistently. Result in homogeneous suspension of the drug. This made injections difficult and the proportion of the drug remaining in the syringe very large. After reviewing the problem with the assistance, Mrs. Valerie Pope, Dr. Beck modified the procedure of preparation of the suspension and the rest of the patients received the complete dose of the drug (see addendums 1 and 2 for details on the changes).
The minor problem determined a delay on the completion of the protocol (approximately 2 month) and the need to receive two extra subjects.

E. Operational Significance

Our protocol has not been completed and the final data on the cycles is not available. However, up to now there is a clear increase in break through bleeding and menstrual irregularity (mainly oligomenorrhea). We don't have hormonal (FSH, LH, P and E_2) values to discuss at this time. We are waiting for the completion of the blood samples to do RIA in all of them.

F. Dissemination and Utilization of Research Results

None up to now.

G. Involvement of Minority Personnel and Women

Two research nurses both of them females and of Mexican American origin participant activity in the protocol.
Connie Lejia - Health Care Counselor in charge of patient recruitment, blo samples and patient records. Also, assistance of physician or physical exams.

H. Work Plan for Coming Reporting Period

Our immediate objectives are to complete the protocol.
Perform one endometrial biopsy.
Eight blood samples to collect
None
None

H. Con't.

a. Presentation of preliminary report on the PARFR Meeting, May 1983.

b. After analysis of the complete data (hormonal values, endometrial samples and cycles) we will publish the results on a prior review journal.

I. Budget Forecast

None

JPB:lg

PROGRESS REPORT

Project Number: PARFR - 301 C

Project Title: Clinical Evaluation of the Bioabsorbable Subdermal Contraceptive Norethindrone Pellets Implants.

Period: November 1st, 1982 to January 20th, 1983.

Activities performed until January 20, 1983 are summarized in Table 1.

TABLE 1

Status of the Study

Control cycle completed	11
RIA of control cycle samples	11
Pretreatment sampling for lipoprotein study	10
Pellet insertion	10
8 months of treatment completed	10
RIA of treatment sampling periods	
days 0 - 42	10
days 90 - 132	10
days 180 - 222	9
Sampling for lipoprotein study at the end of treatment	10
Removal of implants	10
Post-treatment sampling period	
initiated	9
completed	6
not done	1
RIA of post-treatment sampling period	6

All implants have been removed though removal was difficult and led to fragmentation of the pellets.

Progesterone RIA have been completed for 10 pretreatment and 6 post-treatment sampling periods and results are listed in Table 2. All Control cycles were ovulatory with an average highest progesterone value of 28.6 ± 16 nmol/l. Progesterone was measured in 29 treated sampling periods. Values compatible with ovulation were found in 25 (86.2%) cases. Progesterone was clearly non ovulatory in 3 cases and uncertain in one. The average highest progesterone value for treated periods considered ovulatory was 36.6 ± 18 nmol/l.

Clinical record forms, results of RIA performed, pellets removed and samples for RIA of NET hab been delivered to Dr. Saxena during January '83.

TABLE 2

Highest Progesterone Value (nmol/l)

Case	Pre-treatment	Sampling Period Treatment 1°	2°	3°	Post-treatment
1	12.3	6.7	34.7	86.8	35.0
2	39.1	28.4	61.4	28.6	39.4
3	17.5	37.5	36.2	62.6	56.9
4	20.7	35.6	25.1	--	34.3
5	46.7	14.5	4.3	11.1	35.6
6	18.8	59.5	39.1	39.5	23.3
7	43.6	43.6	34.0	45.6	P
8	16.5	9.0	13.3	24.2	P
9	23.4	9.8	21.9	36.2	P
10	54.5	6.0	33.4	51.8	-

P = Pending

Comprehensive Technical Report

Project: Phase 1 clinical studies with Bioabsorbable Subcutaneous Contraceptive Norethindrone (NET) pellets.

PARFR subcontract No: 229

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Narrative Summary

The overall objective of our studies can be visualized in the establishment of pharmacokinetic, pharmacologic, hormonal and clinical parameters for the evaluation of subcutaneous Norethindrone (NET) fused pellets as bioabsorbable, safe, longterm and low cost contraceptive device in women volunteers. In the phase 1 clinical study the experiment were designed to establish daily release rate and levels of NET in the serum upto a period of 12 months from two NET-pellets of 35 mg each implanted subcutaneosly in the forearm of sexually protected women. The phase 1 clinical studies were performed in 10 volunteers each at Cornell University Medical College-The New York Hospital as well as in Chile, Brazil, Mexico and Texas. The phase 1 clinical study has been completed at Cornell for a period of 12 months as well as in San Antonio, Mexico and Brazil upto a period of 6 to 7 months. The study in Chile was started later and will be completed by Oct. 31, 1982.

Sufficient number of ellipsoidal shape, sterile NET-pellets, of 5.5 mm x 3 mm x 2.4 mm dimension with a surface-area 58 mm^2 and a weight of 34.4 $\pm$ 0.6 mg each were prepared in our laboratory by a simple fusion method. The pellets were individually packed in teflon tubes and stored in small glass-vials. Similarly a sufficient number of stainless steel trocars and plungers, with sharp stylets, were manufactured. The NET-pellets were implanted subcutaneously by the use of the trocar.

The volunteers were selected according to the clinical protocols approved by the Institutional Review Board and by Federal Drug Administration, Washington. A single blood sample was obtained per week from each subject by venepuncture duri

pretreatment (control) and post pellet implantation menstrual cycles. The sera were analyzed for FSH, LH, E_2, P and for NET-levels by radioimmunoassay techniques. The ovulation was established on the basis of basal body temperature, bleeding patterns and hormonal (FSH, LH, E_2 and P) levels. All the serum-NET levels during treatment period and analysis of NET-content in pellets retrieved at the end of the study were performed at Cornell. All the subjects were frequently interviewed for any clinical or systemic or local reactions during the entire study period. The clinical and laboratory data were collected at Cornell to prepare the comprehensive report presented hereunder.

The results indicated that subcutaneous implants of NET-pellets did not cause any necrotic or carcinogenic reaction at the site of the implantation. The procedures used in the implantation and retrieval of the pellets caused no scar and showed high degree of acceptability by the volunteers. The pellets, however, sometimes broke into two fragments, which did not affect the study. However, pellets of modified design have already been prepared to avoid fragmentation.

Pharmacokinetic observations at Cornell showed that the mean ($\pm$ SEM) daily NET release rate from two pellets per subject was 73.8 $\pm$ 5.2 ug/day. The NET release rate per cm length of pellets, was 67.0 $\pm$ 5.2 ug/day which is 6 or 7 times more efficient than the silastic-capsules. This study, therefore, clearly demonstrates that the fused pellet per unit length is one of the best among the currently available devices for a sustained delivery of NET in humans. The NET release rate remained fairly constant and comparatively better than the silastic-capsules, silastic-rods and polylactic polymeric formulations. The calculated mean ($\pm$ SEM) daily NET release per pellet and per 100 mm^2 surface-area were 36.9 $\pm$ 2.6 ug and 63.6 $\pm$ 4.5 ug, respectively. Thus the fused pellets provided an efficient mode of controlling the release rate of NET either by number of pellets or their surface-area. Our observations showed individual

variations in serum NET-levels. Nonetheless a sustained and relatively constant, serum NET levels were maintained in all subjects upto 11-12 months. The mean (± SEM) serum NET levels in women initially were 1.0 ± .34 ng/ml, which slowly declined to 0.36 ± .04 ng/ml during a period upto 343 days.

Clinical observations indicated that the pretreatment (control) cycles were ovulatory in all the subjects. After implantation of the pellets, the LH and FSH levels were suppressed during initial 3-4 cycles, after which, the LH and FSH levels gradually rose to a higher level and were sustained throughout the study period. The ovarian response to the continuous low dose of NET in terms of serum E_2 and P levels, was expressed as anovulatory, mixed and ovulatory. At Cornell, the percent distribution of ovulatory, mixed and anovulatory patterns during initial cycles was 40%, 20% and 40% respectively, which changed to 50%, 10% and 40% in later cycles. It may be pointed out that these observations are similar to the effect of progestin-only low-dose oral NET-minipills. Average days of bleeding and spotting were 3.8 days. Of the total subjects 40% bled for 5.2-5.6 days, 30% bled for 3.5-4.2 days and 30% bled for 0.6-2.6 days.

In conclusion, the Phase 1 study has shown that 1) the NET-pellet implants produced neither local and systemic reactions nor any side effect. The NET-pellets provided a high degree of acceptability by all the volunteers. 2) The serum NET-levels were sustained, and fairly constant and efficacious over periods of 6 to 11 months. There were slight differences in the daily serum NET levels in the subjects among various centers. The concentration of serum NET levels obtained at the five centers could be arranged in the following order: Cornell = Mexico > Chile > San Antonio = Brazil. In Mexico, and Chile the percent distribution of ovulatory and anovulatory cycles was

approximately 56% and 44%, respectively. In Brazil, the percent distribution of ovulatory and anovulatory cycles were 63% and 37%. The percent of ovulatory cycles in Brazil was higher than the other centers, which may be attributed to the lower serum NET levels in volunteers in Brazil.

These results indicate that most of the objectives of the Phase 1 clinical study have been accomplished. Based on these data we have proposed a Phase 2 clinical study with 3 and 4 NET pellets per subject for a period of 12 months in 15 fertile and unprotected volunteers each at New York, Michigan, Los Angeles and San Antonio. The phase 2 studies are proposed to establish the efficacy of the NET-pellets as a contraceptive device. The use of 3 and 4 pellets is proposed to increase serum NET levels to be contraceptive in the majority of women of various socio-economic background. The probabilitay of any side-effects with 3-4 small pellets may not be expected, since the maximum levels of serum NET levels are anticipated to be in the range of 1 to 2 ng/ml, which is significantly lower than obtained by the daily oral pills or the injectable contraceptives.

SUBSTANTIVE TECHNICAL RESEARCH REPORT

A. Statement of Objectives

1. Preparation of a sufficient number of fused norethindrone (NET) pellets for clinical investigations at Cornell at New York, USA, and at 1) Texas, 2) Mexico, 3) Brazil and 4) Chile.
2. Manufacture of a sufficient number of trocars and plungers for all centers to implant the NET-pellets.
3. Phase 1 NET-pharmacokinetic studies each at Cornell and at the four centers on 10 healthy women volunteers implanted subcutaneously with two NET-pellets of 35 mg each.
4. Determination of daily NET release from two pellets implanted and serum NET levels in each subject over a period of 11-12 months.
5. Levels of LH, FSH, Estradiol-17B (E_2) and Progesterone (P) during pretreated (control) and treated (post-implanted) cycles.
6. Evaluation of follicular and lutel response during the study period.
7. Evaluation of bleeding patterns of the women volunteers.
8. Evaluation of the Phase 1 studies at Cornell and the other four centers.

B. Progress to Date

1. Subjects for Phase 1 Clinical Studies:

Women volunteers were recruited for the study according to the consent form approved by our Institutional Review Board. Initially, twelve normally menstruating women volunteers of proven fertility were enrolled for the study. Subsequently, eleven subjects were selected for the Phase 1 clinical pharmacological study. Each of them had two sterile NET-pellets inserted in the left ventral forearm by means of a specially designed trocar after proper sterilization and anesthesia. The list and background information of the participating women are given in Table 1. After about 78 days post-implanted period, subject R.N. dropped out of the study. The Phase 1 clinical study in 10 women has been completed at Cornell for a period of 11 to 12 months. Similar studies have been completed over a period of 6 to 7 months in San Antonio, Mexico and Brazil. The study in Chile was started later and will be completed by Oct. 31, 1982. The number of subjects at other centers were 10, 9, 8 and 9 at San Antonio, Mexico, Brazil and Chile, respectively.

2. NET-pellets:

Sufficient number of the NET-pellets were prepared in our laboratory for clinical studies at Cornell and at four additional centers by a simple fusion method which has been patented (1). The sterility of pellets was confirmed in the department of Microbiology at Cornell University Medical College. The ellipsoidal shape, NET-pellets of 5.5 mm x 3 mm x 2.4 mm dimension, with a surface-area 58 mm^2 and a weight of 34.4 $\pm$ 0.6 mg each were individually sterile packaged in teflon tubes and stored in small glass-vials under aspectic conditions (Fig. 1).

3. Trocars and Plungers:

Similarly, a sufficient number of steel trocars and plungers, (Fig. 2) were manufactured with a sharp stylet, to allow easy and rapid subcutaneous implantation of the NET-pellets.

4. Implantation of NET-pellets:

A NET-pellet (Fig. 1) was taken out carefully from the teflon-tube as described before in the procedural detail of the manufacture and packaging of the pellets. In our hands, a pellet split into two pieces about 20% of the time while it was being taken out of the teflon tube. In these cases, both pieces of the pellet along with a second pellet (i.e. three pieces) were inserted. The insertion of the pellets by means of a specially designed trocar and the plunger (Fig. 2) was simple and quick. No infection, skin reaction or inflammation was observed in any of the eleven women. The insertion site of the skin healed normally and there was not visible mark or scar in any of the subjects.

5. Estimation of NET in serum samples:

NET was estimated using specifically ^3H-labeled NET in 6, 7 positions with specific activity of 55 Ci/mmol and antiserum raised against NET-11-succinoyl-oxy-BSA in rabbit, obtained from Schering AG., Berlin. The RIA procedure has been described in detail in the first progress report. The interassay coefficient of variation was 9 per cent. The serum NET levels in samples of all other centers were also analyzed in our laboratory.

6. Determination of E_2 and P in serum samples:

The standards, labeled hormons and antisera for E_2 and P were obtained from commercial sources. The E_2 and P were analyzed in serum samples after ether extraction similar to the NET as described in detail in the first report.

7. Determination of FSH and LH in serum samples:

Serum FSH and LH were estimated using specific radioimmunoassay methods. The results are expressed in mIu/ml of serum.

8. Bleeding pattern record:

Menstrual bleeding and spotting records were maintained for each subject and are depicted in charts 1 to 11 (Appendix-d) for the elven subjects.

9. Pharmacokinetic observations:

Daily absorption of NET from the pellets

a) As shown in Table 2 the mean (± SEM) daily NET absorption from two pellets per subject was found to be 73.8 ± 5.2 ug/day and b) As shown in Table 3, the NET-release rate per cm length of pellets, was 67.0 ± 5.2 ug/. day, and the NET release rates from the silastic-capsules and silastic-rods were much lower. This study therefore, clearly demonstrates that the fused pellets per unit length are at least 6 to 7 times more efficient than the silastic-capsules for a sustained delivery of NET in humans. The NET release rate remained fairly constant over a period of 11-12 months.

c) As shown in Table 4, the calculated mean (± SEM) daily NET release per pellet and per 100 mm^2 surface-area in five women are 36.9 ± 2.6 ug and 63.6 ± 4.5 ug, respectively. In the solid dosage design of a drug in the pellet form, the drug release is directly proportional to the surface -area. Hence, the NET release can be accurately controlled by either the size or number of pellets or their surface-area over a short or or long-term period in animals and humans.

NET-concentrations in serum at Cornell:

i) Serum NET concentrations in individual subject

The individual serum NET concentration in all subjects upto 247 to 372 days are shown in Figs. 3-6. The subject (RN) dropped out of the project

for personal reasons. Hence, the NET serum concentrations of this subject are shown only up to 80 days in Fig. 4. Our observations show that there are individual variations in the serum NET levels. Nonetheless, a sustained and relatively constant, NET level was observed in all subjects upto 11-12 months. A few unexpected high levels of NET were found during the treatment periods. Such samples were repeated to confirm the results. Recent funding of these unexpected high levels of NET may be explained by a Croxatto et al. (6) that plasma levels of norgestrel in women differed significantly when the serum samples were analyzed from the antecubital vein ipsilateral or contralateral to the implant site in the same subject. In our study, the contralateral and ipsilateral serum samples were not differentiated.

ii) Mean Serum NET levels in 10 women

As shown in Fig. 7, the mean (± SEM) serum NET levels in 10 women in our study were 1.0 ± .34 ng/ml during an early 14 day period, which continued at a sustained level with a slow decline to the levels of between 0.36 ± .04 ng/ml to 0.34 ± .08 ng/ml upto 247 to 372 days.

Serum NET levels at other centers:

a) Serum NET levels in the subjects in Mexico

The mean (± SEM) serum NET levels curve over an extend period of 189 days is shown in Fig. 8. The mean serum NET level was 1.0 ± .32 ng/ml initially after implantation, which continued at a steady state with slight decline to a level of 0.40 ± .08 ng/ml at 189 day of the treatment period. The results from Mexico are quite similar to ours at Cornell.

b) Serum NET levels in the subjects in San-Antonio, Texas:

The mean (± SEM) serum NET levels curve over a period of 161 days is shown in Fig. 9. The mean serum NET level was 0.62 ± .14 ng/ml initially,

level of 0.46 ± 0.04 ng/ml at 161.

day. In this study, the levels during initial periods are slightly lower than ours and to that obtained in Mexico.

c) Serum NET levels in the subjects in Brazil

The mean (± SEM) serum NET levels curve for 8-women over a period of 204 days is shown in Fig. 10. The mean NET level was 0.61 ± .22 ng/ml initially at day 4, which continued at a steady state to a level of 0.34 ± .03 ng/ml at 204 day. There is no surge or a "burst effect" of NET in an early period, commonly observed with the silastic capsules.

d) Serum NET levels in the subjects in Chile

The mean (± SEM) serum NET levels curve for 9 women over a period of only 41 days is shown in Fig. 11. The initial peak level was 0.75 ± .12 ng/ml at day 4, which continued at a fairly constant rate to a level of 0.46 ± .05 ng/ml at 41 day. At the onset there is no surge or a "burst effect" of NET.

e) Comparative serum NET levels curves of all five centers

Fig. 22 depicts the time-course curves of the mean serum NET levels in all five centers. The highlights of all the results from all the centers are as follows: 1) the NET-levels are sustained, and fairly constant over periods of 6 to 11 months. There are however, slight differences in the daily serum NET levels in the subjects among the centers. The sustained serum NET levels obtained at the five centers could be arranged in the following order: Cornell = Mexico > Chile > San-Antonio = Brazil. These differences could be explained by recent reports by Prasad et al. (7) that there are significant differences in plasma NET levels between high and low socio-economic groups of women. The differences were reported due to the nutritional status of these women, the concentration of specific steroid binding proteins and the metabolic clearance rates of steroids.

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10. Clinical observations

a) Hormones levels and their patterns

The pituitary and gonadal hormones levels and profiles of LH, FSH, E_2 and P including NET levels during control and treatment periods of upto 11 months in 10 women at Cornell are shown in Figs. 13 to 22. The pretreatment (control) cycles were ovulatory in all the 10 subjects. After implantation of the pellets, the mid cycle FSH and LH surges and follicular and luteal phase LH and FSH levels were suppressed during initial 3-4 cycles (Figs. 13 to 22). After which, the LH and FSH levels gradually rose to a higher level and were maintained steadily throughout the treatment periods of 11-12 months. Due to infrequent measurement of LH and variations in the day of ovulation, ovulatory LH surges were not recognised during subsequent cycles.

b) Ovarian response and E_2 and P levels

The ovarian response to the continuous low dose of NET in terms of serum E_2 and P levels, in the 10 subjects was classified into three groups essentially according to Landgren and Diczfalusy et al. (8). 1st group- exhibited no signs of follicular or luteal activity as evidenced by the low progesterone levels and was designated as anovulatory; the 2nd group- (Mixed type) showed either marked cyclic follicular activity but no luteal function or normal follicular activity but a little luteal activity; and 3rd group-(Ovlatory type) and was designated as mixed (Table 5).

11. Menstrual bleeding, spotting and cycle lengths:

Average days of bleeding and spotting assessed per 28 days interval during the treatment period of 247 to 372 days in 10 women are depicted in Table 6. Average days of bleeding and spotting are 3.8 days. Percent distribution of women experiencing bleeding and spotting during control and treatment

periods are shown in Table 7. In general, the subjects could be distributed into three groups for different period of bleeding and spotting, namely, a) 40% of the subjects like normal; b) 30% lower than normal; and c) 30% very low as shown in Table 7. Figs. 13 to 22 also illustrate bleeding days shown by hatched areas, spotting by the solid bar and the cycle lengths. There is relatively little spotting in these subjects as shown in Figs. 13 to 22. There are some variations in the cycle lengths during initial treatment periods. These variations are similar to the effect of progestin-only low-dose oral NET minipills.

12. Reversibility of implanted pellets:

Because of the solid dosage design of the progestin in the pellet form, it was easy to first palpate the pellets under the skin and mark its exact position with a sterile dye before injecting local anesthetic. The pellets were easily taken out after a minor incision. The edges of incision then were closed with 6.0 absorbable chronic sutures. There was no infection in any subject. In our study, we have taken out the pellets from five subjects. The retrieved pellets with and without the surrounding fibrous membranes from the two subjects (i.e. G.M. and M.C.) are shown in Fig. 23.

13. Histopathological examinations of the local-tissue at pellet-site:

The histopathological examinations revealed the formation of a thin fibrous capsule with slight chronic inflammation, which was a completely typical reaction. The tissue was associated with different degree of inflammatory cell reaction, mainly mononuclear cells and foreign body giant cells. There were no other morphological changes and excessive local changes. The pathological examination reports for the two subjects (i.e. G.M. and M.C.) are included.

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14. Summary of Ovarian responses during treatment period and percent distribution of ovulatory, mixed and anovulatory cycles at five centers:

The estimation of ovarian response as reflected by E_2 and P during the treatment periods and expressed in terms of percent (%) of ovulatory, mixed and anovulatory cycles at the five centers is shown in Table 8. In our study at Cornell, the percent distribution of ovulatory, mixed and anovulatory patterns during initial and later cycles was 40%, 20% and 40%, respectively, which slightly changed to 50%, 10% and 40% in later cycles. This pattern is very similar to that obtained by the oral NET minipills reported recently by Landgren et al. (9).

In Mexico, the percent distribution of ovulatory and anovulatory cycles was approximately 56% and 44%, respectively. Similar distribution pattern was also observed in Chile.

In Brazil, the percent distribution of ovulatory and anovulatory cycles were 63% and 37%. The ovulatory percent is slightly higher than the other centers. However, it may be due to the lower serum NET levels obtained there.

The patterns of ovulatory and unovulatory cycles for the subjects in San Antonio are not available at the present time.

15. Summary of different dosage forms, NET-dose, approximate duration of effectiveness and the serum NET levels:

An updated summary of different dosage-form, NET-doses, approximate duration of effectiveness and the serum NET levels is shown in Table 9. The data of Table 9 indicate that only three dosage forms are bioabsorbable, namely, i) the daily pills, ii) NET-en injectable and iii) the NET-pellets. The NET-en injectable provides a long-term effectiveness of about 65 days with a wide fluctuation of high and low serum NET levels.

In contrast, the NET-pellets provide a serum NET level at a fairly constant level over a period of many months. Furthermore, the release rate through the pellets can be precisely controlled and the pellets are retrievable.

C. Unanticipated Results

The results obtained in the present Phase 1 clinical pharmacological study in 10 women corroborate with our earlier long-term studies in animals and humans with the pellet method. There has been no unexpected complication either in the clinical study here at Cornell or at any other center.

D. Research Design

In the present study, it was possible to collect blood samples once a week from each subject and not three times a week as mentioned in the original protocol. Our experience gained in this study suggests that a weekly collection of blood will provide a sufficient amount of significant data toward evaluation of hormonal profiles, follicular and luteal functions and the menstrual bleeding pattern over a long-term study of 11 to 12 months. Besides, there has not been any other change in the study.

E. Operational Significance

The accomplishments are described above in the narrative summary. Their relationship to the overall research objectives of the project are the following:

a) The sustained, fairly constant and efficacious NET release from the two NET-pellets were obtained in women over extended periods of 6 to 11 months here at Cornell and other four centers. The results corroborate the earlier findings of long-term sustained release of NET from the pellets in animals and humans (13-15).

b) Further, the results of this study provide substantial evidence in that the

daily release per unit surface-area of a pellet and its constancy are far better than other drug delivery systems, namely, silastic-capsule, rods and various polylactic polymeric formulations (16-19).

c) Furthermore, the release rate can be precisely controlled by either the size or number of pellets or their surface-area.

d) Still further, the pellets have other useful characteristics, viz., they are easily and quickly prepared, molded and packaged in a sterile condition in a single teflon tube in one operation. Besides, they are bioabsorbable and easily retrievable.

e) Moreover, the solid dosage design of NET contraceptive in the pellet form is modeled on the well established and proven system of the system of the sex-steroid hormone pellets used clinically over the last 40 years in the USA and England. Based on this background and in keeping with the objectives of PARFR in developing a simple, safe, effective and inexpensive contraceptive method, there is a urgent need to continue the present study into Phase 2 clinical studies here and at other centers. The results of the present studies have been promising. The contraceptive pellet method has a potential for wide clinical uses both in the USA and in other countries.

F. Dissemination and Utilization of Research Results

1. Project output: The results of the present clinical study seem to be promising for the fertility regulation in women. The preliminary results of the study has been presented before the American Fertility Society in March, 1982. A copy of the abstract is attached in the Appendix-e.

2. An abstract of this study has been sent to be presented at "International Symposium on Hormone Receptors in Growth and Reproduction" on Nov. 18, 1982, in Bombay, India.

3. A full paper is being prepared for publication in a suitable journal.

G. Involvement of Minority Personnel and Women

1. Two of the technicians for the hormonal analyses and laboratory experimental works are women.

2. Secondly, one of the technicians employed is of a Korean nationality. She had some experience with radioimmunoassay in Korea.

H. Work Plan for coming period

1. Anticipated accomplishments

Based upon the progress made to date in the Phase I clinical pharmacological studies here and other centers, a Phase 2 clinical study is proposed, which will establish the contraceptive efficacy and the usefulness of the NET-pellet method in women with proven fertility.

2. Proposal and Procedures

a) This proposal is for a phase 2 clinical evaluation of the subcutaneous Norethindrone (NET) pellet implants as a long-term contraceptive method.

b) Description of the study and the budgets are given in "The Proposal for Phase 2 Study" (Appendix-h).

3. Factors which may promote accomplishments

The fused NET-pellets used in the Phase 1 studies were found to break easily when taken out of the teflon tube. Now, the NET-pellets of higher strength have been prepared by slightly increasing the width, height and decreasing the length of pellet by using a slightly larger diameter of the teflon tubing. These pellets do not break. Because, the strength of the solid dosage form of a drug is dependent upon dimensions of its width, height and length. The other properties of pellets, for example, weight, surface-

FIRST TECHNICAL REPORT

ELECTROEJACULATION OF PRIMATES FOR SHUG EVALUATION, AND LDH IMMUNIZATION

PARFR - 317

February 18, 1983

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Starting Date: July 1, 1982

Ending Date: June 30, 1983

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Overview of Results to Date

This is a two part project aimed at: 1) evaluating the efficacy of the reversible vas deferens device (Shug) in the absence of a tissue wrap around the vas in primates; and 2) collecting blood samples from the baboons for the PARFR project with Dr. E. Goldberg (Northwestern University) to evaluate the contraceptive efficacy of the LDH peptide antibodies.

In regard to project 1, five male rhesus macaques were ordered and have been isolated for a two month period. Their baseline semen data have been established. In collaboration with Bivona Surgical Co., Indiana, the Shug has undergone some further modifications which were needed to improve device implantation and removal. With the devices tested previously, implantation was difficult because the silicone device was so soft that entry through a small hole in the vas deferens was difficult. Removal was somewhat complicated because the thread which held both parts of the device together, came loose easily. To solve these problems, the tip of the device was constructed of stainless steel, conical in shape, and covered by a thin layer of silicone. The stainless steel cone has a small identation in its flat end in which a small stylus (inserter) fits. Thus, the device has rigidity at its tip and the stylus cannot perforate the device when the Shug is pushed through the opening in the vas. The ends of the thread are incorporated into the metal so that they are difficult to dislodge on device removal. These modifications should greatly aid the clinical applicability of the device. A sample of the improved device was given to Ms. Diane Krier-Morrow at PARFR.

Device implantation was tested in two primates, one a primate left over from the previous experiments (at present not funded by PARFR) and the other, one of the five animals to be tested under the present contract. Because of the metal tip the device could not be constructed with a diameter of 0.70 mm, generally used previously, but was constructed with a 1.0 mm diameter. Even so, device implantation

was much easier than previously. To date, neither one of these animals have ejaculated spermatozoa after device implantation and a 4 week recovery period.

While performing the second implantation, the stylus was bent and a new one is being constructed. As soon as it is completed, the other 4 primates will be implanted. Thus, the project is going according as planned except for some time delays due to the construction of the improved device. No changes in protocol are anticipated.

In regard to project 2, details need to be obtained from Dr. Goldberg. Our efforts are only to collect weekly blood samples from the 30 baboons and to prepare sera. These are than picked up by a person from Dr. Goldberg's laboratory. Once organized, this has been proceeding well. No changes from the protocol are anticipated.

MAR 3 1983

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DYNATECH

PARFR-320

February 23, 1983

Ms. Diane H. Krier-Morrow
Program for Applied Research in
Fertility Regulation
875 N. Michigan Avenue
Suite 1525
Chicago, IL 60611

Dear Ms. Krier-Morrow:

We have received your letter (special delivery) of February 16th. It seems that some review of our mutual situation is in order.

* We have prepared the required Levonorgestrel/PLGA rods at Toxical Laboratories. The preparation was conducted under Good Manufacturing Procedures and the records of the process were maintained in GMP format. These rods have been radiation sterilized and are in cold storage in England, awaiting certain confirmation testing before being approved for human testing. Note: All rods made were part of the manufacturing carried out at Toxical.

* Some analytical work remains to be done. The major tests are for sterility, verification of composition of the rods after extrusion, and the assessment of the aminal tests (which PARFR is to arrange).

* As we informed you in our letter of December 10, 1982, the preparation for the extrusion of the rods was begun in England on November 1, as scheduled. The completion of the extrusion work was delayed due to problems getting the mold press through customs, and other problems associated with deliveries of supplies to the laboratory. We pointed out in this letter of 12/10/82 that the delay would entail extra expenses for us (Mr. Crooker's salary) and for Toxical.

* On December 21, you and Dr. Goldsmith visited Dynatech. We discussed the arrangements for continuing the work required to get the rods to clinical testing in U.K.; and we reached an agreement for a revised budget. The revisions included moving funds from other categories into the Engineering Aide Category, a separate arrangement for $5,000 to cover the additional expense of report preparation and final analytical work on the rods. This work was

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to be completed by the end of February. We were to request an additional sum to cover work during and after the clinical tests. You also requested and received a sample of rod from the batch made in England on which we were proceeding to do confirmation testing.

* Following a phone call, on December 22, 1982, you wrote us (Special Delivery) ordering that all work be temporarily halted and that no further expenses be allowed. You had identified a concern for the suitability of the rods for implantation. The concern was, that upon examining the rods, they might be too fragile for implantation/removal.

* On January 13, 1983, we sent you rods for animal tests (to be arranged by you) to resolve this question of fragility - it is our understanding that resumption of any work rests on the outcome of these tests. We have done no further work on this project although several aspects of the analysis of the rods have been left hanging. We have not prepared a technical report. These tasks, as we have discussed in the past, are related to the need for additional funds.

* We agreed much earlier that PARFR would pay for the freight costs of shipping the mold press to and from England, as well as the air fare of Dr. Wise and Mr. Crooker, i.e. all international air costs were to be paid by PARFR. You will recall that items dealing with air fare were excluded from our budget. Our accounting office is preparing a statement for this expense ($1,036.32).

We are as frustrated as you must be with the delays and expenses associated with getting this product into clinical testing. We are anxious to get the work underway again.

We request that you again consider the budget figures (Table 1) we prepared in December for your visit but which you did not wish to take with you. Please consider this present letter as another request for the additional sums for completion of analysis and reporting for this preparative work($8,264 - see Table 1). We also request again additional funds to follow through with the analysis and reporting on the rods after they are removed from human subjects ($7,253 - see Table 1). The budget prepared on 12/21/82 is attached (Table 1). Not all projected December expences were charged and only a few small expenses came in in January. Column 5 (Projected January Expenses) represents the work delayed by your order to stop work. It could be renamed Projected March/April Expenses. Column 9 might be renamed Expenses May to Project End.

When we have the authorization and funding from you we will resume completion of the Preparative Phase of this Program.

Finally, in order to cover these past expenses, we request formal permission to reallocate our present budget as follows:

Move from:

Materials (Precursors)	$ 966.24
Materials (Glassware)	916.89
Telephone	399.10
Xerox	322.40
Polymer testing	1,545.00
Salary - Staff Engrs.	194.29
Salary - Tech. Typist	25.91
TOTAL	$4,369.83

Move to:

Engineer Aide	$1,842.93
Engineering Overhead	2,526.90
TOTAL	$4,369.83

Your approval of these changes will leave a balance of zero in each category.

As you previously requested, my resume is enclosed.

Sincerely yours,

Judith P. Kitchell, Ph.D.
Principal Scientist
Chemical Engineering Dept.

JPK/ph

cc: Ms. Dewer
Dr. G.I. Zatuchni

FEB 0 9 1983

I. PROJECT REPORT SUMMARY

A. General Information

1. "Guaiacol peroxidase levels: Predictors of ovulation in women"
PARFR 321

2. John C.M. Tsibris, Ph.D. Principal Investigator
Associate Professor
Department of Obstetrics and Gynecology
University of Illinois College of Medicine
840 South Wood Street
Chicago, Illinois 60612 Phone: 312-996-9617

William N. Spellacy, M.D. Co-Investigator
Professor and Head
Department of Obstetrics and Gynecology
University of Illinois College of Medicine
840 South Wood Street
Chicago, Illinois 60612 Phone: 312-996-7430

3. Project duration: August 1, 1982-July 31, 1983

4. This is the first technical report covering the period 8/1/82-2/1/83

I. B. Narrative Summary

In the first 6 months of this project we have accomplished a great deal: a) trained an excellent medical technologist (MT), a medical student and a nurse (all female) to sample volunteers on weekdays and weekends, b) trained the MT to assay guaiacol peroxidase (G-Px) in whole saliva, vaginal secretions and cervical mucus, c) established RIA assays for plasma hormones (estrogen (E_2), progesterone, LH and FSH), d) established procedures, criteria and a "pool" of health center employees and health professions students to serve as volunteers in the study and e) have completed assays of G-Px in 9 women-cycles (the serum hormone levels are now being analyzed). It appears that G-Px levels in vaginal secretions are the most promising parameter in predicting ovulation because of the easy accessibility of vaginal secretions. G-Px levels in cervical mucus are as good predictors of impending ovulation although cervical mucus is not as easily sampled. G-Px levels in whole saliva are unsatisfactory because they do not show a consistent periovulatory change.

II. SUBSTANTIVE TECHNICAL RESEARCH SUPPORT

A. Statement of Objectives

We had agreed to study the levels of guaiacol peroxidase (G-Px) in whole saliva, vaginal secretions and cervical mucus of volunteers for thirty (30) menstrual cycles; some volunteers were to be tested in more than one cycle (sampling starts at the end of menses until the next menses). The time of ovulation was to be determined by the serum LH, FSH surges and ovulation verified by the progesterone rise. The volunteers were also to record their basal body temperature for comparison purposes.

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B. Progress to Date

a. Methodology

All volunteers are screened by Drs. Spellacy and Thomason and sign an informed consent form for the study. The volunteers arrive at the Diagnostic Clinic 20 min. apart starting from 7:00 AM until 8:30-9:00 AM; on weekends we are now insisting on this 2-hour gap, otherwise the weekend nurse or medical student would spend 5-6 hours to sample the same number of women. The blood is coagulated, spun in a centrifuge and the serum kept at -20^{0}C until assayed. The 3 samples from each volunteer are weighed, G-Px extracted with the Ca^{++}-Tris) pH 7.4 buffer and assayed with a spectrophotometer. Although the MT is extremely well organized she can collect and test no more than 5 volunteers a day at a "neck-breaking" pace. The calculations of G-Px activity are also time-consuming. The RIA assays of plasma hormones are done with kits which greatly expedite the process. The weekend G-Px samples are kept frozen and assayed on Monday with no apparent loss of enzymatic activity.

b. Research Findings

Right after menses, the specific activity (G-Px units extracted per g of secretion) ranges from 800-5000 in cervical mucus, 20-100 in vaginal secretions and 5-20 in whole saliva. Although we have to await the RIA data to pinpoint the time of ovulation, it is clear that in 8 of 9 cycles tested, cervical mucus G-Px drops substantially at midcycle whereas salivary G-Px fluctuates in seemingly random fashion. G-Px of vaginal secretions also shows a distinct dip at midcycle in all 9 cycles but in 4 of 9 cycles it remained at zero values for longer than 4 days at midcycle which makes us suspect that we should be sampling deeper, near the cervix, using a longer Q-tip. It should be emphasized that the key to the method's success in natural family planning is a) the timing of the drop in G-Px specific activity, it should be 3-6 days before ovulation, and b) the steepness of the drop, the steeper it is the easier it would be for the woman to be alerted of impending ovulation and abstain from unprotected intercourse It looks from the 9 cycles that vaginal secretions look very promising based on criterion (b) above, and most likely will also prove very promising on criterion (a). We have not encountered any constraints in the project during this reporting period except for the tightness of available funds.

C. Unanticipated Results

Salivary G-Px does not look as a suitable parameter to predict ovulation. Although the simplicity of sampling whole saliva is most attractive as a method for mass self-screening of the fertile period in the third world, there are many unknown factors interfering with the G-Px levels to warranty any further investment of time in improving it. For example, any attempt to improve the sampling, collection of submaxillary saliva by special containers will increase the complexity of the method and reduce compliance by the subjects. Sampling the deep vaginal secretions with a plastic 10 cm Q-tip is simple enough and, I believe, not unacceptably awkard to women of many different cultures.

D. Research Design

Except for the increased length of the Q-tip used in vaginal sampling, there are no other changes in our protocols. Interestingly, we found that the volunteers mind only the venipuncture and none of the sampling procedures.

E. Operational Significance

In this reporting period there are no publications that we are aware of, pertaining to this project and its objectives. One of the great advantages of our method is that it depends on the concentration of something (G-Px) in a body fluid and not on the ability of a subject to collect semi-quantitatively a body-fluid (e.g. vaginal secretion volume as informally outlined by one of our Chicago colleagues recently); the size of the tip of the Q-tip avoids the danger that the subject would collect, and assay, too much of vaginal secretions.

F. Publications: None yet

G. Diane Washington, a black MT student assisted us in the laboratory for a few weeks during the early, developmental stages of the project. Unfortunately, we could not continue with her, due to fund limitations.

H. Work Plans for Coming Reporting Period

This month we are testing 4-7 women; they are not "synchronized", and will complete the RIA tests to evaluate the first 9 volunteers. We will continue at the same pace until all 30 cycles are tested.

FIRST TECHNICAL REPORT

EFFICACY OF QUINACRINE AND TETRACYCLINE IN THE PRIMATE

PARFR - 323

February 18, 1983

Prepared by:

L.J.D. Zaneveld, D.V.M., Ph.D.

Professor

Departments of Physiology and Biophysics, and Obstetrics and Gynecology

University of Illinois

Chicago, Illinois

(312) 996-7151

Starting Date: September 1, 1982

Ending Date: August 31, 1983

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Summary

The objectives of this project were to compare the tubal occlusive effects of different types of quinacrine HCl pellets and of tetracycline pellets in the primate (cynomolgus macaque). Quick and slow release quinacrine HCl pellets were to be studied. So far, the ability of the cynomolgus macaque uterus to hold the pellets was tested. It was found that by laparotomy and uterine incision, two pellets, approximately 0.3 cm in diameter and 0.5 cm in length can readily be inserted into the uterus. These pellets are both located in the uterine cavity without putting undue pressue on the uterine wall. The animal has shown no side effects to the surgery or the continuous presence of the pellets over a 2-3 month period. Twenty cynomolgus macaques were ordered and arrived at the Primate Center of the University of Illinois. They are presently in their two month isolation period. Quick release pellets have been obtained from Dr. R. Weeler at the IFRP. Slow release quinacrine pellets are being constructed by Southern Research Institute under the direction of John W. Gibson. The tetracycline pellets are prepared under the direction of Dr. N. Dubin at Johns Hopkins University. Implantation of the pellets will ensue as soon as the animals are out of isolation and the pellets have arrived. No changes from the original protocol are anticipated.

The starting time of the project was delayed by several months due to a problem in obtaining the agreement and signatures from the University. However, these problems were solved completely and the project is fully active.

PARFR 324

1.a. PROGRESS REPORT TO

THE PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION, NORTHWESTERN UNIVERSITY

1.b. Investigator and Institution

Richard L. Dunn, Ph.D.
Head, Polymer Division

Southern Research Institute
2000 Ninth Avenue South
P. O. Box 3307-A
Birmingham, Alabama 35255

1.c. Title of Project

Development of a Fibrous IUD Delivery System for Estradiol/Progesterone

1.d. Period Covered by Report

September 1, 1982, to January 26, 1983

February 1, 1983

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DEVELOPMENT OF A FIBROUS IUD DELIVERY SYSTEM FOR ESTRADIOL/PROGESTERONE

I. PROJECT REPORT SUMMARY

A. General Information

1. Title and PARFR Subcontract Number: Development of a Fibrous IUD Delivery System for Estradiol/Progesterone, PARFR-324.

2. Principal Investigator: Richard L. Dunn, Ph.D., Southern Research Institute, 2000 Ninth Avenue South, P. O. Box 3307-A, Birmingham, Alabama 35255, (205) 323-6592.

3. Institution: Southern Research Institute, 2000 Ninth Avenue South, P. O. Box 3307-A, Birmingham, Alabama 35255.

4. Subcontract Period: September 1, 1982, to August 31, 1983.

5. Period Covered by Report: September 1, 1982, to January 26, 1983.

B. Narrative Summary

The major accomplishments during this reporting period have been 1) the selection of suitable fiber-forming polymers for an estradiol delivery system, 2) the preparation of monolithic and coaxial fibers loaded with estradiol, 3) the determination of the basic mechanical properties of the coaxial fibers loaded with estradiol, 4) the in vitro evaluation of the fibers, and 5) a review of work done earlier on the progesterone fibrous system.

We selected polycaprolactone (PCL), a biodegradable polymer, as a core matrix for the estradiol delivery system. Polyethylene (PE) and polypropylene (PP) were selected as sheath materials or rate-controlling membranes. Our experience with the progesterone fibrous system indicates that these polymers are the best candidates to provide good mechanical properties for long-term delivery systems.

We prepared monolithic fibers comprising 50 wt % of estradiol and 50 wt % of PCL by melt-spinning. We also prepared coaxial fibers with a PE sheath and a core matrix containing equal quantities of estradiol and PCL. During these spinning operations, we damaged our spinning equipment and were unable to spin any fibers with a PP sheath. However, we did spin two sizes of the coaxial fibers with a PE sheath. Both were uniform and relatively strong.

The coaxial fibers followed approximate zero-order kinetics in the release of estradiol when agitated in 0.5 wt % sodium dodecyl sulfate (SDS) solution at 37 °C. The release results were used to calculate the apparent permeability product, DC_s, for estradiol in PE. This value for DC_s was used to design a fibrous system that would release estradiol at a rate of 0.5 μg per centimeter of fiber per day for 3 years.

We reviewed the in vitro release results of progesterone fibers from the earlier program. Based on these results, we selected PE as a sheath and a core matrix comprising 75 wt % of progesterone and 25 wt % of PCL for the progesterone component of the fibrous IUD delivery system. The release results were also used to calculate the apparent permeability product, DC_s, for progesterone in PE. This value was used to design a fibrous system that would release progesterone at a rate of 1.0 μg per centimeter of fiber per day for 3 years.

II. SUBSTANTIVE TECHNICAL RESEARCH REPORT

A. Statement of Objectives

The primary objective of this project is to develop and evaluate a fibrous IUD system for the combined delivery of estradiol and progesterone to the female reproductive tract. Five main tasks were outlined as the necessary steps to achieve this objective.

1) Preparation of coaxial fibers of polyethylene, polypropylene, and polycaprolactone loaded with estradiol.

2) Determination of the mechanical properties of the estradiol coaxial fibers.

3) Determination of the in vitro release characteristics of the most promising coaxial fibers.

4) Correlation of in vitro release rates of the estradiol coaxial fibers with those obtained in vivo in rabbits.

5) Fabrication of T-shaped IUDs with fibers containing various ratios of estradiol to progesterone.

B. Accomplishments to Date

We have prepared monolithic and coaxial (sheath-core) fibers from PCL and PE with estradiol as the active agent. The mechanical properties of the coaxial fibers show great potential for intrauterine applications. They are comparable to those obtained with progesterone coaxial fibers. The release of the steroid from the fibers has been evaluated in vitro. The coaxial fibers followed approximate zero-order release kinetics in the release of estradiol. These results were used to calculate an empirically based value of DC_s. This value was used to design a fibrous delivery system that would release estradiol at a rate of 0.5 μg/cm/day for 3 years.

We reviewed the in vitro release results of progesterone fibers from the previous study and used these values to calculate the apparent permeability product, DC_s, for progesterone in PE. Based on this value, we designed the progesterone component of the fibrous IUD delivery system. A detailed description of the experimental work is given in the following sections.

1. Selection of suitable fiber-forming polymers

Because of our past success in preparing other steroid-loaded fibers by melt-spinning, we used the same method to produce estradiol-loaded fibers. Also, melt-spinning is the most convenient and economical technique to prepare fibers. β-Estradiol purchased from Sigma Chemical Company, St. Louis, MO, was the drug used in melt-spinning of the fibers. Based upon our experience in developing fibrous systems that release progesterone and other steroids, we limited our selection of fiber-forming polymers to those that were biocompatible and low melting. Biocompatible polymers are needed because of the intrauterine application, and low-melting polymers are desired because of the relatively low melting point of estradiol (173 to 179 °C).

For these reasons, we selected PE, PP, and PCL as fibrous excipients for the delivery of estradiol. These polymers can be extruded into high-strength fibers at temperatures in the range of 180 to 200 °C. From our work with progesterone and other steroids, we expected that estradiol would be quite soluble and permeable in PCL and much less so in PE and PP. Therefore, we selected PCL as a core matrix for the estradiol delivery system and PE and PP as sheath materials or rate-controlling membranes. Our experience with the progesterone fibrous system indicates that PE and PP are the best candidates for long-term delivery systems.

2. Preparation of monolithic fibers

We prepared monolithic fibers loaded with estradiol from PCL only. These fibers contained 50 wt % of the drug and were melt spun on a ram extruder. The ram extruder was designed and built at Southern Research Institute. The most essential part of the extruder is a 4 in. diameter, 8 in. long cylindrical, electrically heated, metal block with a 0.6 in. diameter bore through the center. A spinneret and a metal screen filter are fitted to the bottom of the block. A ram with a nominal diameter of 0.6 in. and driven by a variable-speed motor is used to force the sample through the spinneret. A pressure gauge located directly above the ram is used to measure the applied ram force. A take-up winder is located about 3 ft below the spinneret for collection of the fibers onto paper spools.

For our experiments, a single orifice spinneret was used to produce the fibers. The drug/polymer sample was charged into the barrel of the extruder and equilibrated at the spinning temperature. Then it was forced through the spinneret and collected on spools. Various block temperatures, ram drive rates, ram drive forces, and fiber take-up rates were used to obtain fibers with suitable properties and uniformity.

Normally, we prepare monolithic fibers from each candidate polymer and evaluate them in vitro to obtain the permeability product, DC_s. D is the diffusion coefficient of the steroid through the polymer and C_s is the saturation solubility of the steroid in the polymer. By applying Fick's first law of diffusion to devices with cylindrical geometry, a graphical solution for the permeability product, DC_s, can be obtained from the in vitro release data for monolithic fibers. This product can then be used to design optimum drug-release systems. However, experience with other drug-release systems show that the product of DC_s derived from monolithic fibers is not necessarily indicative of the true molecular diffusion of the drug in the polymer because of other possible mechanisms of release such as leaching through solvent-filled

pores. Thus, our primary reason for preparing monolithic fibers of PCL was to compare the relative permeability of estradiol in PCL to that we had obtained in earlier work for progesterone. The estradiol/PCL monolithic fiber that we prepared was spun at 120 °C and had a diameter of 0.478 mm.

3. In vitro evaluation of monolithic fibers

We determined the in vitro release of estradiol from the PCL monolithic fiber by immersing triplicate samples in separate containers of 50 mL aliquots of 0.5 wt % sodium dodecyl sulfate (SDS) solution. We selected 0.5 wt % SDS as the receiving fluid because it shows less interference at the absorption maximum of β-estradiol (201 mm) than do the aqueous ethanol solutions. Like the ethanolic solution, 0.5 wt % SDS is an excellent receiving fluid for in vitro release studies because it approximates serum in its ability to solubilize various drugs. Therefore, the SDS solution should also be indicative of in vivo release rates that we can expect for the steroid.

The sealed containers were incubated at 37 °C and agitated on low speed in an Eberbach shaker bath. Periodically, the receiving fluid from each container was exchanged for fresh SDS solution and assayed for estradiol by measuring the absorption peak at 201 nm on a Perkin Elmer 575 spectrophotometer. The quantity of estradiol released was determined from a Beer's law plot (Figure 1) of absorbance versus estradiol concentration.

Figure 2 shows the cumulative fraction of estradiol released as a function of time for each fiber sample. These plots show that the rate of release is first order with a fairly constant release after the initial burst.

The observed release profiles of the fibers can be described mathematically by Fick's Law of diffusion for devices with cylindrical geometry:

$$\frac{d(M_t/M_\infty)}{dt} = \frac{-4DC_s}{r_o^2 C_o \ln(1-M_t/M_\infty)}$$

where

$\frac{d(M_t/M_\infty)}{dt}$ = fractional rate of release of drug

D = diffusion coefficient of the drug in the polymer, cm^2/s

C_s = saturation solubility of the drug in the polymer, g/cm^3

r_o = radius of the cylinder

C_o = drug loading, g/cm^3

M_t/M_∞ = fraction of initial mass released at time t

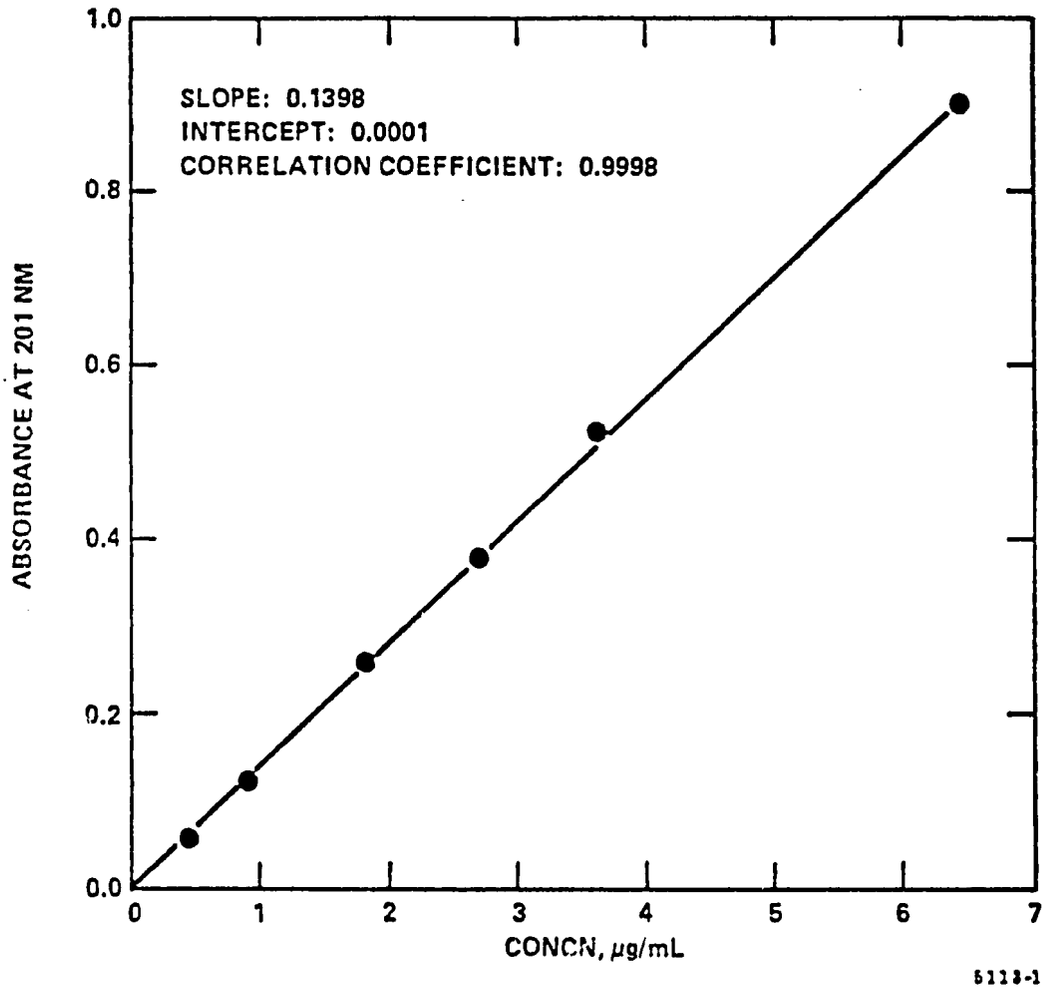

Figure 1. Beer's law plot for absorbance versus estradiol concentration in 0.5 wt% SDS at 37 °C.

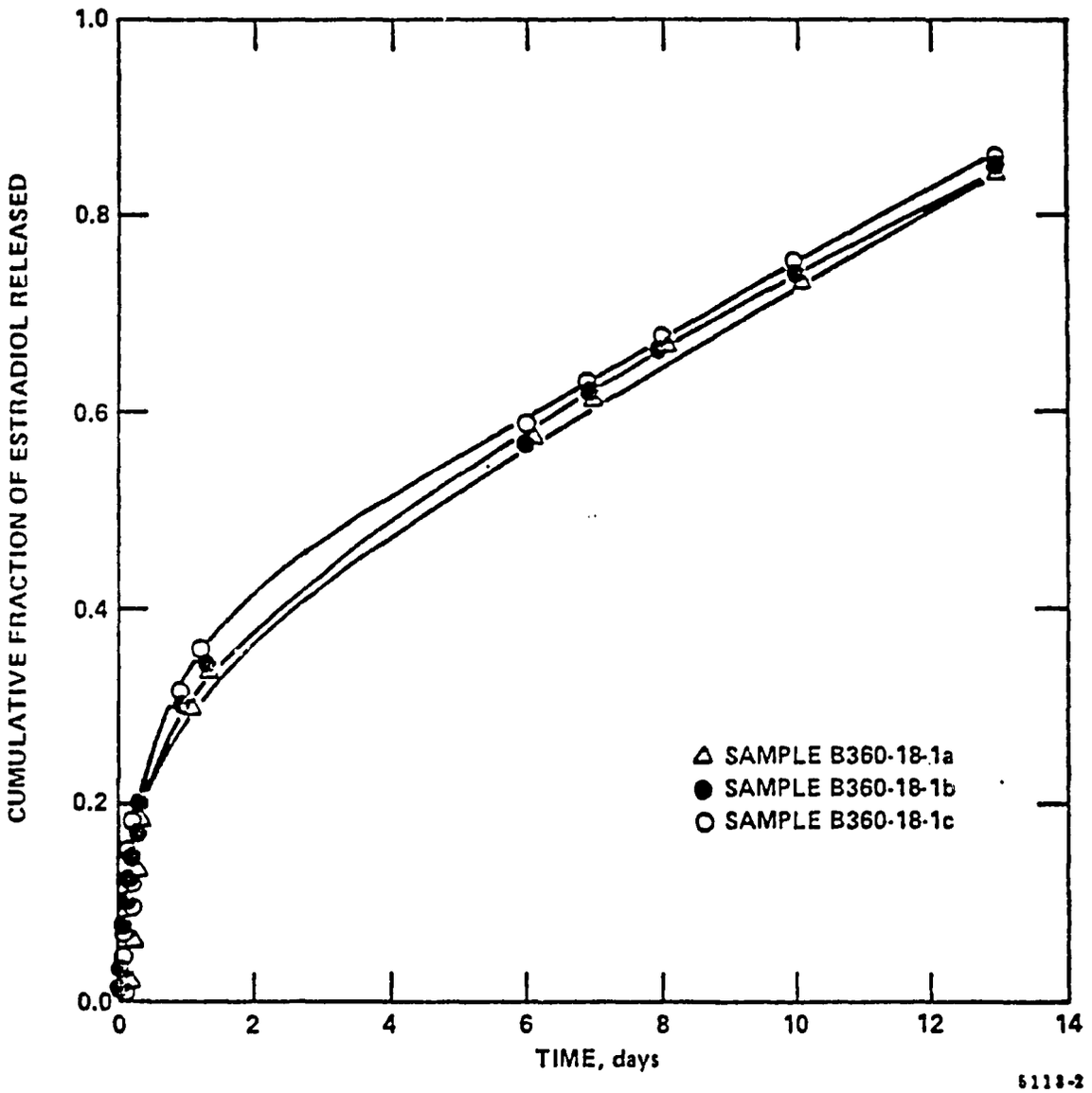

Figure 2. In vitro release of estradiol from 50%-loaded PCL monolithic fibers.

By integrating the above equation and rearranging the terms, the following expression is obtained:

$$\left(\frac{C_o \cdot r_o}{4}\right)\left([1-M_t/M_\infty][\ln(1-M_t/M_\infty)] + M_t/M_\infty\right) = DC_s \cdot t$$

A plot of the terms on the left side of the equation versus time in seconds will yield a straight line of which the slope is the permeability product, DC_s. These calculations were done for the PCL monolithic fibers and the results are given in Table 1.

The results in Table 1 indicate that estradiol is quite permeable in PCL. The permeability product for progesterone in PCL was determined in the earlier program to be 6.0×10^{-11} g cm^{-1} s^{-1}. This value was also derived from a graphical analysis of the in vitro release data of monolithic fibers. The value of DC_s obtained for estradiol in PCL is slightly lower than the one for progesterone. This same order of permeability has been reported for these steroids in silicone rubber. From both our data and that in the literature, we would expect to observe a similar trend in PE and PP, the other candidate polymers.

4. Preparation of coaxial fibers

To obtain a constant or zero-order rate of drug release, we prepared coaxial (sheath-core) fibers in which estradiol located in the core of a fiber is surrounded by a sheath of a rate-controlling, drug-free polymer. These fibers were prepared by coaxial melt spinning. In this process, the steroid is combined with a polymer in which it is quite permeable. The steroid/polymer mixture is melted in the barrel of a ram extruder and forced through the center of a special coaxial spinneret designed and fabricated at the Institute.

The coaxial spinneret consists of two concentric rings positioned such that material passing through the center ring forms the core of the fiber, and material passing through the outer ring forms the sheath. The sheath polymer is melted in a separate chamber and metered with a melt pump to the outer concentric ring. Both polymer streams emerge simultaneously from the spinneret and cool to form the coaxial fiber. Manipulations in extrusion temperatures, extrusion rates, and fiber take-up speed are used to obtain fibers with different diameters and different ratios of sheath-to-core dimensions. The sheath-to-core ratio is optimized to control the rate of release of steroid from the fiber.

We prepared coaxial fibers with a PE sheath and a core matrix comprising 50 wt % of estradiol and 50 wt % of PCL. We experienced some difficulty during the spinning process which damaged our equipment. As a result, we could not prepare any fibers with a PP sheath. But, we did prepare two sizes of the coaxial fibers with a PE sheath before the damage occurred. Both were uniform with an average diameter of 0.4 mm. Table 2 describes the coaxial fibers that we prepared.

TABLE 1. VALUES OF DC_s FOR ESTRADIOL IN PCL

Sample B360-18	DC_s, g cm^{-1} s^{-1}
-1a	3.6×10^{-11}
-1b	3.5×10^{-11}
-1c	3.6×10^{-11}

TABLE 2. COAXIAL FIBERS WITH POLYETHYLENE SHEATH, 50:50 ESTRADIOL/PCL CORE

Sample B360-15	Melt-spinning temp, °C	Extrusion rate, cm^3/min	Overall diam, mm	Core diam, mm	Sheath thickness, mm	$r_o:r_i$	Core loading, μg/cm
-1	135	0.46	0.381	0.253	0.064	1.51	281.8
-2	135	0.46	0.390	0.188	0.101	2.08	154.8

The values for r_o and r_i were obtained by measuring cross sections of the fibers under a microscope equipped with a calibrated scale. The total amount of drug per centimeter of fiber was obtained as follows: A weighted density of 50:50 estradiol/PCL was calculated. This weighted density was multiplied by the core loading and the core volume of each fiber to obtain the weight of estradiol per centimeter of fiber. The duration of release can then be calculated by dividing the total amount of drug by the daily rate of release of estradiol.

5. Mechanical properties of coaxial fibers

The mechanical strength of the estradiol loaded fibers is an important aspect of the fibrous delivery system. For intrauterine applications, the fibers should have sufficient strength and flexibility to be easily coiled around a standard T-shaped IUD without breaking or collapsing. For these reasons, we determined the basic mechanical properties of the estradiol loaded fibers. We also plan to determine the flexibility of the fibers by wrapping them around a T-shaped IUD, inspecting them under a microscope for any stress marks or cracks, and removing the fibers to measure their percentage of recovery (memory). The method we used to determine the basic mechanical properties is given below.

Five 10-in. samples were cut from each fiber. The samples were tested on an Instron Tensile Tester according to standard ASTM procedures. The force required to break the fibers (load at break) and the percent of elongation of the fibers at break were recorded. The strength measurements were converted to tenacity values by dividing the load at break in grams by the fiber denier. Fiber denier is the weight in grams of 9000 meters of the fiber. Another value commonly used to compare the basic strengths of fiber is tensile factor. This value is derived from the product of the tenacity and square root of the elongation for a particular fiber.

Table 3 summarizes the mechanical properties of the coaxial fibers. These values are relatively low, but they are comparable to those obtained with progesterone coaxial fibers. Also, the estradiol-loaded fibers were not optimized for maximum strength. We plan to prepare additional coaxial fibers with greater mechanical strength by drawing them over heated godets to several ratios of initial to final length. This orientation process will enhance the strength and flexibility of the coaxial fibers.

6. In vitro evaluation of coaxial fibers

For the in vitro release experiments, we cut triplicate samples of each fiber. To prevent the release of estradiol from the fiber ends, we sealed the ends of each sample with poly(methyl methacrylate), a denture-base resin. This is a solventless, two-component system that cures at room temperature. One component is the liquid methyl methacrylate monomer; the other is a mixture of powdered poly(methyl methacrylate) and a catalyst. The two components were mixed together to form a viscous paste, and the fiber ends were repeatedly dipped in the mixture until a visible coating had been applied. Then, the in vitro release rates of the sealed fibers were determined in SDS solution as described earlier.

TABLE 3. MECHANICAL PROPERTIES OF COAXIAL FIBERS WITH POLYETHYLENE SHEATH, 50:50 ESTRADIOL/PCL CORE

Sample B360-15	Load at break, g	Elongation at break (E), %	Tenacity (T), g/d	Tensile factor, [$\sqrt{E}$ (T)]
-1	170	81	0.18	1.6
-2	170	65	0.16	1.3

Figure 3 shows the cumulative release of estradiol as a function of time for representative fiber samples. The fiber samples followed approximate zero-order kinetics in the release of estradiol as shown by the mass released being a linear function of time. Each graph has been normalized to show the mass of estradiol released per centimeter of fiber. Thus, the slope of the graph is the release rate in micrograms of estradiol per centimeter of fiber per day. Table 4 summarizes the in vitro results for the coaxial fibers.

The results in Table 4 show the effect of the different values for $r_o:r_i$ on the release rates of the fiber samples. We used the release rates of the coaxial fiber samples to calculate an empirically based product of DC_s for estradiol in polyethylene. These calculations were based on the following expression for steady-state release of a drug by diffusion through the rate-controlling wall of a cylinder:

$$\frac{dM}{dt} = \frac{2\pi hDC_s}{\ln(r_o:r_i)}$$

where

dM/dt = rate of release, g/s

h = length of cylinder, cm

D = diffusion coefficient of the drug in the polymer, cm^2/s

C_s = saturation solubility of the drug in the polymer, g/cm^3

r_o = outer radius of cylinder (sheath + core), cm

r_i = inner radius of cylinder (core), cm

By changing the value of $r_o:r_i$, we can design a controlled-delivery system for estradiol at almost any rate. As shown in Table 4, durations up to 4 years are possible with the estradiol coaxial fibers. But, the slow release rate of these fibers is undesirable because long lengths of fiber would be needed to release a sufficient amount of estradiol for intrauterine application. As stated in our proposal, the Cu-T-IUD, which we plan to use as a carrier vehicle for the intrauterine evaluation of the combined estradiol/progesterone fibrous system will hold approximately 90 cm of fiber. Therefore, we must limit the length of fiber needed to give the desired delivery rate. To achieve this goal, the rate of release per centimeter of fiber can be increased by changing the ratio of $r_o:r_i$. We used the average permeability product for estradiol in PE, 1.6×10^{-13} g cm^{-1} s^{-1}, to design a fibrous delivery system that would release estradiol at a rate of 0.5 μg/cm/day for 3 years. The fiber specifications are given in Table 5.

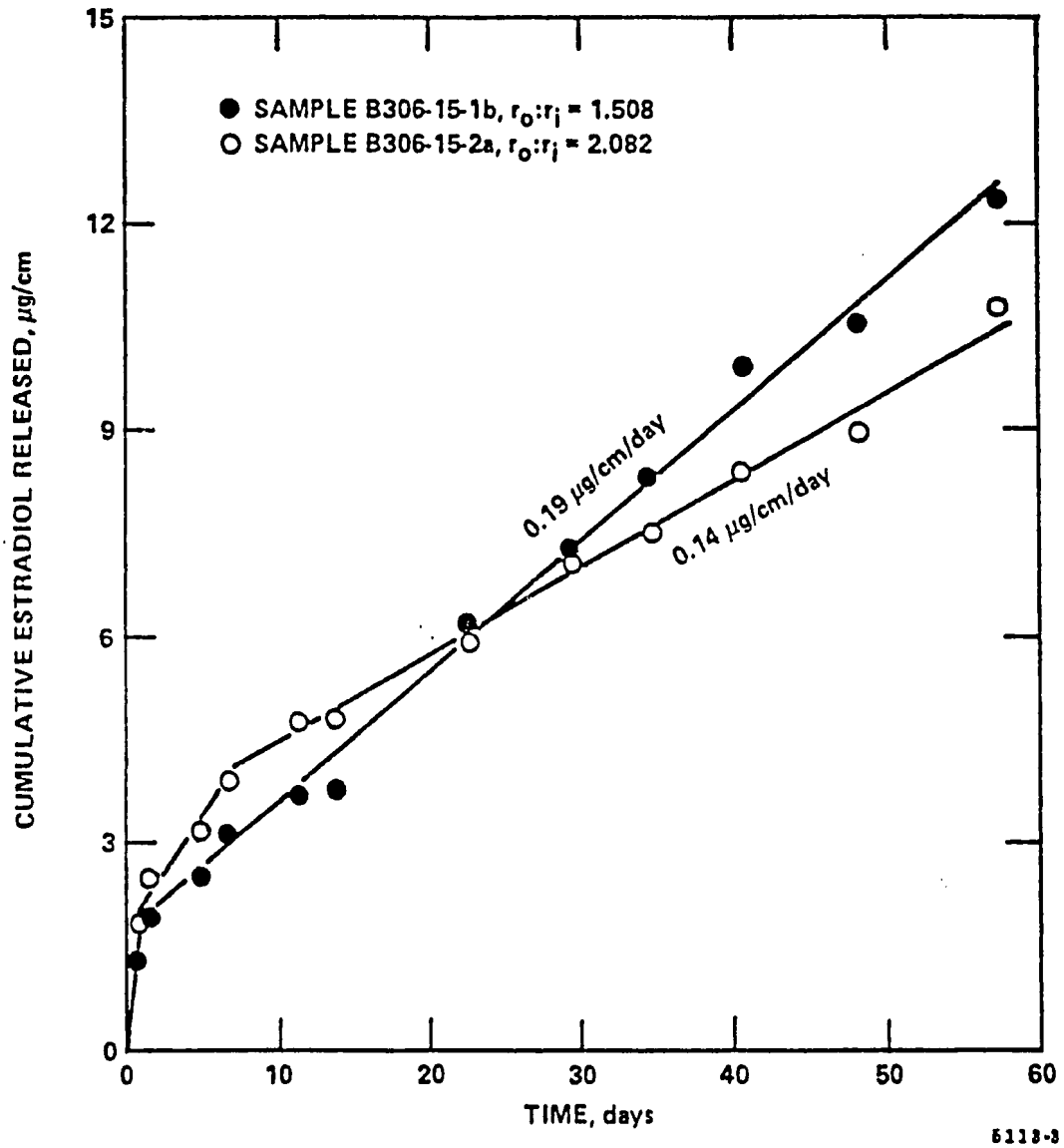

Figure 3. In vitro release of estradiol from coaxial fibers with P.E. sheath, 50:50 estradiol/PCL core.

TABLE 4. IN VITRO RELEASE OF ESTRADIOL FROM COAXIAL FIBERS WITH POLYETHYLENE SHEATH, 50:50 ESTRADIOL/PCL CORE

Sample B360-15	$r_o:r_i$	Release rate, μg/cm/day	Estimated duration of release, days	DC_s, g cm^{-1} s^{-1}
-1b	1.51	0.19	1483	1.4×10^{-13}
-2a	2.08	0.14	1106	1.9×10^{-13}

TABLE 5. DESIGN SPECIFICATIONS FOR ESTRADIOL/PROGESTERONE FIBROUS IUD DELIVERY SYSTEM

Specification	Estradiol	Progesterone
Sheath material	Polyethylene	Polyethylene
Core composition	50:50 Estradiol/PCL	75:25 Progesterone/PCL
Core loading, μg/cm	548	1095
Release rate, μg/cm/day	0.5	1.0
Overall diam, mm	0.420	0.670
Core diam, mm	0.350	0.400
Sheath thickness, mm	0.035	0.135
$r_o:r_i$	1.20	1.67

7. Review of progesterone fibers

We reviewed the work that was done on the progesterone fibrous system during the earlier program, "A Fibrous Polymer for the Delivery of Contraceptive Steroids to the Female Reproductive Tract". First, we examined the permeability products of DC_s (Table 6) that were determined for progesterone in PE, PP, and PCL. These values of DC_s were derived from a graphical analysis of the in vitro release results of monolithic fibers. As stated earlier, the permeability products obtained from monolithic fibers may not necessarily be indicative of the true molecular diffusion of the polymer because of other possible mechanisms of release such as leaching through solvent-filled pores. For these reasons, we calculated the apparent permeability products for progesterone in these polymers from previous in vitro release results of coaxial fibers in which PE, PP, or PCL were used as sheaths or rate-controlling membranes.

We analyzed coaxial fibers with either poly(ethylene glycol) (PEG) or PCL in the core matrix. While analyzing the in vitro release results of the coaxial fibers, we discovered that the core composition of the fibers also influenced the apparent permeability of progesterone in the sheath polymer. Therefore, we calculated the values of DC_s for each core composition. These values are also given in Table 6. As shown in Table 6, the values of DC_s obtained from each method of analysis are slightly different for each polymer. However, based on these results and the desired rate and duration of release for the progesterone component of the fibrous IUD delivery system, we selected PE as the sheath or rate-controlling membrane.

The results in Table 6 show that fibers with PEG in the core matrix released the steroid at a faster rate than those with PCL in the core matrix. Progesterone is highly permeable in both PEG and PCL. But, PCL adds strength and flexibility to the fiber, whereas PEG adds essentially nothing to the fiber. Therefore, we have selected a PE sheath and a core matrix comprising 75 wt % of progesterone and 25 wt % of PCL for the progesterone component of the fibrous IUD delivery system. The value of DC_s for the steroid in PE that was determined from coaxial fibers with PCL in the core matrix was used to design the fibrous system. This system should release progesterone at a delivery rate of 1.0 μg/cm/day for 3 years. The fiber specifications are given in Table 5.

C. Operational Significance

Findings during this study are especially significant because coaxial fibers with a polyethylene sheath have been shown to release estradiol at rates suitable for long-term delivery systems.

D. Unanticipated Results

Additional information beyond that described in the statement of work has not occurred during this reporting period.

TABLE 6. VALUES OF DC_s FOR PROGESTERONE IN CANDIDATE FIBERS

Fiber-forming polymer	DC_s, g cm^{-1} s^{-1}		
	Monolithic fibers	Coaxial fibers	
		75:25 P_4/PEG core	75:25 P_4/PCL core
PCL	6.0×10^{-11}	6.0×10^{-10}	NA[a]
PE	1.2×10^{-12}	2.3×10^{-12}	9.4×10^{-13}
PP	3.3×10^{-13}	2.5×10^{-13}	4.1×10^{-14}

[a]NA = Not applicable.

E. Research Design

No significant modifications have been made in the research design during this reporting period. However, based on the results of the release experiments with estradiol coaxial fibers, it is not necessary to examine polypropylene as a possible excipient for the estradiol delivery system.

F. Dissemination and Utilization of Research Results

No papers or publications were developed during this reporting period.

G. Involvement of Minority Personnel and Women

Ms. Brenda H. Perkins, a black female biomathematician, is involved in all aspects of this project. She is a full-time employee of Southern Research Institute. The Institute is an equal opportunity employer and has a number of minorities on its staff.

H. Work Plan for the Upcoming Reporting Period

During the next reporting period, we expect to prepare additional coaxial fibers with PE sheaths and a core matrix containing 50 wt % of estradiol and 50 wt % of PCL. These fibers will be optimized for increased mechanical strength and a delivery rate of 0.5 μg/cm/day. The most promising coaxial fibers will be evaluated in rabbits to correlate the in vitro release rates with those obtained in vivo.

We will also prepare coaxial fibers with a PE sheath and a core matrix containing 75 wt % of progesterone and 25 wt % of PCL. Likewise, these fibers will be optimized for increased mechanical strength and a delivery rate of 1.0 μg/cm/day.

The mechanical properties of both estradiol and progesterone coaxial fibers will be determined. These properties will include strength, elongation and flexibility. The in vitro release rates of the steroids from the fibers will be determined also. After the in vivo release rates of the estradiol coaxial fibers have been established and correlated with those measured in vitro, we will fabricate T-shaped IUDs with various ratios of estradiol- to progesterone-loaded fibers. We expect that these devices will then produce a controlled delivery system for estradiol/progesterone that can be used in the uterus for effective contraception.

I. Budget Forecast

Category	Subcontract budget 9/1/82 - 8/31/83	Expenditures for reporting period 9/1/82 - 12/31/82	Projected Expenditures for next reporting period 1/1/83 - 5/1/83
Personnel	$19,755.00	$3,666.21	$8,044.00
Supplies	3,750.00	842.36	1,454.00
Reports and Communication	225.00	6.96	109.00
Facilities cost of capital	2,732.00	507.60	1,112.00
Indirect costs:			
Burden	17,384.00	3,230.30	7,077.00
G & A	5,427.00	1,086.74	2,170.00
Fee	2,792.00	529.95	1,131.00
TOTALS	$52,065.00	$9,870.12	$21,097.00

J. Acknowledgments

Mr. W. C. Stoner, Jr., Senior Chemist, Polymer Engineering Section, was responsible for the spinning of the fibers.

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NB: B360, B380
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"Development of a Fibrous Delivery System for Estradiol"
Richard L. Dunn, PhD

This is a progress report on the development of a fibrous IUD delivery system for estradiol/progesterone. The investigator has selected polycaprolactone as the core matrix and polyethylene as the rate controlling membrane for both estradiol and progesterone. Using the same polymers for both steroids will simplify the manufacturing of the final system. The investigator has tested the basic mechanical properties and the vitro release of the co-axial fibers.

Based on these experiments the investigator has calculated the desired ratio between the controlling membrane and the core matrix to release 0.5 μg of estradiol per cm per day. In the next reporting period he will fabricate such fibers, test their mechanical strength and the release rates characteristics in vitro and correlate this with in vivo release in the rabbit. The investigator is well on the way in the development of a fibrous IUD delivery system.

In Table 3 the investigator gives the mechanical properties of his co-axial fibers. It is evident that the fibers are not too strong. In Table 5, the proposed estradiol fibrous system has a sheath thickness which is half as thick as the fibers tested in Table 3 (see Tables 1 and 2). The decreased sheath thickness may further contribute to the fragility of the fibers.

In my first evaluation I raised some questions: what is the shape of the platform to be used for the delivery of the steroids and will the fibers make the rigid support of the delivery platform. I am not sure I understand the plans completely. The final form of the device may well affect the release rates.

For example, if the fibers are placed in a bundle the release rate is sure to diminish. Thus, it is essential that the platform question be addressed promptly.

Antonio Scommegna, M.D.

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1.a. PROGRESS REPORT TO

THE PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION, NORTHWESTERN UNIVERSITY

1.b. Investigator and Institution

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1.c. Title of Project

Development of a Vaginal Ring Delivery System for Norgestimate

1.d. Period Covered by Report

September 1, 1982, to January 31, 1983

March 9, 198:

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DEVELOPMENT OF A VAGINAL RING DELIVERY SYSTEM
FOR NORGESTIMATE

I. PROJECT REPORT SUMMARY

A. General Information

1. Title and PARFR Subcontract Number: Development of a Vaginal Ring Delivery System for Norgestimate, PARFR-325.

2. Principal Investigator: Richard L. Dunn, Ph.D., Southern Research Institute, 2000 Ninth Avenue South, P. O. Box 55305, Birmingham, Alabama 35255-5305, (205) 323-6592.

3. Institution: Southern Research Institute, 2000 Ninth Avenue South, P. O. Box 55305, Birmingham, Alabama 35255-5305.

4. Subcontract Period: September 1, 1982, to August 31, 1983.

5. Date of Last Technical Report for this Project: None.

6. Period Covered by this Report: September 1, 1982, to January 31, 1983.

B. Narrative Summary

The major accomplishments during this reporting period have been (1) a review of work done earlier with the norgestimate (NGM) fibrous delivery system, (2) the determination of the apparent permeability product for NGM in candidate polymers, (3) the design of both a fibrous and triaxial ring delivery system for NGM, (4) the selection of the base ring material, and (5) the fabrication of a human-size vaginal ring mold and vaginal rings.

We reviewed the data from our earlier studies, which were directed toward the development of a three-year delivery system for NGM. The fibers prepared during that study were based on a polycaprolactone (PCL) core matrix and a polyethylene (PE) rate-controlling sheath. From the earlier data, we calculated dimensions for fibers that would deliver NGM at the desired rate of 500 μg/day. For acceptable fiber lengths, we found that coaxial fibers with extremely thin sheaths were required, but we were unable to prepare these fibers by our usual methods. Therefore, we decided to identify other materials that would be more suitable for the three-month delivery system.

We selected eight alternative polymers as candidate materials for the NGM delivery system. Measurements of the apparent permeability product for NGM in each of these materials were made. The best materials for use in the three-month system were then selected on the basis of the preferred dimensions and the observed permeabilities. We subsequently calculated design parameters for the fibrous and triaxial ring delivery systems.

We also used the permeability data to select the base ring material. We found that NGM was significantly less permeable in Silastic MDX-4-4210 than either Estane 5714 or the softer ethylene/vinyl acetate copolymers. Therefore, we selected Silastic as the most desirable material for use as the base ring material because it should show better storage stability than the other materials considered.

Finally, we designed and fabricated a two-cavity mold to prepare human-sized vaginal rings. The mold was designed for use with RTV silicone elastomers as well as thermoplastic elastomers. Also, we are now modifying our existing baboon-size ring mold for use with RTV silicone elastomers.

II. SUBSTANTIVE RESEARCH REPORT

A. Statement of Objectives

The objective of this research program is the development of a vaginal ring delivery system for NGM. The system that is sought should provide a constant dose of NGM of up to 500 µg/day for a period of 90 days. To achieve this objective, we outlined three principal tasks:

1. Fabrication of vaginal rings that provide a controlled dose of NGM.

2. Determination of the in vitro and in vivo rates of release of NGM from the rings.

3. Fabrication of nonmedicated vaginal rings for preclinical acceptability studies.

B. Accomplishments

After a thorough review of the data obtained in our previous study (Subcontract PARFR 235 SRI), we designed a coaxial fibrous delivery system for NGM. The system was designed with a PE sheath and a PCL core matrix. However, we have not been successful in preparing these fibers because of the thin sheaths of PE required for proper release of NGM. Consequently, we have determined the permeability of NGM in several other polymers that we outlined as candidate materials in our proposal. From these data we have calculated design parameters for both a fibrous system and a triaxial ring system. Finally, we have prepared human-sized vaginal rings of a medical-grade silicone rubber, and we have fabricated a mold for the preparation of vaginal rings from thermoplastic materials.

1. Design of the NGM delivery system

Our first choice of a vaginal delivery system for NGM is that of NGM-releasing coaxial fibers wrapped around an elastomeric vaginal ring. The second design is similar to the vaginal ring device developed by the Population Council for release of levonorgestrel. In both systems, three polymeric materials are needed to achieve the combined objectives of mechanical reliability, tissue compatibility, and drug efficacy. The materials are the base ring polymer, the drug matrix, and the rate-controlling membrane. Initially, we compiled a list of candidate polymers that are tissue compatible, have good mechanical properties, and exhibit a wide range of permeabilities to most steroids. These materials are polyurethanes, polysiloxanes, styrene/butadiene copolymers, ethylene/vinyl acetate (EVA) copolymers, PCL, and PE.

The actual designs are based on results obtained from in vitro release studies and measurements of the apparent permeability product for NGM in the various materials. These results are presented in the following sections.

a. Review of previous results

We reviewed the results obtained on our earlier program, which was directed toward the development of a three-year delivery system for NGM. Fibers containing a core of NGM/PCL and a sheath of either PCL, Estane 5714 (a segmented polyurethane), or Vynathene 903 (a 55/45 EVA copolymer) exhibited first-order release kinetics. Because the release of NGM from these fibers was first-order, we were unable to determine the permeability of NGM in any of the sheath polymers. However, first-order release from sheath-core sys typically indicates that the permeability of the drug in the sheath polymer is similar in magnitude to the permeability of the drug in the core matrix. And we could calculate the apparent permeability product (DC_s) in PCL from the in vitro release profiles for monolithic fibers of NGM in PCL. The apparen permeability product is the product of the diffusivity (D) and the saturation solubility (C_s) for a drug in a polymer under specified conditions such as the temperature and the solvent system in which the release profile was determined.

We also evaluated coaxial fibers comprising a core of an equal mixture of NGM/PCL and a sheath of PE. These fibers behaved as a true reservoir system in that the release of NGM was zero-order. For three of the fiber samples, Table 1 shows the design parameters, the in vitro rate of release of NGM, and the calculated value of DC_s for NGM in PE. We determined the permeability product by a graphical analysis of the in vitro release data and the following expression of Fick's Law for diffusion through the wall of a cylinder:

$$\frac{dM_t}{dt} = \frac{2\pi h \, DC_s}{\ln(r_o : r_i)}$$

TABLE 1. IN VITRO RELEASE OF NGM FROM COAXIAL FIBERS WITH PE SHEATHS

Sample No. A713-	Release rate, μg/cm·day	$r_o:r_i$	DC_s, g/cm·s
67-3	0.39	1.24	2.47×10^{-13}
67-2	0.23	1.41	1.46×10^{-13}
67-4	0.11	1.52	8.48×10^{-14}
		Avg. =	1.59×10^{-13}

where

$\frac{dM_t}{dt}$ = rate of release, g/s

h = length of fiber, cm

DC_s = apparent permeability product, g/cm·s

r_o = radius of core and sheath, cm

r_i = radius of core, cm

The mean value of DC_s was then used to design a fibrous delivery system based on a PE sheath and a PCL core matrix. Table 2 shows design parameters for several fibrous systems capable of maintaining a rate of release of 500 µg/day for a period of 90 days, which is the maximum rate and duration desired. Because of the extremely low permeability of NGM in PE and the desired high flux, all of the fiber designs require very thin sheaths. This restriction combined with the particular materials used have led to several difficulties in the preparation of the coaxial fibers.

b. Preparation of NGM fibers

We most frequently prepare coaxial fibers by melt spinning. However, for extremely thin sheaths we use a solution coating method to apply the sheath to a monolithic core. We prepared monolithic fibers from a blend of 25 wt % NGM (Ortho Pharmaceutical Corporation, Raritan, NJ) and 75 wt % PCL 700 (Union Carbide, New York, NY). The blend was prepared by codissolving the NGM and PCL in dichloromethane and then evaporating the solvent. The dried blend was next cut into small pieces and extruded on a Tinius Olsen extrusion plastometer to produce monolithic fibers.

The chamber of the plastometer, with a 0.0508-cm diameter spinneret inserted, was heated to 130 °C. Next, the NGM/PCL blend was charged into the heated chamber. After the sample had reached thermal equilibrium, a ram force of 1369 kPa was applied to extrude the molten blend through the spinneret. As the extrudate cooled, a uniform fiber with a nominal diameter of 0.0416 cm was collected on a cardboard spool.

We attempted to coat the monolithic fiber with PE by a solution coating process. A solution containing 4 wt % of PE (LD 600, Exxon Chemical Americas, Houston, TX) in toluene was prepared by heating the materials to 75 °C. This solution was then placed in the outer chamber of a strand-coating die also heated to about 75 °C. Next, the monolithic fiber was fed through the center opening of the die. Although we made several attempts to coat fibers of both NGM/PCL and 100% PCL, the fibers always melted upon contact with the hot toluene solution. Other solvents were considered, but we were unable to identify a less aggressive solvent system than 75 °C toluene for this grade of PE.

TABLE 2. DESIGN PARAMETERS FOR 90-DAY FIBERS--NGM/PCL CORE, PE SHEATH

Fiber length, cm	Core loading, wt %	Core diam, μm	Sheath diam, μm	Sheath thickness, μm	$r_o:r_i$
100	50	320.4	326.0	2.8	1.017
200	40	250.0	258.8	4.4	1.035
200	25	320.0	331.2	5.6	1.035
400	25	225.0	241.0	8.0	1.071
400	15	270.0	289.2	9.6	1.071
600	15	240.0	266.2	13.1	1.109

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We had also intended to prepare the thin sheath system by coaxial melt extrusion. However, in a spinning trial to prepare coaxial fibers containing β-estradiol for another project (PARFR-324), we damaged our coaxial spinneret and have not yet tried melt spinning with the NGM blend. Consequently, we have not been successful in preparing the PE/PCL fibrous system.

2. Determination of DC_6 in Other Polymers

In our proposal, we listed several other polymers that are amenable to the development of steroid delivery systems. We proposed to investigate the use of these materials in the event that we were unable to prepare a fibrous system from PE and PCL capable of delivering NGM at the desired rate. To minimize the time required to identify alternative polymers, we decided to determine first the permeability of NGM in the proposed materials. These measurements were made by determining the rate of desorption into 50:50 ethanol/water solution of NGM from either fibers or films of the polymers.

a. Preparation of samples

We chose to use fibers of two of the ethylene/vinyl acetate (EVA) copolymers for the desorption studies because films of the materials are difficult to handle. Blends of 10 wt % NGM in either Vynathene EY-903 (55/45 EVA) or Vynathene EV-902 (50/40 EVA) (U.S. Industrial Chemicals Company, New York, NY) were prepared by codissolving the materials in dichloromethane and evaporating the solvent. Fibers were next prepared from the blends on the Tinius Olsen extrusion plastometer at a block temperature of 100 to 120 °C and a ram force of 2273 kPa. The fibers were collected on glass tubes and stored over desiccant until use.

Because we had already prepared monolithic fibers of NGM/PCL, we also included samples of these fibers in the desorption studies. For our other materials, we used thin films to determine the permeability product. These included four other EVA compositions, Estane 5714 (a segmented polyurethane, B. F. Goodrich Company, Cleveland, OH), and Silastic MDX-4-4210 (an RTV silicone elastomer, Dow Corning Corporation, Midland, MI). The films were prepared by solvent casting using centrifugal film-casting methods. For each polymer, a solution was prepared at a polymer concentration of 15 to 20 wt %. A weighed amount of solution was then placed into a centrifugal film-casting cup rotating at approximately 3600 rpm. After most of the solvent had evaporated, the films were removed from the cups and dried under vacuum for several days to remove the remaining solvent.

Three circular samples 35 mm in diameter were cut from each film for the desorption studies. The thickness and weight of each sample were recorded. Each film sample was then placed in a saturated solution of NGM in 50:50 ethanol/water containing excess NGM. The samples were equilibrated with the saturated solutions at 37 °C for one week to allow the concentration of NGM in the film to reach saturation. Next, the samples were removed from the NGM solutions, briefly rinsed in ethanol, blotted to remove solvent droplets, and dried for several days at 37 °C.

b. Desorption studies

We determined the desorption kinetics for NGM from each polymer by immersing triplicate samples in separate containers of 150-mL aliquots of 50 wt % aqueous ethanol solution. The sealed containers were incubated at 37 °C and agitated on low speed in an Eberbach shaker bath. Periodically, the receiving fluid from each container was exchanged for fresh aqueous ethanol and assayed for NGM by measuring the absorption peak at 247 nm on a Perkin-Elmer 575 spectrophotometer. The quantity of NGM desorbed was determined from a Beer's law plot (Figure 1) of absorbance versus NGM concentration.

The release of NGM from the monolithic fiber samples can be described mathematically by Fick's law of diffusion for devices of cylindrical geometry:

$$\frac{d(M_t/M_\infty)}{dt} = \frac{-4DC_s}{r_o^2 C_o \ln(1-M_t/M_\infty)}$$

where

$\frac{d(M_t/M_\infty)}{dt}$ = fractional rate of release of drug

D = diffusion coefficient of the drug in the polymer, cm^2/s

C_s = saturation solubility of the drug in the polymer, g/cm^3

r_o = radius of the cylinder

C_o = drug loading, g/cm^3

M_t/M_∞ = fraction of initial mass released at time t

By integrating the above equation and rearranging the terms, the following expression is obtained:

$$\left(\frac{C_o \cdot r_o}{4}\right)\left([1-M_t/M_\infty][\ln(1-M_t/M_\infty)] + M_t/M_\infty\right) = DC_s \cdot t$$

The slope of a plot of the terms on the left side of the equation versus time is the permeability product, DC_s. These calculations, summarized in Table 3, were done for the PCL and Vynathene fibers.

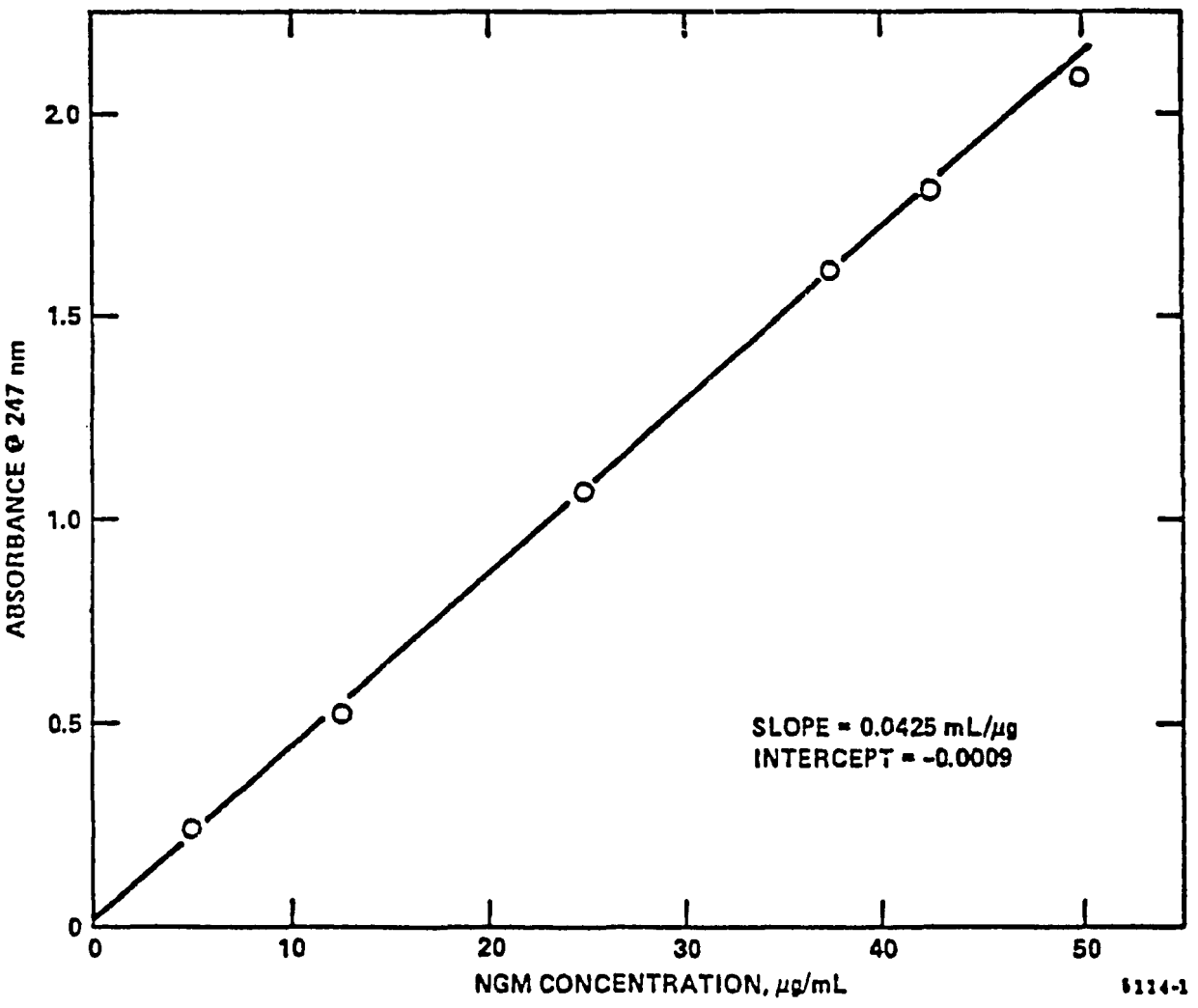

Figure 1. Beer's law plot for NGM in 50:50 ethanol/water solution.

TABLE 3. SUMMARY OF PERMEATION DATA FOR NGM IN CANDIDATE POLYMERS*

Material	Form	Replicates	$D, cm^2/s$	$C_s, g/cm^3$	$D \cdot C_s$, $g/cm \cdot s$*
Vynathene EY903-00 (55:45 EVA)	Monolithic fiber	3	--	--	7.67×10^{-8}
Vynathene EY902-00 (60:40 EVA)	Monolithic fiber	3	--	--	5.21×10^{-8}
Polycaprolactone (PCL-700)	Monolithic fiber	1	--	--	3.79×10^{-8}
Estane 5714 (Segmented polyurethane)	Film	3	6.87×10^{-8}	5.03×10^{-12}	3.47×10^{-9}
Elvax 150 (67:33 EVA)	Film	3	3.36×10^{-8}	1.21×10^{-2}	4.05×10^{-10}
Elvax 250 (72:28 EVA)	Film	3	2.50×10^{-8}	7.71×10^{-3}	1.92×10^{-10}
Elvax 350 (75:25 EVA)	Film	3	4.22×10^{-8}	9.58×10^{-3}	4.04×10^{-10}
Elvax 450 (82:18 EVA)	Film	3	6.12×10^{-9}	3.85×10^{-3}	2.36×10^{-11}
Silastic MDX-4-4210 (Polydimethylsiloxane)	Film	3	2.74×10^{-8}	9.19×10^{-3}	2.43×10^{-11}
Polyethylene (LD600)	Coaxial fiber	3	--	--	1.59×10^{-13}

*Values of DC_s shown are the mean of the individual DC_s determined for each replicate. Thus, the product of the average D and average C_s may differ slightly from the reported value of DC_s.

For the film samples, the desorption of NGM is approximately described by the following expression of Fick's law:

$$\frac{M_t}{M_\infty} = 4\left(\frac{Dt}{\pi \ell^2}\right)^{\frac{1}{2}} \text{ for } 0 < \frac{M_t}{M_\infty} < 0.6$$

where

$\frac{M_t}{M_\infty}$ = fraction of drug released at time t

D = diffusion coefficient, cm^2/s

ℓ = film thickness, cm

Figure 2 shows the fraction of NGM desorbed from samples of an EVA copolymer as a function of the square root of time. The diffusion coefficient can be obtained from the slope of the initial part of the curve and the film thickness (Table 3).

Because the film samples were initially saturated with NGM, we could also calculate values of the saturation solubility and permeability product. We calculated the saturation solubility by dividing the total mass of NGM desorbed by the film volume. From the values of C_s and D, we could then calculate DC_s, which is also shown in Table 3. The slight discrepancies between DC_s and the product of D and C_s shown are a result of the method used for averaging the individual values.

3. Alternative Designs for Fibers and Rings

a. Fibers

From the permeability data in Table 3, we developed alternative designs for the NGM delivery system. First, we selected the most suitable materials for use as the drug matrix. To achieve true zero-order release from a reservoir device, the permeability product for the drug in the core matrix must be substantially higher than in the rate-controlling membrane. Therefore, we chose the two Vynathene copolymers and PCL as the best-suited matrix polymers because of the high values of DC_s ($\approx 10^{-8}$ g/cm·s).

Next, we calculated the DC_s for NGM in the rate-controlling sheaths of fibers that would be needed to achieve the desired release of 500 μg/day. These values, shown in Table 4, are for three practical fiber designs that we should be able to fabricate in the laboratory. The values indicate that the desired sheath material should be an EVA copolymer having a low vinyl acetate content. As a first approximation, we selected the EVA composition by plotting the log of the permeability product versus copolymer composition for the four Elvax samples and PE (Figure 3). We then selected commercial materials having a composition in which the DC_s for NGM would be about 2×10^{-12} g/cm·s. Two samples, Elvax 650(88:12 EVA) and Elvax 750 (91:9), that meet this criteria were obtained for use as sheath materials. We plan to prepare by melt spinning coaxial fibers that contain the Elvax 750 as a sheath and PCL as the core.

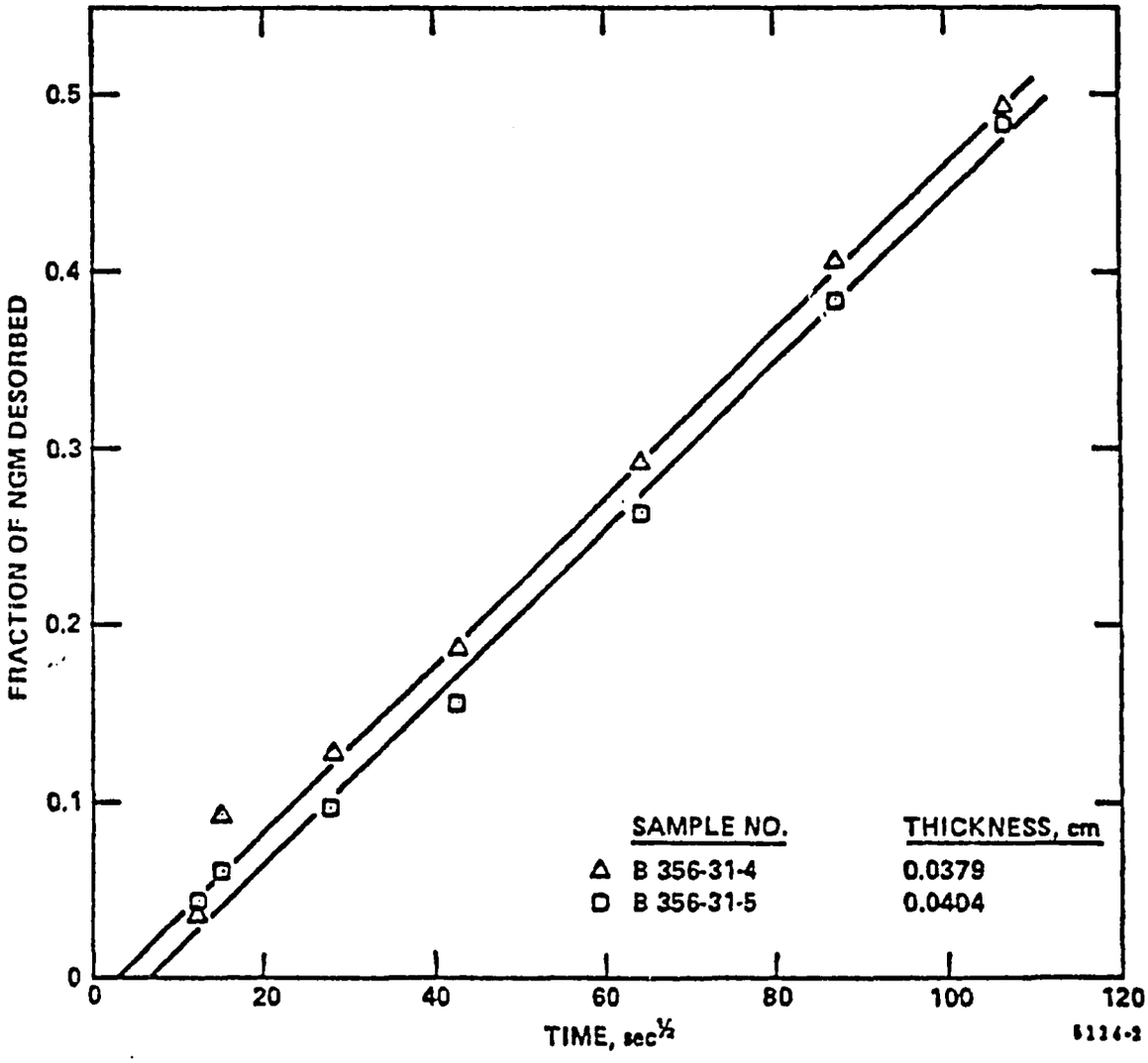

Figure 2. Desorption of NGM into 50:50 ethanol/water from Elvax 250.

TABLE 4. CALCULATED VALUES OF DC_s FOR FIBROUS DELIVERY SYSTEMS[a]

Length, cm	Core loading, wt %	Core diam, μm	Sheath thickness, μm	$r_o:r_i$	DC_s, g/cm·s
100	25	230	40	1.35	2.74×10^{-12}
200	25	160	40	1.50	1.86×10^{-12}
300	15	170	40	1.47	1.18×10^{-12}

[a] $\frac{dM_t}{dt}$ = 500 μg/day.

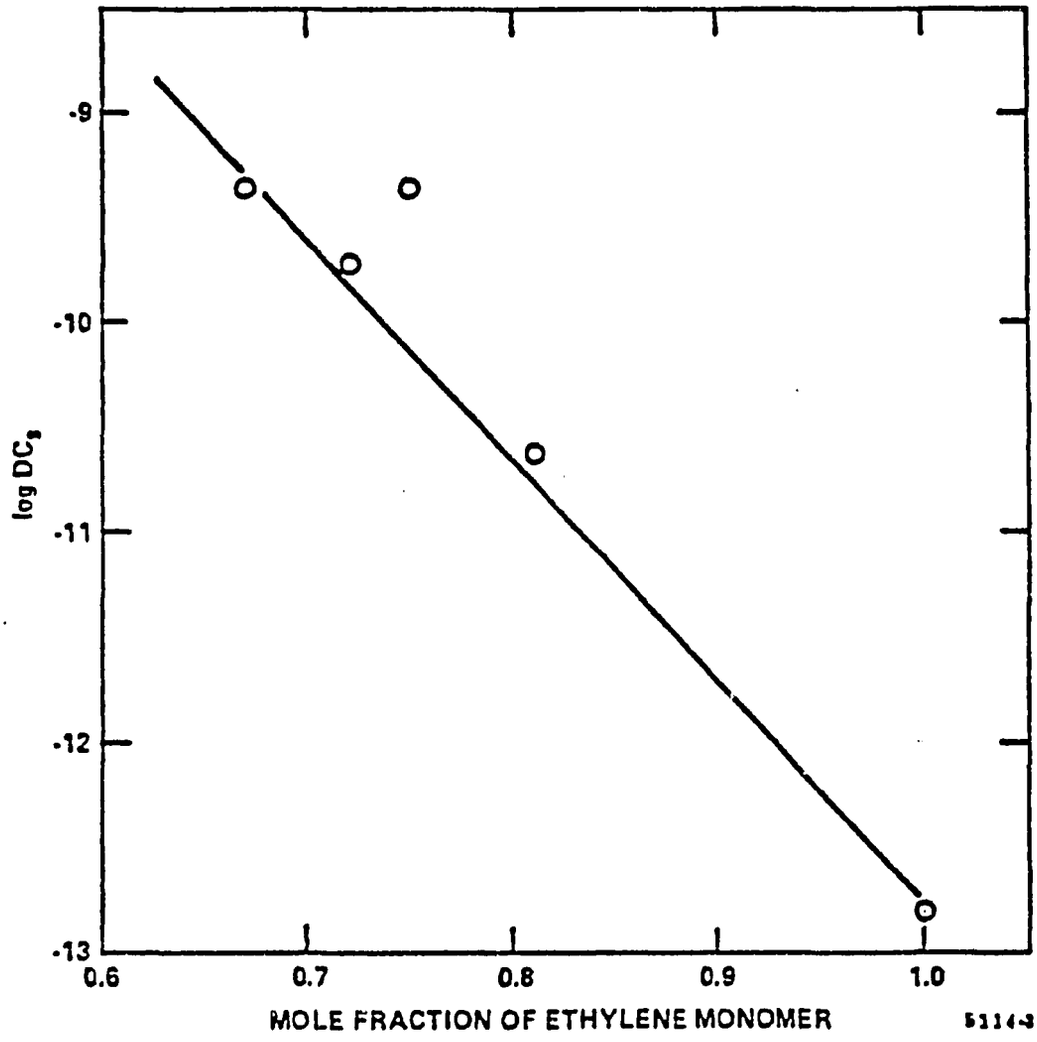

Figure 3. Effect of the composition of EVA copolymers on the permeability of NGM.

b. Triaxial rings

We have also designed a triaxial vaginal ring system based on the permeability data in Table 5. We selected Silastic MDX-4-4210 as the base ring material because it has good flexibility and is not very permeable to NGM. The low permeability of NGM in the base ring is needed to minimize the amount of NGM that will migrate from the drug-loaded layer into the ring during storage. A 10 wt % mixture of NGM in Vynathene EY 903-00 was chosen as the drug reservoir. Silastic MDX-4-4210, PE, and an EVA were chosen for the rate-controlling sheath materials. The dimensions for both baboon- and human-size triaxial ring systems are shown in Table 5.

c. Preparation of vaginal rings

In Task 3 of our proposal, we outlined the manufacture of nonmedicated vaginal rings for preclinical acceptability studies. We initiated this work early to allow ample time for preparing the Good Manufacturing Practices (GMP) protocol. Initially, we borrowed a mold from Dr. Harold Nash at the Population Council, Inc., that was designed for preparing rings from RTV silicone elastomers. The rings have an overall diameter of 58 mm and a cross-sectional diameter of 8 mm. From this mold, we designed a two-cavity mold suitable for use with RTV silicones that will also fit our injection molder. This mold has been prepared and is now ready for use.

The baboon-size vaginal ring has an overall diameter of 30 mm and a cross-sectional diameter of 5 mm. We have previously prepared a two-cavity mold to produce these rings by injection molding. Since we have selected Silastic as the base ring material, we are currently modifying the mold to accept a standard Luer syringe tip for injection of the uncrosslinked elastomer.

C. Operational Significance

Findings during the current reporting period are significant because we have identified materials suitable for the preparation of both the coaxial fibers and the triaxial ring system. Furthermore, the permeability data show that our initial selection of a base ring polymer, Silastic MDX-4-4210, is the most suitable material with respect to the storage stability of the medicated devices.

D. Unanticipated Results

That we would be unable to apply the thin PE sheath by solvent coating was not anticipated.

E. Research Design

No major changes in the proposed research design were required. However, we were unable to prepare the fibers with a thin PE sheath and found it necessary to evaluate several of the alternative polymers outlined in the proposal. While this work was included in the original outline, it has slightly delayed the in vitro release studies. This delay has not affected the project budget, and we do not anticipate any effect on the completion of the proposed work.

TABLE 5. TRIAXIAL RINGS FOR DELIVERY OF NGM[a]

Base ring dimensions, cm OD/CSD[b]	Drug Reservoir				Rate-Controlling Membrane	
	Matrix	Loading, wt %	Vol, cm^3	Thickness, μm	Material	Thickness, μm
3.0/0.5	Vynathene EY903-00	40	0.102	68	Elvax 460	701
3.0/0.5	Vynathene EY903-00	40	0.102	68	MDX-4-4210	725
5.8/0.8	Vynathene EY903-00	10	0.409	88	PE LD-600	13
5.8/0.8	Vynathene EY903-00	10	0.409	88	MDX-4-4210	2,523

[a]Estimated $\frac{dM_t}{dt}$ = 500 μg/day for 90 days.

[b]CSD = Cross-sectional diameter.

SOUTHERN RESEARCH INSTITUTE

F. Dissemination and Utilization of Research Results

Dr. Richard L. Dunn presented a paper entitled "Controlled Delivery of Contraceptive Steroids from Fibrous Polymers" at the International Symposium on Reproductive Health Care in Maui, HI, in October 1982. The paper included only results obtained on Subcontract PARFR-235 SRI. None of the data from the current subcontract have been presented.

G. Minority Personnel and Women

Ms. Brenda H. Perkins, a black female Assistant Biomathematician, helped with the data treatment and analysis. Due to the potency of NGM, the manufacturer has cautioned against females being directly involved with the fiber work. Male chemists were therefore used in all work directly involved with handling the drug and drug-loaded fibers.

H. Statement of Expenditures

Category	Subcontract budget 9/1/82-9/31/83	Expenditures for reporting period 9/1/82-1/31/83	Projected expenditures for next reporting period 2/1/83-4/30/83
Personnel and Benefits	$11,089	$5,770	$2280
Supplies	2,600	997	686
Baboon Services	12,000	—	3000
Reports and Communications	50	9	14
Liability Insurance	5,013	5,013	—
Indirect costs:			
Burden	9,758	5,018	2030
G & A	5,347	2,382	1270
Facilities cost of capital	1,604	818	337
Fee	2,751	1,151	685
TOTALS	$50,212	$21,158	$10,302

I. Acknowledgments

Mr. Kenneth R. Bynum, Assistant Chemist, conducted the laboratory experiments. Ms. Brenda H. Perkins, Assistant Biomathematician, assisted in the data analysis.

Submitted by:

John W. Gibson / by RLD

John W. Gibson
Senior Chemist
Membranes Section

Approved by:

Richard L. Dunn

Richard L. Dunn
Head, Polymer Division
Principal Investigator

SoRI-EAS-83-190
Project 5114-I
NB:B354, B416
(3::10:5) baj

EVALUATION IN SHEEP OF ORABASE® AS A VAGINAL BARRIER CONTRACEPTIVE (PARFR-326).

Quarterly Report

Submitted to:

Program for Applied Research on Fertility Regulation
Northwestern University
Suite 1525
875 North Michigan Avenue
Chicago, Illinois 60611

Submitted by:

J. P. Polidoro, Ph.D.
Arthur D. Little, Inc.
Acorn Park
Cambridge, Massachusetts 02140

March 1, 1983

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I. PROJECT REPORT SUMMARY

A. General Information

1. Title: Evaluation in Sheep of Orabase® as a Barrier Contraceptive (PARFR-326)

2. Principal Investigator:

John P. Polidoro, Ph.D.
Arthur D. Little, Incorporated
Memorial Drive Complex
Cambridge, Massachusetts 02140
(617) 864-5770, extension 2022

3. Institution: Arthur D. Little, Incorporated
Acorn Park
Cambridge, Massachusetts 02140

4. Subagreement Period

   a. Starting Date: December 1, 1982
   b. Ending Date: May 31, 1983

5. Date of Last Technical Report

   None. This is the first Quarterly Report.

6. Inclusive Dates of This Technical Report

   December 1, 1982 - February 1, 1983.

B. Narrative Summary

During this initial contract period December 1, 1982 - February 1, 1983), the following accomplishments were achieved:

1. Three animal rooms were modified to accommodate the housing of sheep.

2. Twenty ewes and two rams were purchased and acquired for the study.

3. Food, bedding and experimental materials were purchased and received.

4. All ewes were randomly assigned to groups, rooms and pens.

5. Estrous cycle lengths were established in most of the ewes.

6. Rams were evaluated for reproductive soundness (electro-ejaculation and semen evaluation).

7. Contraceptive testing and mating studies (control and treatment groups were initiated in all animals with confirmed estrous cycle lengths.

These accomplishments, in relationship to the overall research objectives of this project, equal or exceed the proposed timetable of the study.

II. SUBSTANTIVE CHEMICAL RESEARCH REPORT

A. Statement of Objectives

The objective of the proposed research is to assess, in sheep, the contraceptive efficacy of Orabase® as a novel, barrier vaginal contraceptive. The specific objectives and workscope for the contract are outlined on page 3 of the subagreement and are listed below:

1. Perform a short-term feasibility study in sheep;

2. purchase and maintain 20 ewes and 2 rams to evaluate Orabase® as a vaginal contraceptive in a series of mating studies;

3. establish normal estrous cycle lengths in all ewes over a 1-2 month period prior to initiation of the study; at estrus administer, intravaginally Orabase® paste (containing no spermicide) at the cervial os and mate the ewes to a ram of proven fertility;

4. use a total of 20 ewes in the study: one untreated control group (n=4) and four experimental groups (n=4/group). Experimental groups will be mated at 0, 2, 4 and 8 hours after Orabase® administration. Confirmation of the presence of Orabase® administration at the cervical os will be made prior to mating using a speculum and portable light source. Only animals showing complete coverage of the cervical os with Orabase® will be used at the mating study;

5. assess pregnancy by the presence of subsequent anestrus (during the anticipated estrous period) as well as by surgical confirmation (laparotomy) and examination of the reproductive tract 2-4 weeks after missed uterus;

6. compile, record and analyze all data relevant to contraceptive efficacy (pregnancy rate/group) and compare data to control animals; and

7. provide a written report of all studies to PARFR upon completion of the above mentioned experiments.

B. Progress to Date

During this initial contract period, substantial progress was made to accomplish the task outlined in the Statement of Work as described in Section II, A. of this Technical Report. Following the authorization of the subagreement by PARFR (December 1, 1982), all animals and materials necessary for the conduct of the study were acquired.

1. Animal Room Modification

Three consecutive rooms on the second floor of the newly renovated animal facility (134 Main Street) were modified to house a total of 22 sheep. A total of six sheep pens were fabricated (two pens per room) as shown in Figure 1. Pens were constructed of cyclone fencing supported by 2" x 4" wood framing. Epoxy floors of each pen were covered with closely aligned 4' x 8' sheets of particle board to facilitate sanitation procedures and increase the "footing" necessary for normal mobility of the sheep.

Mangers (hay), feeder trays (grain) and water buckets (5 gal.) were purchased or fabricated and installed in each pen. All bedding covering the particle board floors consisted of fine, pine shavings distributed in amounts sufficient for proper sanitation and husbandry. All sheep housing and modifications were inspected and approved by the Regional USDA veterinarian (Waltham, Mass.) and are in compliance with the proper farm management practices as described in the Animal Welfare Act (Title 9) and "Guide for the Care and Use of Laboratory Animals" (PHS No. 1024).

2. Animals and Maintenance

On December 20, 1982, twenty (20) mature, cycling ewes and two (2) mature, proven rams of the Dorset breed were obtained from Thomas Morris, Incorporated, a licensed breeder in Reistertown, Maryland. Prior to shipment, all sheep were Q fever tested (negative), eartagged and vaccinated for enterotoxemia, tetanus and sore mouth. A supplemental iron injection preceded shipment of the animals from Maryland to Boston. Upon arrival at Arthur D. Little, Inc., the sheep were shorn and administered a prophylactic, topical application of Paradex®; a medication for "foot rot."

Using a Table of Random Numbers (Document Geigy Scientific Tables, 6th Edition, 1968, page 131), each of the twenty ewes was randomly assigned to one of five groups: one control and four treatment groups (4 ewes each). Groups were then randomly assigned to one of five separate pens (Figures 2 and 3).

All sheep are maintained on free-standing hay (Timothy) supplemented by "cracked corn" (1 lb./sheep/day) and fresh water ad libitum. Floor bedding is changed one or two times weekly, or as needed. Room temperature and relative humidity are maintained at 65-68°F and 50%, respectively. Since sheep naturally begin "cycling" in the fall, room light is maintained on a 10 hour light/14 hour dark schedule designed to mimic the "short-day" length of fall-early winter natural lighting.

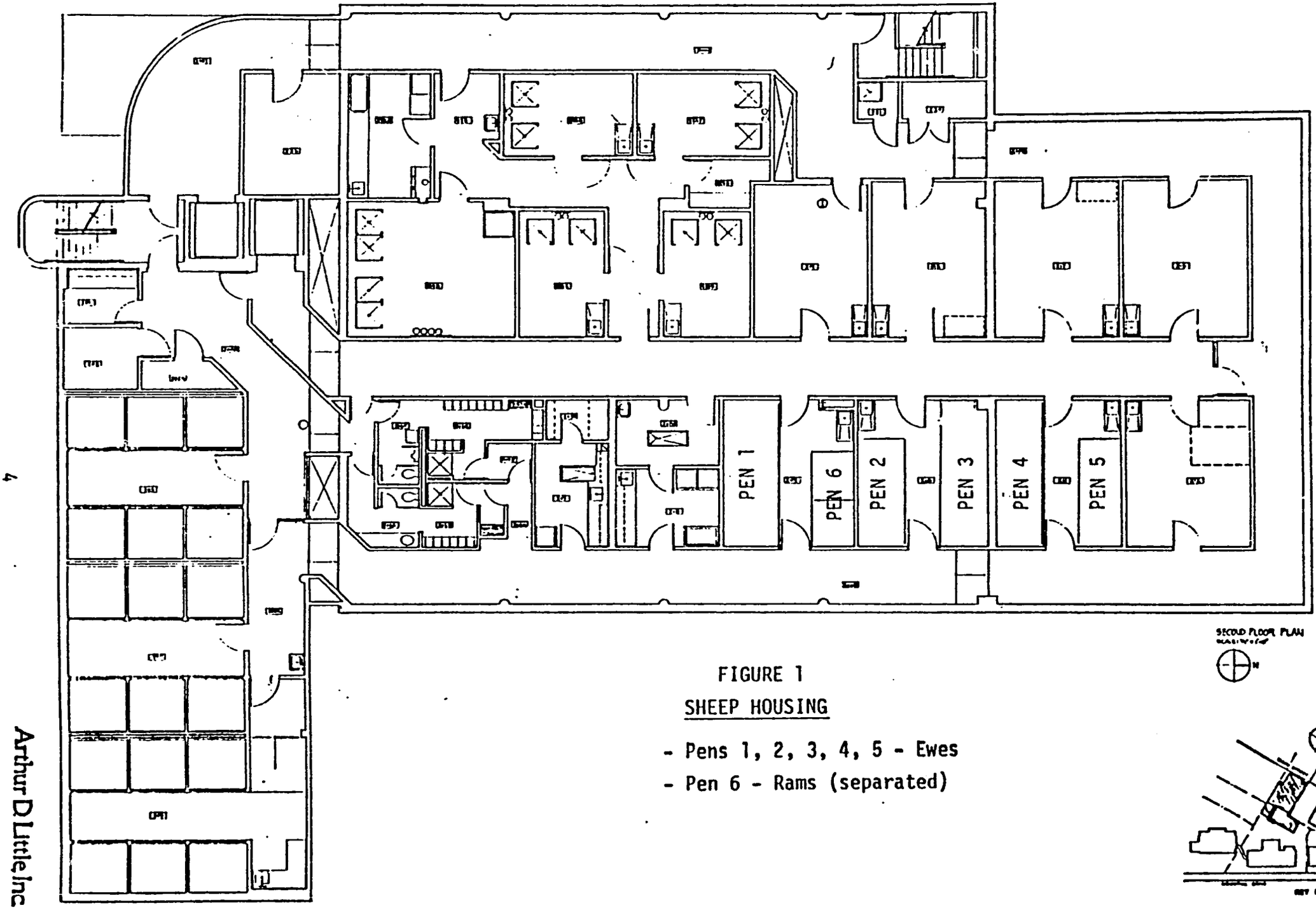

FIGURE 1

SHEEP HOUSING

- Pens 1, 2, 3, 4, 5 - Ewes
- Pen 6 - Rams (separated)

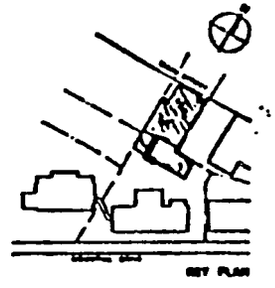

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FIGURE 2

SHEEP I.D. AND RANDOMIZATION

Sheep Ear Tag I.D.	Room Number	Pen Number	Treatment Group (hrs)
704 (ewe)	211	5	Control
706 (ewe)	209	1	2
707 (ewe)	210	3	8
711 (ewe)	210	3	8
714 (ewe)	211	4	0
715 (ewe)	210	3	8
716 (ewe)	211	4	0
718 (ewe)	210	2	4
719 (ewe)	209	1	2
720 (ewe)	211	5	Control
722 (ewe)	211	5	Control
726 (ewe)	211	4	0
733 (ewe)	209	1	2
734 (ewe)	210	3	8
735 (ewe)	211	4	0
736 (ewe)	210	2	4
737 (ewe)	210	2	4
738 (ewe)	210	2	4
739 (ewe)	211	5	Control
744 (ewe)	209	1	2
359 (ram)	209	6	-
750 (ram)	209	6	-

FIGURE 3

SHEEP GROUP AND PEN ASSIGNMENT

(2 hrs.) Pen No. 1	I.D.
	706
	719
Room 209	733
	744

(0 hrs.) Pen No. 4	I.D.
	714
	716
Room 211	726
	735

(4 hrs.) Pen No. 2	I.D.
	738
	718
Room 210	736
	737

Control Pen No. 5	I.D.
	704
	720
Room 211	722
	739

(8 hrs.) Pen No. 3	I.D.
	707
	711
Room 210	715
	734

Rams Pen No. 6	I.D.
	359
	750
Room 209	

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3. Estrous Cycle Length Determination ("heat" detection)

In general, sheep maintain a fairly regular estrous cycle length of 16-17 days. Ewes remain in estrus for 17-24 hours. Ovulation occurs generally at the end of estrus. All ewes assigned to either the control or treatment groups are checked for estrus on a daily basis (8:30-9:00 a.m.). This is achieved by bringing the ram (on a halter) into each pen and observing the ram and ewe's behavorial patterns. Naso-vaginal examination of the ewe vulval area by the ram results in "lip curl" and a desire to "mount" the female. Any ewe that will stand to be mounted is considered to be in estrus. Ewes in estrus may also show other behavioral or physiological signs of estrus e.g., "tail-wagging", general interest in the ram and cervical mucus presence in the vagina.

The ram is withdrawn from the estrous ewe after mounting to prevent penile insertion. "Heat" detection records of estrous cycle length are maintained on a daily basis for each ewe and the next anticipated "heat" is calculated and estimated 16-17 days in advance for future mating studies. Only "normally-cycling" ewes are used in the experimental contraceptive studies.

4. Ram Reproductive Soundness

Prior to the initiation of the study, a reproductive soundness examination of each ram (Nos. 359 and 750) was conducted in conjunction with the veterinary staff of the Division of Comparative Medicine at Massachusetts Institute of Technology (MIT) in Cambridge, Massachusetts. The scrotal area of each ram was examined for scrotal circumference and firmness and semen samples were collected by electroejaculation (standard Precision Electronics, Denver, Colorado) and evaluated for motility and general sperm morphology. The results are described in detail in the Appendix and indicate viable sperm in both rams. With the exception of reduced sperm motility in ram No. 750 (attributed to poor sperm collection and possible urine contamination), all other results of the evaluation appeared to be within normal limits (e.g., concentration, quality, sperm morphology and scrotal circumference). Ram No. 359, who was used for the majority of matings to date, had greater than 80% motility in this examination.

5. Experimental Design and Methods

The experimental design for the evaluation of the vaginal barrier contraceptive Orabase® consists of one control group and four experimental groups. In the control and treatment groups, ewes with established estrous cycle lengths are mated on the day they return to estrus. The experimental design for this study is shown in Table 1. With the exception of the control animals which receive no Orabase® application, each animal in a treatment group undergoes the same procedure for dose administration and mating. Estrous ewes are manually restrained and the cervix located by vaginal examination using a disposable speculum (Kleen-Spec #58501 Welch-Allen) and portable light source (Fiberoptic Illuminator #78500, Welch-Allen). A small amount of K-Y jelly (Johnson and Johnson) is applied to the speculum to facilitate insertion into the vagina. Using dry, cotton-tipped applicator sticks

TABLE 1

EXPERIMENTAL DESIGN

Group	n	Dose	Hour Mated Post Orabase® Treatment
Control	4	0	No treatment; mated within 2 hrs. of heat detection.
Orabase®-o hr.	4	1.0 ml	Immediately after dosing
Orabase®-2 hrs.	4	1.0 ml	2 hrs. after dosing
Orabase®-4 hrs.	4	1.0 ml	4 hrs. after dosing
Orabase®-8 hrs.	4	1.0 ml	8 hrs. after dosing

(Scientific Products #A5002-1), the cervix is swabbed of any cervical mucus prior to administration of the Orabase®. A 1.0 ml dose of Orabase® (Hoyt Laboratories, Needham, Mass.), pre-measured in a disposable syringe, is expressed onto the tip of a cotton swab and applied directly to the cervical os and the periphery of the cervix. Once the dose is adhered to the cervix, the speculum is gently withdrawn to avoid disturbing the placement of the formulation. The time of Orabase® application is then recorded. Prior to mating, the ewe is examined again to confirm retention of the material in the cervico-vaginal area. Orabase®-treated animals are then mated at 0, 2, 4 or 8 hours after the formulation has been in place. Each ewe is mated to a ram once or twice, depending upon the length of time of initial mounting. A second mating is generally required to observe the thrusts necessary for complete ejaculation. Following the initial mount, the time of mating is recorded. Immediately after mating, the vagina is examined for the presence of the ejaculate. This, generally, does not require a speculum and can be visualized in the exterior vaginal area.

Control animals undergo the same procedures as the treated groups except that no Orabase® is administered prior to mating.

6. Post-Coital Followup

The first indication of pregnancy or contraceptive efficacy in sheep can be determined approximately two-three weeks after mating. This would be the time of an expected return to estrus (in a normally-cycling ewe). Depending on individual estrous cycle length, ewes in which the vaginal barrier contraceptive was successful should theoretically return to estrus 16-17 days post coitum. Those ewes which become pregnant would no longer "cycle" since corpus luteum (CL) development following ovulation would prevent the return to estrus.

All ewes which do not return to estrus will undergo a mid-ventral laparatomy one month after missed "heat" to examine the uterus for the presence of a fetus. Details of the surgical procedure will be outlined in the next quarterly technical report.

7. Results to Date

The research progress and results generated during the first two months of the subagreement are shown in Table 2. As can be seen in this table, estrous cycle lengths were established in 16 of the 20 ewes. The mean estrous cycle length for the 16 ewes was 16.9 days. Of these animals, 13 or the 20 have been mated to date. Animals mated thus far represent the following control and treatment groups:

Group	n
- Controls	(4)
- Orabase®	
- 0 hr.	(3)
- 2 hr.	(3)
- 4 hr.	(1)
- 8 hr.	(2)
	13

As can be seen in Table 2, two of the four control animals (Nos. 720 and 722), which were mated, did not return to heat on the projected dates of estrus and will undergo laparatomy early in March, 1983. Four Orabase®-treated animals (Nos. 735, 706, 733 and 737), expected to return to heat during this contract period, will also undergo a laparotomy in February and March (one month following the missed heat). The remaining Orabase®-treated animals to date are scheduled to return to heat during the next contract period. These animals are Nos. 704, 739, 714, 726, 719, 711 and 734.

8. Discussion

The six ewes (2 controls, 4 treated) which did not return to heat are suspected to be pregnant and this will be confirmed or negated by surgery during the next contract period. The only animals which were anticipated to become pregnant were the two controls. The remaining four Orabase®-treated animals would be expected to return to heat if the following conditions were met:

- Proper placement of the Orabase® dose at the cervix; and
- Sufficient amounts of Orabase® applied to the cervix to act as a barrier.

Factors which cannot be controlled but would result in induction of pregnancy or the lack of "heat" are the following:

- Disturbance or removal of the dose by the ram during mating; and
- Return of the animals to "seasonal anestrus" (an event which occurs in the Spring).

Animals which were mated were examined vaginally after mating to confirm the presence of the ejaculate in the vagina. The examination was restricted to exterior vagina to avoid disturbance of the Orabase® or ejaculate in the distal cervical/vaginal area.

During this time of year (late Winter-early Spring), some sheep may become "seasonally anestrus" and the lack of "heat" could generate "false pregnancy" indications. The Dorset breed, used in this study, is known to show anestrus in some ewes as early as February and March of the normal calendar year. Since this is a factor over which we have no control, our studies were initiated as soon as possible after the subagreement was approved. Only surgical examination of the uterus which is planned in the next contract period will confirm or negate the suspected pregnancy data to date.

9. Technical Constraints

The only technical constraints on the program during the initial contract period involve some unanticipated problems with the husbandry of the sheep. The specific problems during the initial phase of this contract are listed below.

TABLE 2

SHEEP ESTROUS CYCLE AND MATING RECORD

Treatment Group	Ewe I.D.	1st Heat	2nd Heat	3rd Heat	Additional Heats	Cycle Length	Date Mated	Ram #/ # Matings	Date Expected Heat Return	Heat Yes/No	Tentative Surgery Date (preg. check)
Control											
	704	01/22/83	02/08/83			17	02/08/83	359 (2x)	02/25/83		03/25/83
	720	12/29/82	01/15/83			18	01/15/83	359 (1x)	02/03/83	No	03/02/83
	722	12/29/82	01/16/83			17	01/16/83	359 (1x)	02/01/83	No	03/01/83
	739	01/11/83	01/27/83			16	01/27/83	359 (2x)	02/14/83		03/14/83
Orabase®											
0 Hour	714	01/22/83	02/08/83			17	02/08/83	359 (2x)	02/25/83		03/25/83
	716	01/16/83	02/05/83			20					
	726	01/08/83	01/25/83			17	01/25/83	359 (2x)	02/11/83		03/11/83
	735	12/30/82	01/17/83			18	01/17/83	359 (1x)	02/03/83	No	03/02/83
2 Hour	706	12/22/82	01/08/83			17	01/08/83	359 (1x)? 750 (2x)	01/25/83	No	02/25/83
	719	01/19/83(s)	02/02/83			14	02/02/83	359 (2x)	02/16/83		03/16/83
	733	12/27/82	01/12/83			16	01/12/83	359 (2x)	01/28/83	No	02/28/83
	744	01/23/83									
4 Hour	718	01/12/83	01/27/83			15					
	736	12/22/82	01/09/83	01/27/83		18					
	737	01/01/83	01/18/83			17	01/18/83	359 (2x)	02/04/83	No	03/04/83
	738	01/03/83(s)									
8 Hour	707	01/18/83									
	711	01/23/83	02/08/83			16	02/08/83	359 (2x)	02/24/83		03/24/83
	715	01/19/83									
	734	01/08/83	01/25/83			17	01/25/83	359 (2x)	02/11/83		03/11/83
						$\bar{x}$ = 16.9					

(s) = suspect heat.

Arthur D. Little, Inc.

a. Ram No. 750 appeared to detect estrus in ewes but showed little desire to mate. Ram No. 359 has done well in both tasks and has serviced most of the ewes in the initial phase of the contract. We intend to utilize No. 359 for the 7 remaining animals to be mated.

b. Ewe No. 710, which arrived with the initial flock at the beginning of the study, showed chronic, irregular estrus signs and was returned to the Maryland breeder and replaced by ewe No. 738.

c. Ewe No. 738, 18 days after arrival, became ill and was diagnosed by the veterinarian to have a thiamine (Vitamin B_1) deficiency. Hematology and clinical chemistries were performed on the serum from No. 738 as a precaution. No adverse values were noted. A Vitamin B_1 injection (600mg IM) corrected the problem and the animal now appears healthy.

d. Ewe No. 716 came into estrus, was treated with Orabase®, but failed to allow the ram to mate. This generally occurs if the animal is "coming into" or "going out of" full estrus. Attempts will be made to mate her at a later date.

e. Although many sheep will show some nasal discharge (from shipping and transport), two ewes (711, 737) had chronic respiratory problems which required antibiotic therapy for three weeks (Bicillin). Nasal cultures showed growth of E. coli and Pasturella, both of which were sensitive to Bicillin treatment.

f. Two ewes (Nos. 716 and 718) showed psychic signs of estrus but did not undergo complete vaginal relaxation for the speculum. They were considered not to be in complete estrus and will be mated at a subsequent date.

C. Unanticipated Results - None to date.

D. Research Design

There have been no significant modifications in the research design during the current reporting period. The use of laparoscope in lieu of surgery, to examine the ewes for pregnancy, was proposed by our veterinarian for consideration. Should we decide to use this method, prior approval would be acquired from PARFR.

E. Operational Significance

No results have been confirmed to date relevant to the contraceptive efficacy of the Orabase® in the treated sheep. This data should be initially available during the next quarter of the subagreement and will be compared to relevant studies in the literature at that time.

F. Dissemination and Utilization of Research Results

1. Project Output - none to date.

2. Dissemination - none to date.

G. Involvement of Minority Personnel and Women

Personnel: Pamela Durant
Involvement: Secretary
Future Involvement: secretarial tasks, reports
Special Problems: None

H. Work Plan for Coming Reporting Period

The work plan for the upcoming reporting period will be a continuation of the original statement of work already in progress. The anticipated accomplishments will be: 1) the mating of the remaining sheep; 2) followup pregnancy checks (lack of estrus; surgery) in sheep already mated; and 3) collation of data relevant to contraceptive efficacy.

Procedures to be used during the remainder of the study will remain the same as described earlier in this report.

The only factor which could impede the proposed accomplishments would be the ceasation of reproductive "cycling" due to "seasonal anestrus."

We anticipate the additional involvement of personnel and institutions would only concern specialized veterinary personnel at MIT should any unusual problems arise in the health of the sheep. The additional veterinary help would be on an ad hoc basis.

No dissemination or utilization of findings are planned during the next quarterly contract period.

I. Budget Forecast*

Direct Materials	$2,500.00
- Food, bedding, miscellaneous husbandry	
- Pharmaceuticals	
- Surgical supplies	

Direct Labor	5,600.00
- Principal Investigator - Biological Technician - Animal Caretaker	
Other Direct Costs	100.00
- Veterinary services	
	$8,200.00 Total

*Additional financial ramifications might occur should veterinary diagnostic tests be required in specialized health problems of the sheep. Extraordinary veterinary services (hematology, cultures and diagnostic procedures) were not anticipated for or budgeted for in the original proposal.

APPENDIX

BREEDING SOUNDNESS EXAMINATION: ADL 1/4/83

Ram No. 359: No physical abnormalities detected
Scrotal circumference = 13.5 inches
Semen collection: satisfactory
Gross appearance of semen: satisfactory
Microscopic examination of unstained, undiluted semen: good wave pattern, ≥80% motile

(DCM #83-06)* Stained smear (100 cells counted):

	91 normal
I° abnormal	1 head
	5 midpiece
	2 tail
II° abnormal	1 tail

occ., normal epithelial cells

Ram No. 750: No physical abnormalities detected
Scrotal circumference = 14 inches
Gross appearance of semen: less viscous than normal, satisfactory color
Microscopic examination of unstained, undiluted semen: fair to good wave pattern, ~50% motile

(DCM #83-07)* Stained smear (100 cells counted):

	87 normal
I° abnormal	3 head
	6 midpiece
	1 tail
II° abnormal	3 tail

occ., normal epithelial cells

Interpretive Note: Ram #359 fulfills all criteria examined for breeding soundness. The primary, midpiece abnormalities observed were mostly broken midpieces, which may be an artifact in smear preparation. Semen is judged satisfactory in this regard since there are fewer than 10% abnormalities.

Ram #750 is more difficult to evaluate due to the less successful ejaculation. Semen was obtained without complete erection, and therefore likely contaminated with preputial fluids and urine. This may account for the lower viscosity, motility, and morphology values. As the obtained values are not severely abnormal, this animal may perform adequately (especially if the semen sample was not representative).

*M.I.T. Div. Comp. Med. Report No.

S.M. Niemi, DVM

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FEB 14 1983

Family Health International • Research Triangle Park, NC 27709 USA • 919/549-0517 • Telex 579442

February 10, 1983

Ms. Diane Krier-Morrow
PARFR
Northwestern University
Suite 1525
875 N. Michigan Avenue
Chicago, Illinois 60611

Study: 780
Centers: 026, 298, 907

Dear Ms. Krier-Morrow:

As promised, I am attaching a copy of the final report on the collagen sponge. The numbers have not changed significantly from the previous report but at least one can now say all processable data has been analyzed.

A copy of this same report will be sent directly to Dr. Chvapil today.

with best regards,

Susan

Susan L. McIntyre
Regional Coordinator
International Projects Department

SLM/cw/39
Attachment

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EVALUATION OF THE COLLAGEN SPONGE
AT THREE CENTERS

Prepared for

Dr. Alfredo Goldsmith

PARFR

Centers 026, 298, 907

Study 780

January 1983

International Fertility Research Program

(a program of Family Health International)

Research Triangle Park, North Carolina 27709

USA

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TABLE VII

Reasons for Discontinuation

Reason	Hungary (N = 14) No.	England (N = 55) No.	United States (N = 61) No.	Total (N = 130) No.
Accidental pregnancy				
Method related	1	5	4	10
Patient related*	0	3	2*	5
Unknown	0	0	1	1
Planning pregnancy	0	2	0	2
Allergic reaction/vaginal infections/pain				
Soreness	0	1	0	1
Other medical				
Cystitis	0	1	0	1
Product-related				
Vaginal dryness	0	6	3	9
Troublesome, messy	0	1	2	3
Insertion difficulty	1	1	0	2
Unspecified use difficulty	1	1	0	2
Discomfort				
Male	2	2	4**	8
Unspecified	6	0	2	8
Other personal				
No confidence in method	0	5	11	16
Prefers another method	0	1	2	3
Moving from clinic	0	0	2	2
No partner	0	0	1	1
Partner objects	0	0	1	1
Total Discontinuations	11	29	35	75

*One of these pregnancies was conceived before admission to the study and is excluded from the life-table rates in Table VI.

**One woman who discontinued due to male discomfort subsequently became pregnant while using a diaphragm.

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EVALUATION OF THE COLLAGEN SPONGE
AT THREE CENTERS

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I. INTRODUCTION

In June 1981, a study of the Collagen sponge was initiated by the Program for Applied Research in Fertility Regulation at three centers--the Noi Klinika in Debrecen, Hungary, the Margaret Pyke Center in London, England and the Center for Reproductive Health in Chapel Hill, North Carolina. Devices were given to 14 women in Hungary, 55 women in England and 61 women in the US. In order to participate in the study, volunteers had to be generally healthy women between 18 and 40 years of age who were not currently pregnant, not known to be sterile or infertile, were sexually active, had no anatomic abnormalities that might prevent successful use of the sponge and had previous experience with a contraceptive method other than withdrawal or condoms.

The study was designed to evaluate the effectiveness, safety and acceptability of the Collagen sponge. The sponge is made from collagen fibrous protein isolated from bovine skin and exerts its contraceptive effect by acting as a physical barrier to the sperm and through its ability to absorb semen much in excess of its own weight.

Women wishing to participate and meeting the eligibility requirements were given a complete pelvic examination. Each woman received a supply of two 7 cm and two 6 cm sponges with instructions to begin use with the larger sponge, along with detailed instruction on the proper insertion, removal and maintenance of the sponges. Follow-up of the women was scheduled at monthly intervals. Contact at 2, 4, 5, 7, 8, 10 and 11 months was to be made by telephone; clinic visits and laboratory tests were scheduled for 1, 3, 6, 9 and 12 months after admission to the study.

Analysis is based on sociodemographic and medical data recorded on standard reporting forms by the staff of the centers. Data were sent to the International Fertility Research Program (IFRP, a program of Family Health International) for scanning, keypunching and computer analysis.

II. PATIENT CHARACTERISTICS

Sociodemographic Characteristics

Selected characteristics of the women in each of the three centers are shown in Table I. The majority of the women were well-educated, employed and resided in urban areas. Women in Hungary were generally older, of higher parity and more likely to be married than those in the England and US centers. The overall mean age was 28.6 years and overall mean parity was 0.5 live births.

Contraceptive Practice

Volunteers reported the contraceptive method used prior to acceptance of the Collagen sponge (Table II). Overall, 44.7% of the women had been using a diaphragm or cervical cap, 19.2% were using orals and 14.6% were using an IUD. IUD use was prevalent in the Hungarian center (85.7%) and less common in the other two centers. In England, 16.4% of the women had been using the Collatex sponge, a barrier device containing spermicide. The highest prevalence of pill use was in England (29.1%). The great majority of women in England and the US (89.1% and 96.7%, respectively) had prior experience with a female barrier method. Most of these women had used a diaphragm or cervical cap at some time in the past and described this experience as extensive. Only three (21.4%) women in the Hungarian center had experience with a female barrier method.

Medical History and Physical Examination

Medical history and physical examination data appear in Table III. The majority of the women (64.6%) reported no vaginal infections in the preceding year. Approximately one third of the women in the England and US centers had had a vaginal infection in that time.

At physical examination, 34.5% of the women in England and 9.8% of the women in the US tested positive for monilia. Four women in Hungary (28.6%) had a vaginal infection upon examination (Table III). Fourteen women (25.5%) in England had cervical erosion, and seven women in the same center (12.7%) had cervical cysts. One woman in the US center tested positive for gonorrhea; the Pap smear of another woman in that center showed dysplasia. In England, one woman was diagnosed as having endometriosis and another woman had fibroids.

III. RESULTS

Product Evaluation

Product-related problems refer to difficulties experienced by the woman and/or her partner during use of the sponge (Table IV). Insertion problems were reported by 21 (34.4%) women in the US, five (35.7%) women in Hungary and 27 (49.1%) women in England. The most common reason given was that the sponge was too large. Retention problems were reported by 26.2% of the women in the US and 30.9% of the women in England. Chief among the retention problems cited was the tendency of the sponge to slip.

The majority of women at all three centers had method-related complaints. Frequently, women in England complained of excessive absorption of vaginal secretions and resulting dryness or said that the partner had

discomfort. In Hungary, there were four instances of discomfort during use. The overall complaint rate was 62.3% (Table IV).

Sponge removal problems were noted by 58.2% of the women in England and 37.7% of the women in the US, mainly difficulty in reaching the device and tearing of the sponge during removal. Physical changes in the device were commonly reported in England and the US. Fragmentation was reported by 31 women in England (56.4%) and 13 women in the US (21.3%).

Medical Method-related Complications

Medical method-related complications are defined as clinically significant problems that developed during use of the sponge. These are listed in Table V. Twenty-two women in England (40.0%) tested positive for monilia during the follow-up period and eight women (14.5%) had a cervical condition. Three women in the US (4.9%) were diagnosed with monilia during follow-up. Overall, 30.0% of the women had a clinically significant complication.

Discontinuation

One- and three-month cumulative gross life-table rates per 100 women are presented in Table VI. Sample sizes are small so that the center-specific life-table rates are not meaningful and only the pooled data are shown. At one month, the total termination rate was 18.1 with a follow-up rate of 92.1%. The three-month termination rate was 45.5 and follow-up was 83.5%.

As seen in Table VII, there were sixteen accidental pregnancies in these three centers. One woman was unknowingly pregnant at admission. There was only one discontinuation for medical reasons--a woman in England

developed cystitis. Sixteen women terminated due to discomfort. Among the 16 women who terminated for product-related reasons, nine cited excessive absorption and resulting vaginal dryness. There were 23 terminations for other personal reasons; sixteen women withdrew from the study because they had no confidence in the method.

IV. SUMMARY

A study of the Collagen sponge was conducted at three centers in Debrecen, Hungary, London, England and Chapel Hill, North Carolina. A total of 130 women were admitted, most of whom had prior experience with barrier methods. Approximately one third of the women had some sort of vaginal infection at admission.

More than one third of the women reported insertion problems and more than one quarter reported retention problems. The majority of the women had product-related complaints, particularly that the partner felt discomfort during use or that the sponge caused excessive vaginal dryness. Removal problems and physical changes in the device were also common.

Those women with monilia at admission probably contributed heavily to the medical method-related complications noted at follow-up, mainly monilia (20.0% of the women). There was a total of 75 discontinuations (57.7%). The overall life-table termination rate at three months was 45.5 with a follow-up rate of 83.5%. Eleven of the 15 accidental pregnancies after admission occurred within three months. Other personal reasons accounted for the greatest number (23) of discontinuations; the major reason cited was a lack of confidence in the method. There were 16 terminations for

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product-related reasons, mainly vaginal dryness, and 16 terminations due to male or female discomfort.

crx355

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TABLE I

Selected Patient Characteristics

Characteristic	Hungary (N = 14) No.	%	England (N = 55) No.	%	United States (N = 61) No.	%	Total (N = 130) No.	%
Age								
<20	0	-	0	-	2	3.3	2	1.5
20-24	0	-	11	20.0	14	23.0	25	19.2
25-29	2	14.2	20	36.4	29	47.4	51	39.3
30-34	6	42.9	21	38.1	9	14.8	36	27.7
35+	6	42.9	3	5.5	7	11.5	16	12.3
Mean	33.6		28.5		27.5		28.6	
Education (years)								
<6	0	-	1	1.8	0	-	1	0.8
7-9	7	50.0	1	1.8	0	-	8	6.2
10-12	5	35.7	17	30.9	5	8.2	27	20.8
13+	2	14.3	36	65.5	56	91.8	94	72.2
Median	9.5		13.2		13.9		13.4	
Marital status								
Never married	1	7.1	4	7.3	27	44.3	32	24.6
Currently married	13	92.9	21	38.2	19	31.1	53	40.8
Consensual union	0	-	26	47.3	0	-	26	20.0
Separated/divorced	0	-	4	7.2	15	24.6	19	14.6
Gainfully employed								
No	1	7.1	9	16.4	16	26.2	26	20.0
Yes	13	92.9	46	83.6	45	73.8	104	80.0
Residence								
Urban	14	100.0	53	96.4	51	83.6	118	90.8
Rural	0	-	2		None			0
1	3	21.4	8	14.5	8	13.1	19	14.6
2	11	78.6	4	7.3	6	9.8	21	16.2
3	0	-	1	1.8	1	1.6	2	1.5
Mean	1.8		0.3		0.4		0.5	

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Table I (cont'd)

Characteristic	Hungary (N = 14) No.	%	England (N = 55) No.	%	United States (N = 61) No.	%	Total (N = 130) No.	%
Number of additional children wanted								
None	13	92.9	7	12.7	20	32.8	40	30.8
1	1	7.1	12	21.8	12	19.7	25	19.2
2	0	-	25	45.5	18	29.5	43	33.0
3+	0	-	7	12.7	5	8.2	12	9.3
Unspecified	0	-	4	7.3	6	9.8	10	7.7
Mean	0.1		1.7		1.2		1.3	

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TABLE II

Prior Contraceptive Use

	Hungary (N = 14)		England (N = 55)		United States (N = 61)		Total (N = 130)	
	No.	%	No.	%	No.	%	No.	%
Previous method								
Orals	0	-	16	29.1	9	14.8	25	19.2
IUD	12	85.7	0	-	7	11.5	19	14.6
Diaphragm/cervical cap	0	-	21	38.2	37	60.6	58	44.7
Collatex sponge	0	-	9	16.4	0	-	9	6.9
Condom	0	-	6	10.9	3	4.9	9	6.9
Foam/jelly/suppository	2	14.3	1	1.8	2	3.3	5	3.8
Douching	0	-	1	1.8	0	-	1	0.8
Rhythm/withdrawal	0	-	1	1.8	3	4.9	4	3.1
Primary experience with female barrier method								
None	11	78.6	6	10.9	2	3.3	19	14.6
Diaphragm/cervical cap	1	7.1	36	65.5	53	86.9	90	69.3
Collatex sponge	0	-	7	12.7	0	-	7	5.4
Foam	0	-	4	7.3	3	4.9	7	5.4
Jelly	2	14.3	0	-	1	1.6	3	2.3
Suppository	0	-	2	3.6	0	-	2	1.5
Combination	0	-	0	-	2	3.3	2	1.5
Extent of experience with female barrier method								
Limited	2	14.3	6	10.9	11	18.0	19	14.6
Extensive	1	7.1	43	78.2	48	78.7	92	70.8
Unspecified	11	78.6	6	10.9	2	3.3	19	14.6

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TABLE III

Medical History and Physical Examination

	Hungary (N = 14)		England (N = 55)		United States (N = 61)		Total (N = 130)	
	No.	%	No.	%	No.	%	No.	%
Number of vaginal infections in past year								
None	6	42.9	38	69.2	40	65.6	84	64.6
1	7	50.0	13	23.6	13	21.3	33	25.4
2	0	-	1	1.8	5	8.2	6	4.6
3	0	-	2	3.6	1	1.6	3	2.3
4+	0	-	1	1.8	2	3.3	3	2.3
Unspecified	1	7.1	0	-	0	-	1	0.8
Vaginal conditions								
Monilia	2	14.3	19	34.5	6	9.8	27	20.8
Trichomonas	1	7.1	1	1.8	0	-	2	1.5
Nonspecific inflammation	1	7.1	0	-	2	3.3	3	2.3
Cervical conditions								
Eversion	1	7.1	2	3.6	0	-	3	2.3
Erosion	0	-	14	25.5	0	-	14	10.8
Cysts	0	-	7	12.7	0	-	7	5.4
Unspecified	2	14.3	0	-	0	-	2	1.5
Other gynecologic conditions								
Gonorrhea	0	-	0	-	1	1.6	1	0.8
Cervical dysplasia	0	-	0	-	1	1.6	1	0.8
Endometriosis	0	-	1	1.8	0	-	1	0.8
Fibroids	0	-	1	1.8	0	-	1	0.8
Unspecified	1	7.1	0	-	0	-	1	0.8

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TABLE IV

Product-related Problems*

	Hungary (N = 14)		England (N = 55)		United States (N = 61)		Total (N = 130)	
	No.	%	No.	%	No.	%	No.	%
Insertion problems								
Uncomfortable	0	-	2	3.6	2	3.3	4	3.1
Sponge not inserted high enough	0	-	3	5.5	0	-	3	2.3
Slippery, messy	0	-	0	-	2	3.3	2	1.5
Sponge too large	0	-	10	18.2	3	4.9	13	10.0
Sponge too flexible	0	-	0	-	1	1.6	1	0.8
Uncertainty as to position over cervix	0	-	2	3.6	1	1.6	3	2.3
Folds, rolls, curls	0	-	1	1.8	1	1.6	2	1.5
Unspecified	5	35.7	15	25.5	13	21.3	32	24.6
Total women with insertion problems	5	35.7	27	49.1	21	34.4	53	40.8
Retention problems								
Tendency to slip	0	-	15	27.3	11	18.0	26	20.0
Turns over	0	-	5	9.1	2	3.3	7	5.4
Unspecified	0	-	0	-	4	6.6	4	3.1
Total women with retention problems	0	-	17	30.9	16	26.2	33	25.4
Complaints from patient or partner								
Abnormal menstrual bleeding	0	-	0	-	1	1.6	1	0.8
Male discomfort	4	28.6	17	30.9	15	24.6	36	27.7
Less spontaneity of sex	0	-	1	1.8	0	-	1	0.8
Heavy or full feeling	0	-	5	9.1	2	3.3	7	5.4
Discomfort	4	28.6	4	7.3	7	11.5	15	11.5
Excessive absorption/ dryness	1	7.1	19	34.5	5	8.2	25	19.2
No confidence in method	0	-	3	5.5	0	-	3	2.3
Odor	0	-	1	1.8	6	9.8	7	5.4
Unspecified	3	21.4	0	-	2	3.3	5	3.8
Total women with method-related complaints	11	78.6	37	67.3	33	54.1	81	62.3

Table IV (cont'd)

	Hungary (N = 14)		England (N = 55)		United States (N = 61)		Total (N = 130)	
	No.	%	No.	%	No.	%	No.	%
Removal problems								
Difficulty reaching device	0	-	16	29.1	8	13.1	24	18.5
Sponge tore during removal	0	-	17	30.9	12	19.7	29	22.3
Unspecified	1	7.1	9	16.4	7	11.5	17	13.1
Total women with removal problems	1	7.1	32	58.2	23	37.7	56	43.1
Changes in the device								
Fragmentation	1	7.1	31	56.4	13	21.3	45	34.6
Color change	0	-	0	-	6	9.8	6	4.6
Decrease in size	0	-	1	1.8	4	6.6	5	3.8
Device hardened	0	-	1	1.8	4	6.6	5	3.8
Device wrinkled/puckered	0	-	0	-	1	1.6	1	0.8
Molded, mildewed	0	-	1	1.8	7	11.5	8	6.2
Unspecified	1	7.1	1	1.8	2	3.3	4	3.1
Total women with changed devices	2	14.3	32	58.2	29	47.5	63	48.5

*More than one of each type of problem may be recorded for each woman.

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TABLE V

Medical Method-Related Complications

Complication	Hungary (N = 14) No.	%	England (N = 55) No.	%	United States (N = 61) No.	%	Total (N = 130) No.	%
Monilia	1	7.1	22	40.0	3	4.9	26	20.0
Nonspecific inflammation	0	-	0	-	1	1.6	1	0.8
Cervical conditions								
Eversion	0	-	2	3.6	0	-	2	1.5
Erosion	0	-	5	9.1	0	-	5	2.8
Cervicitis	0	-	0	-	2	3.3	2	1.5
Cysts	0	-	1	1.8	0	-	1	0.8
Allergic reaction	0	-	0	-	1	1.6	1	0.8
Other complications								
Cystitis	0	-	1	1.8	0	-	1	0.8
Bladder discomfort	0	-	0	-	1	1.6	1	0.8
Vaginitis	0	-	0	-	1	1.6	1	0.8
UTI	0	-	0	-	1	1.6	1	0.8
Unspecified	0	-	0	-	2	3.3	2	1.5
Total women with medical method-related complications	1	7.1	26	47.3	12	19.7	39	30.0

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TABLE VI

One- and Three-month Cumulative Gross Life-table Rates Per 100 Women, Pooled Data

	One Month		Three Month	
	#Events	Rate ± S.E.	# Events	Rate ± S.E.
Pregnancy	2	1.8 ± 1.3	11	13.4 ± 3.9
Discontinuation	20	16.6 ± 3.4	41	37.0 ± 4.7
Total termination	22	18.1 ± 3.5	52	45.5 ± 4.7
Women at risk	121.5		66.0	
Follow-up rate (%)*	92.1		83.5	

*Percentage of women at risk of termination who were actually observed.

TABLE VII

Reasons for Discontinuation

Reason	Hungary (N = 14) No.	England (N = 55) No.	United States (N = 61) No.	Total (N = 130) No.
Accidental pregnancy				
Method related	1	5	4	10
Patient related*	0	3	2*	5
Unknown	0	0	1	1
Planning pregnancy	0	2	0	2
Allergic reaction/vaginal infections/pain				
Soreness	0	1	0	1
Other medical				
Cystitis	0	1	0	1
Product-related				
Vaginal dryness	0	6	3	9
Troublesome, messy	0	1	2	3
Insertion difficulty	1	1	0	2
Unspecified use difficulty	1	1	0	2
Discomfort				
Male	2	2	4**	8
Unspecified	6	0	2	8
Other personal				
No confidence in method	0	5	11	16
Prefers another method	0	1	2	3
Moving from clinic	0	0	2	2
No partner	0	0	1	1
Partner objects	0	0	1	1
Total Discontinuations	11	29	35	75

*One of these pregnancies was conceived before admission to the study and is excluded from the life-table rates in Table VI.

**One woman who discontinued due to male discomfort subsequently became pregnant while using a diaphragm.

FINAL REPORT PARFR 212(85N)

Testing of a Modified Contraceptive Diaphragm

Milos Chvapil, MD, PhD, DSc

Department of Surgery
University of Arizona Health Sciences Center
Tucson, Arizona 85724
602-626-6637

Signature of Investigator _____

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Summary

Two major lines of testing of the sponge diaphragm have been conducted since the last progress report:

1. In order to explain the high incidence of dripping in women testing the sponge diaphragm containing the spermicide nonoxynol-9 (N-9), we repeated the experiment to quantitate the volume of vaginal secretion formed per 12 hours in the three types of sponges (plain sponges, sponges containing N-9 in the form of a cream, and sponges containing N-9 in the form of a solution) used in Phase B of this program, "Testing of the Release of a Spermicidal Detergent from Collagen Sponge Diaphragm." However, instead of using the sponge diaphragm, we used collagen sponge cylinders (2.5 x 10 cm) in order to quantitate the collection of vaginal secretion. The three types were a) plain cylinders, b) cylinders containing N-9 cream, and c) cylinders containing N-9 solution. No differences in the performance among the sponge cylinders were observed from the approximately 10 ml of secretion collected in 12 hours. Thus, we were not able to reproduce the high dripping rate in users of the diaphragm sponges soaked with the N-9 solution.

2. Phase C of this program, "Postcoital Test," so far has been tested in eleven volunteers, without and with a sponge diaphragm containing 100 mg of N-9. The results show a high incidence (approximately 40%) of motile spermatozoa in either exo- or endocervical mucus with the use of the sponge. I propose that the testing be discontinued for the reasons shown in the following report.

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Determination of Vaginal Secretion after Using Various Types of Collagen Sponges Intravaginally

In our previous progress report, we found that those volunteers using the collagen sponge diaphragm containing a solution of 100 mg of nonoxynol-9 (N-9) complained of intensive dripping when the sponge was retained in the vagina for longer than 12 hours. These complaints were significantly more frequent from the users of the collagen sponge diaphragms containing the solution than from the users of the plain collagen sponge diaphragms or the collagen sponges soaked with 100 mg of N-9 in the form of a cream.

At that time, we felt that the easy accessibility of N-9 in the solute form to the vaginal mucosa might be causing an irritation which manifested an increased volume of vaginal secretion.

In order to obtain quantitative data, the entire testing program was repeated. Instead of using sponge diaphragms, collagen sponge in the form of cylinders (2.5 x 10 cm) were used. These were soaked in a buffer of pH 3.7, impregnated with 100 mg N-9 Ortho cream, or dipped in a solution that placed 100 mg N-9 in the sponge. Five volunteers were asked to insert a preweighed, dry sponge tampon through an applicator and to leave the tampon in the vagina for 12 hours. All volunteers were requested to test the sponge during ovulation (between the twelfth and sixteenth days of their cycle). After removal, the sponge was immediately inserted into a plastic bag, sealed, and delivered to our laboratory where the weight of the sponge was determined. As shown in Table 1, there is no difference in weight which corresponds to an average of 10 g of increase of the weight of the sponge during 12 hours of sponge retention in the vagina. Thus, we conclude that we were not able to reproduce the previous observation, namely that sponges soaked with solution of N-9 produce more intensive vaginal secretion resulting in dripping. The interesting aspect of this study is the observation of 20 ml of vaginal secretion formed per 24 hour time period.

It is my understanding that, so far, no one has presented information on the volume of vaginal secretion formed during different days of the cycle. Collagen sponge, with its enormous fluid-binding capacity, makes it possible to absorb vaginal secretion and to quantitatively evaluate this phenomenon. In the past, it has been our observation that collagen sponges, left in the vagina for a few days, contained at least 60-70 ml of fluids. This observation has been repeatedly made by Dr. Heine who considered these sponges to perform less as a vaginal barrier because their fluid-binding capacity was eliminated.

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Postcoital Test in Eleven Volunteers Using
Using Collagen Sponge with 100 mg of Nonoxynol-9

We recruited a total of sixteen volunteers who used an IUD or had ligated tubes as an alternative contraceptive method. A standard postcoital test, as described by us in a previous report, was used. So far eleven volunteers were tested during the ovulation phase without and with the sponge diaphragm in place. Although we requested that the volunteers visit our clinic no later than 2 hours after intercourse, most of them arrived between 2 and 3 hours later. Only one volunteer came 5 hours after intercourse. The data, summarized in Table 2, clearly indicates that all women accepted had sufficient spermatozoa counts in both exo- and endocervix without the sponge. The table also shows that from eleven volunteers, five had one or more viable spermatozoa in exo- or endocervix after the sponge diaphragm was removed from the vagina. We were rather surprised about such a high failure rate and felt that this might have been caused by contamination of the mucus during the removal of the sponge. However, washing the vagina prior to the sponge removal did not improve the results of the sperm penetration test.

Based on this data, we believe that there is no need to continue with additional volunteers because of the poor performance of the sponge diaphragm in the prevention of the propagation of spermatozoa into the cervical mucus.

During the last five years, when my co-workers Dr. Heine and Dr. Droegemueller, several residents and I enthusiastically tested the sponge in the cylindrical or in the diaphragm form, in many volunteers we obtained much new information about vaginal physiology and the performance of the sponges. I have to admit that a new factor arose which makes the use of any sponge in the vaginal territory rather dubious and risky, namely, the incidents of toxic shock syndrome. Collagen sponge, with its large surface area, would certainly be an ideal environment for staphylococcus to grow and produce exotoxin. We made several tests indicating that the acidity of the sponge (which originally was considered to prevent bacterial growth) is not satisfactory because it is very quickly neutralized by vaginal secretions. Incorporation of other factors such as residual glutaraldehyde and zinc, which are considered bacteriostatic or bactericidal, is again inefficient due to the interference with vaginal secretion which binds with these substances and neutralizes their effect. From this point of view, I consider collagen sponge, as well as any other polymer-made diaphragm with great surface area, a potential risk--a method that could possibly increase the incidence of toxic shock syndrome.

Other aspects of collagen sponges, namely the effectiveness, were not satisfactory either. In our previous postcoital tests with the cylindrical sponge, we obtained a rather high rate of positive postcoital tests, which were later documented by studies conducted by PARFR in two different centers. Finally, even the acceptability of the sponge by the user, as well as her partner, was not ideal. It became clear that, during our studies on acceptibility, our volunteers were too well motivated and excited about this new method therefore disregarding some inconvenience of the sponge, as reported in later studies independent of our involvement.

Based on our own experience with testing collagen sponges in the vaginal territory and the field test reports from PARFR, I propose that all studies on

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this method be discontinued. There are other methods on the horizon of contraceptive technology which deserve more of our attention and possible funding by PARFR.

I would like to thank PARFR for their continuous support, both financial as well as philosophical. I believe that the money spent on my research on collagen sponges was not wasted, as it resulted in many new observations related not only to the sponge barrier method, but safety and performance of surfactant as well.

Table 1

Testing of a Modified Contraceptive Diaphragm

Type of Collagen Sponge	Weight Δ g/12 hours
Buffer pH 3.7	9.67 ± 0.92
Buffer + 100 mg N-9 solution	10.30 ± 1.10
Buffer + 100 mg N-9 cream	10.23 ± 0.84

Five volunteers tested three types of tampons (2.5 x 10 cm cylinder) for 12 hours. Tampons were inserted dry with an applicator. Each tampon was preweighed. After removal, the weight was determined. The table shows the difference between the original and final weight.

Table 2

Amount of Spermatozoa in Endo- and Exocervix of Volunteers Using the Collagen Sponge Diaphragm

Volunteer		Spermatozoa/ High Powered Field without Sponge Endo	Spermatozoa/ High Powered Field without Sponge Exo	Spermatozoa/ High Powered Field with Sponge Endo	Spermatozoa/ High Powered Field with Sponge Exo	Total Time Sponge in Place hr	Total Time Sponge in Place min	Total Time between Intercourse and Test hr	Total Time between Intercourse and Test min	Comments
1	motile	10-15	15-20	0	2	5	15	2	15	
	nonmotile	few	few	0	0					
2	motile	>20	>20	0	0	6	30	2	30	
	nonmotile	>10	>10	0	2					
3	motile	10-15	10-15	1	1	4	20	2	0	
	nonmotile	0	0	2-4	2-4					
4	motile	12-15	12-15	0-2	0	4	10	3	25	Sponge only in for 45 min precoitus
	nonmotile	2-4	few	0-2	0-5					
5	motile	>40	>40	0	0	4	0	2	0	
	nonmotile	10	10	0	4					
6	motile	40	40	1-5	2-5	8	10	2	20	Diaphragm falling apart at removal time
	nonmotile	3	3-5	1	1					
7	motile	6-12	4-10	0	0	4	15	1	45	
	nonmotile	0	0	1-2	2-3					
8	motile	20+	12-20	0	0	7	15	3	30	
	nonmotile	1-2	1-2	1-2	1-2					
9	motile	TNTC	TNTC	4-20	-	6	5	3	5	Exo cx mucus too scant Endo cx only done
	nonmotile	few	few	10-20	-					
10	motile	>10	>20	0	0	8	30	5	30	Sponge irritated vagina
	nonmotile	1-4	1-4	2-4	2-4					
11	motile	6-8	5-10	10-15	>20	5	30	2	30	
	nonmotile	1-2	1-2	>20	TNTC					

Family Health International • Research Triangle Park, NC 27709 USA • 919/549-0517 • Telex 579442

FEB 16 1983

11 February 1983

Diane Krier-Morrow
Director of Administration
PARFR
Northwestern University
Suite 1525
875 North Michigan Avenue
Chicago, Illinois 60611

Dear Diane:

Please find enclosed the interim report on the Wing Sound II studies. The most recent shipment of forms from Dr. Toppozada was received on 7 February 1983 and was not processed in time to be included in this report. However, Dr. Toppozada indicated in his letter that he has completed the study including all follow up.

I have talked with Dr. Hasson and plan to get together with him in the near future to discuss linking the uterine measurement data to termination event rates.

If additional information is required in the meantime, feel free to contact me.

Sincerely,

Sandor A. Balogh

Sandor A. Balogh
Senior Research Analyst

SAB/jt

Enclosure

cc: Harrith Hasson
David Edelman

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Interim Report on the Wing Sound II

(prepared February 1983)

The evaluation of the Wing Sound II uterine sound is being conducted in four centers located in Caracas, Venezuela, Exeter, England, Alexandria, Egypt and Valdivia, Chile. To date, 827 insertions have been performed. The planned follow-up schedule is for visits at 1, 3, 6 and 12 months post-insertion. Additional data, including measurements of total uterine length, insertion depth at 18 and 12 mm wing spreads, pain, difficulties or other complications related to use of the Wing Sound II are collected on a supplemental form. The following report is a summary of the current status of the Wing Sound II studies by center. The results are also highlighted in the attached table.

Caracas, Venezuela (Dr. I. Zighelboim)

Admissions to this study are complete. A total of 100 insertions of the Nova T were performed during September-October 1982. The 1- and 3-month follow rates are 71.0 and 22.2, respectively. A single removal for bleeding has been the only termination to occur. With respect to the Wing Sound II, one case of bleeding during the sounding was reported. There appeared to be considerable difference between insertor's and patient's perception of pain; 63% of the women reported experiencing pain during sounding, compared to 15% of the cases in which the insertor perceived the patient to have some pain. No other complications were noted.

Exeter, England (Dr. R. Snowden)

Data for this study were not collected on standard IFRP forms, and rather than events recorded at several follow-up visits, only a single date of and status at last contact were provided. Data are available on 342 insertions performed between May and November 1982, including primarily CuT 200, Multilo. Cu 250 and Cu7 IUDs, although some loops, Nova T and Progestasert devices were also used. There have been 22 terminations from the study, of which six were expulsions, six were removals for bleeding or pain and five were removals due to pelvic inflammatory disease (PID). The continuation rate at one month is 96.6 with a follow-up rate of 50.9. Wind Sound II measurements were made on 327 women, but information on pain or complications was not recorded.

Alexandria, Egypt (Dr. M. Toppozada)

Eighty-four insertions of the Lippes Loop C have been performed at this center between February and September 1982. One device was removed because of pain. The 1-month follow-up rate is 51.4. Difficulty in maneuvering the sound was a problem in one case. Some degree of pain was experienced by 79.8% of the women.

Valdivia, Chile (Dr. R. Guzman-Serani)

A total of 301 insertions were performed between May and November 1982, and recruitment into this study has stopped. Of the 301 women, 110 were fitted with a CuT 200, 87 a Lippes Loop C and 104 received a Multiload Cu 250. Six expulsions have occurred, all within the first month post-insertion and distributed evenly among the three devices. The 1-month continuation rates are 96.4, 98.5 and 96.7 for the CuT, LLC and ML-250, respectively; the corresponding follow-up rates are 81.1, 68.3 and 81.6. Three difficulties and one complication related to use of the Wing Sound II were reported. All three difficulties involved the third position (12 mm wing spread) of the sound: in one case the measurement was not obtained because the sound passed into the cervical canal; in the others the insertor had trouble moving from the second position (20 mm wing spread) to the third. Bleeding during the procedure was observed in one woman. Pain, whether perceived by patient or physician, was experienced by approximately one third of the women.

Summary

Admission into two studies (Venezuela, Chile) is complete. Follow-up rates among all four centers are very low at this point but are expected to improve with time. There are few difficulties or complications associated with use of the Wing Sound II; some degree of pain appears to be likely in most women, although there is some center variability. Since there have not been many terminations up to this point, it is impractical to relate these to the sound measurements; however, this type of analysis is anticipated in the future. Inter-device comparisons may also be possible.

Evaluation of the Wing Sound II (as of February 1983)

	Venezuela	England	Egypt	Chile
No. of insertions	100	342	84	301
Insertion period	9/82-10/82	5/82-11/82	2/82-9/82	5/82-11/82
Devices	Nova T	ML-250,Cu7, CuT 200,others	LLC	CuT 200,LLC, ML-250
Wing Sound measurements(mean)				
Total uterine length	75.3	74.3	76.8	74.7
Depth at 18 mm spread	61.8	66.3	63.3	65.6
Depth at 12 mm spread	50.3	57.9	52.7	55.6
Patient's perception of pain during procedure (%)				
Mild	53.0	-	67.9	28.6
Moderate	7.0	-	10.7	7.3
Severe	3.0	-	1.2	1.0
One-month continuation rate	100.0	96.6	100.0	97.1
One-month follow-up rate	71.0	50.9	51.4	77.7

Report on Potential of India - U.S.

Cooperation to Develop Contraceptive Vaccines

Nancy J. Alexander, Ph.D.
Erwin Goldberg, Ph.D.
Consultants

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INTRODUCTION

This report describes collaborative programs between U.S. and Indian scientists that are designed to enhance development of contraceptive vaccines. Because of high-level negotiations, USAID has an opportunity to participate in the development of a strong research base in the immunology of reproduction in India and to guide joint research activities. Research, technology transfer and training opportunities represent one area which would result in a strengthening of collaborative research efforts between Indian and U.S. scientists. In many cases the majority of the costs in India for such a program could be paid for in rupees and USAID funds would be necessary to provide only those costs that could not be covered in rupees, i.e. purchase of equipment manufactured in the U.S., per diem expenses of Indian scientists visiting the U.S., or laboratory supplies or services provided on a unit or fee basis through U.S. institutions.

For such a program to be a viable option, a commitment of funding for a period of years is necessary. On the Indian side such a commitment is now available in the term of 5-7 years. With a similar U.S. commitment, rapid development of certain programs described below could be initiated within the next few months.

BACKGROUND

The current population explosion in certain regions of the world demands new approaches to fertility regulation, and vaccines offer a potentially safe and effective answer to the problem. The reproductive systems of both men and women contain specialized cells that produce unique molecules; these could provide suitable antigens for contraceptive vaccines. Documented cases of infertility attributable to natural autoimmunity to reproductive antigens further indicate that immunization can provide a safe and effective method of fertility regulation in human beings.

Background on Vaccines and Immunization

Vaccines are classically comprised of inactivated organisms (bacteria, virions) or antigen extracts that stimulate the immune system to respond to and eliminate organisms bearing these antigenic determinants. The unique characteristics of the immune system that permit the success of this approach are: (a) precise specificity of the response, (b) amplification of specific immune-cell populations after immunization, and (c) long-term memory. Most vaccines are administered by injection with or without adjuvants to induce a systemic humoral immune response. However, oral administration of live attenuated organisms (i.e., polio vaccine) can also result in a local secretory immune response.

Passive immunization (administration of preformed antibodies to nonimmune persons) is also widely used in the control of certain infectious diseases and to manage other pathological phenomena such as Rh sensitization after pregnancy. Antibodies are obtained from animals or human beings that have been vaccinated or that have a natural immunity to the infectious agent. This technique provides immediate but relatively short-term (a few weeks) protection because the antibodies are gradually cleared from the system. Frequent administration of specific antibodies for long-term protection in humans has not been possible because animal antisera induce sensitization and serum sickness, and human antisera are expensive and potentially hazardous because of transfer of pathologic viruses. The recent development of human monoclonal antibodies, a breakthrough in this area, may soon provide an economical source of well-defined human antibodies for this procedure.

Prerequisites of a Contraceptive Vaccine for Use in Humans

First, an appropriate antigenic determinant that is unique to the target (is not found on any other tissues or molecules) must be identified. The target antigen must have a physiological function relevant to fertility that can be blocked by antibody, or be located on a cell that can be lysed by complement. The reproductive hormones and several antigens isolated from the zona pellucida, and sperm appear to qualify.

Second, the antigen must be immunogenic and available in large quantities at reasonable cost. This requirement originally was a major obstacle to the development of human contraceptive vaccines because human reproductive antigens are weaker immunogens than the pathogens commonly used in vaccines, and are not available in large quantities. However, several of the reproductive antigens appear to have been evolutionarily conserved, i.e., found in many species, and can be extracted from a more accessible species for subsequent use in humans. The most exciting advance in this area is the recent development of genetic engineering technology, which offers a means to produce large quantities of specific proteins at low cost. Once appropriate antigen(s) are identified, large scale production of human proteins for use in vaccines could begin.

Administration of Contraceptive Vaccines

Since most reproductive antigens are weak immunogens, contraceptive vaccines will probably be administered with adjuvants, agents that act nonspecifically to achieve effective systemic immune responses. The mechanisms underlying the immunopotentiating effects of adjuvants are: (a) slowing of antigen release; (b) denaturation of antigens and a consequent increase in immunogenicity; (c) recruitment of reactive cells; and (d) stimulation of proliferation and differentiation in immunocompetent cells. The macrophage appears to be the cell directly affected by many of the adjuvants: adjuvants may facilitate macrophage-B cell interactions and thus bypass the need for helper T lymphocytes in B cell differentiation and antibody production, and they may increase the efficiency of macrophage stimulation of helper T cells. However, one well-known adjuvant, bacterial endotoxin lipopolysaccharride (LPS), is a B cell mitogen, and its adjuvant effect is due to direct stimulation of B lymphocytes. When cultured with endotoxin, B cells produce antibodies to T-dependent antigens without the assistance of helper T cells.

Freund's adjuvant is one of the most powerful immunopotentiators known. Incomplete Freund's adjuvant primarily stimulates antibody formation, and complete Freund's adjuvant (containing mycobacteria) elicits both cell-mediated and humoral immune responses. Unfortunately, Freund's adjuvant cannot be used in humans because the mineral oil is not degraded; it persists at the injection site and elicits the formation of granulomas. Furthermore, the complete adjuvant also sensitizes the recipient to mycobacterial antigens. Such a powerful adjuvant might induce autoimmune reactions, especially if the immunizing antigen is related to other molecules found in host tissues.

Two adjuvants show promise for use with human contraceptive vaccines: (a) liposomes, biodegradable nonimmunogenic concentric spheres of phospholipids separated by aqueous compartments, which target antigens to macrophages and greatly enhance the immunogenicity of an antigen preparation, and (b) N-acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide or MDP), a synthetic glycopeptide structurally similar to a component of the mycobacterium cell wall that can substitute for mycobacterium in Complete Freund's Adjuvant.

The nature of the immune response is affected by the type of antigen, the type of adjuvant, the route and timing of immunization, and host factors. Considerable experimentation with various antigen/adjuvant combinations and immunization protocols may be required to achieve effective immunocontraception in people.

MEETINGS AND BRIEFINGS:

January 18, 1983

1. USAID Office, New Delhi
   Gary Merritt; Rogers Beasley; Dennis Johnsen

2. ICMR
   Gary Merritt; Rogers Beasley; Minister of Indian Department of Science and Industry, Dhar; Secretary of Indian Department of Science and Technology, Vadarajhar; Additional Secretary, Rachamandran; Dr. G.P. Talwar, All India Institute of Medical Research (AIIMR)

3. U.S. Embassy
   Dennis Johnsen, Scientific and Technological Attache

January 19, 1983

1. AIIMR
   G.P. Talwar and Associates

January 20, 1983

1. National Institute of Health and Family Planning
   Dr. Roy, Director

2. AIIMR (cont'd)

January 21, 1983

1. AIIMR (cont'd)

January 23, 1983

1. Gary Merritt; Rogers Beasley; Dennis Johnsen with Dr. Krause, NIH-NIAAMD

January 24, 1983

1. Bangalore, India Institute of Sciences

January 25, 1983

1. Bombay, Institute for Research in Reproduction

January 27, 1983

1. Dr. Badri Saxena, Head, Indian Council of Medical Research, New Delhi

January 28, 1983

1. ICMR
   Dr. Shoba Sehgal of Post Graduate Institute in Chandigarh; Dr. B. Saxena; Moudgal, et. al.

2. AIIMR
   G.P. Talwar

3. USAID
   Gary Merritt; Rogers Beasley; Priscilla Boughton

February 4, 1983

1. USAID - Washington
   Dr. James Shelton; Mr. Jeff Spieler

DESCRIPTION OF CURRENT INDIAN PROGRAMS ON REPRODUCTIVE IMMUNOLOGY

All Indian Institute of Medical Sciences and National Inst. of Immunology, New Delhi

A vigorous effort to develop contraceptive vaccines is directed by Dr. G.P. Talwar, Director of the National Institute of Immunology. It is apparent that studies by Dr. Talwar have received the highest level priority approval in India and a commitment for long term financial support has been made. If an immunocontraceptive vaccine can indeed be developed, and we believe that it can, then it is likely that major contributions toward this goal will result from support provided to Talwar's program. This program is described in detail below:

Projects on immunoreproduction:

1. Zona Pellucida Antigens: female monkeys have been immunized with fragments of porcine zona. The animals were rendered infertile and ammenorrhaeic for three to six months. Since only three animals were tested, further immunizations are planned with more monkeys. In addition, hybridoma technology has been employed to develop six clones that secrete potent anti-zona pellucida antibodies (five are IgG_1 and one is IgG_2). Work is planned or in progress to establish the specificity of these monoclonal antibodies; to test both in vitro and in vivo binding, especially to follicles; to examine antifertility properties of these antibodies injected into females and to check for abnormalities in endocrine, metabolic, and organ functions.

2. Adjuvant development and evaluation: work is now focused on three adjuvants for primary immunization following studies on approximately 30 compounds. The chemical nature of these compounds was not revealed to us. In addition, a potent nontoxic adjuvant has been used successfully for secondary immunization.

3. HCG vaccine: The most extensive program associated with development of a contraceptive vaccine involves the beta subunit of HCG. Much of this work has been described in the literature. Basically, the vaccine consists of the beta subunit of HCG conjugated with tetanus toxoid. Antibodies developed that were biologically

effective in a variety of in vitro and in vivo assays in which the HCG production associated with pregnancy was suppressed. Monkeys and baboons actively immunized with the vaccine continued normal ovulatory cycles. Autopsies of hyperimmunized animals that had been challenged with the vaccine repeatedly, did not reveal immune complex deposition in kidney, pituitary or choroid plexus. Phase I clinical trials were done in Delhi, Bombay, Helsinki, Uppsala, Bahia, and Santiago. Endogenous HCG did not boost the immune response although a secondary rise in antibody could be generated by injection of the vaccine and the response was always reversible. There was individual variability of antibody response and women who developed only low titers were prone to pregnancy.

Studies of beta HCG are continuing in at least two areas. One involves a combined vaccine of beta HCG and ovine LH (in collaboration with Moudgal, see below). The second consists of synthetic vaccine development. In this project, the beta HCG core peptides of about 20 amino acid residues have been synthesized with intramolecular disulfide bonds retained intact. Such peptides will be tested for immunological properties of antibody binding and antigenicity. Since the HCG vaccines involve abrogation of postfertilization events they will not be considered further in the context of this report, except as models of vaccine development.

4. LHRH: monoclonal antibodies to LHRH have been prepared. Injections into females will interrupt pregnancy by reducing CG levels.

5. Motility promoter in human seminal plasma: three low molecular weight components have been isolated. They enhance motility of spermatozoa from the mouse, human and water buffalo. The latter is of interest because artificial insemination procedures in water buffalo are of great agricultural importance.

6. Other projects: involve monoclonal antibodies to choriocarcinoma and isolation of mycobacteria membrane antigens primarily for adjuvant development.

CRITIQUE: Future Focus of Scientific Projects

At present, there is no work being done by this group on sperm antigens. Of the sperm antigens that would most likely be considered for investigation, only LDH-C_4 (LDH-X) is at a stage of development that suggests immunocontraceptive potential. Therefore, Talwar proposed that a collaborative project with Goldberg could expedite development of LDH-C_4 as a contraceptive vaccine. The rationale is that our efforts at present be confined to the antigen that satisfies the requirements of absolute specificity to sperm and demonstrated utility in suppressing fertility. Other sperm antigens at an earlier stage of experimental development for vaccines may be identified for future studies. These subjects will be considered further under recommendations.

Work on zona pellucida antigens needs to be amplified. Promising results have been described which involve both active and passive immunization with fragments of porcine zona. Dose response studies need to be done. It is essential that studies in this area be directed toward precise identification and isolation of zona antigens. A collaborative effort in which Talwar's monoclonal antibodies are tested with identifiable fractions prepared by one or more of the U.S. investigators working with this material, should be pursued.

Immunopathology needs development as an essential component in vaccine evaluation. Collaborative efforts with Dr. Alexander would be in order, especially since such analyses should be undertaken at sites other than where the initial work was conducted. Furthermore, for maximal acceptance of the data, long term safety studies should be conducted in the U.S. as well as other sites.

Talwar suggested that Alexander's work with monoclonal antibodies against human sperm could be exploited in a collaborative venture by utilizing the clones that produce antibodies against a determinant that is conserved in several species. Studies of infertility are more easily conducted in rodents and small animals. These monoclonal antibodies would be used as probes to isolate specific sperm antigens.

Needs expressed by Talwar in the area of technology transfer included such things as upgrading their in vitro fertilization capabilities to, for example, conduct primate studies. Such studies either with the hamster egg system or with primate eggs would be useful to screen effects of antibodies to sperm or the efficacy of contraceptive vaccines.

Cryopreservation of both eggs and sperm was suggested as another area where technology transfer would be useful. Frozen gametes could be used at a later date for evaluation in vitro of potential vaccines. Semen cryopreservation, particularly with veterinary species could be a critical support component in the development of Indian agriculture, particularly in the utilization of water buffalo for food animals and for developing AI methods that are more efficacious than those which are presently available.

Acquiring expertise in genetic engineering, would also be desirable. This fast moving area provides the potential for production of specific antifertility antigens.

Biostatistical expertise for experimental design is also lacking at present in the Talwar program. Such expertise must be strengthened in the program in order for the Indian studies to have world-wide acceptance. It should be available locally.

When one evaluates the field of contraceptive vaccines, it is obvious that a major deficiency exists in the use of conjugation procedures and adjuvant development. Conjugation of peptides to carrier proteins is at the core of synthetic vaccine construction. Similarly vaccination will ultimately require adjuvants that are compatible with human use. Talwar's group has made important advances in this arena. This work requires open scientific exchange in order to foster collaborative studies on a contraceptive vaccine composed of defined antigens of either spermatozoa or zona pellucida.

Facilities and Environment

Facilities which are available to the Talwar program are well suited to the studies that are underway. Laboratory space is crowded but contains the most modern

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items of equipment for RIA, peptide synthesis, microscopy, cell culture, chemistry, tissue analysis, and fractionation, etc., thus providing a first rate research enterprise. Animal facilities for rodents and primates are excellent at least for short term studies, as is the animal husbandry. The scientists and students associated with the various projects are interested, bright and hard-working.

Collaborative Interactions

Talwar indicated that he will provide a central facility that is available for the following:

1. Conjugation Technology: This presumably involves the use of tetanus toxoid for conjugation to antigens which are then tested for immunogenicity. The need for conjugation technology is to obviate the use of FCA which would never be acceptable for human use.

2. Peptide Synthesis Capability: This is an area that cannot as yet serve as a central facility. It is adequate only to provide peptides for Talwar's use in the programs which he has defined and described. It requires expansion at least in terms of personnel in order to become a central facility. Such an expansion is planned, however.

3. Adjuvant Development: Talwar's group has made considerable progress, based on the data that has been shown to us toward developing adjuvants. In order, however, for any collaborative efforts to be forthcoming, the formulation of the adjuvant, the nature of the response, etc. needs to be shared with potential collaborators.

As noted previously with this plan of a central facility, the work that is presently in progress, and the obvious support of the government to Talwar's program for development of a contraceptive vaccine, it seems highly likely that this research will in fact be an exceedingly strong and viable program. If indeed an immunocontraceptive vaccine can be developed, Talwar's group has the necessary funding, personnel and motivation to make great progress in this area. Whether collaborative programs

can be established remains to be seen; however, it does appear that input from other scientists working in this area would be very useful to this program. Talwar was asked what channels were available for the implementation of new programs. Apparently this has been considered and The Department of Sciences and Technology has a special section to deal with so called thrust areas. These can be investigator initiated projects which fall within the guidelines that have been formulated for contraceptive vaccine development. It is clear, however, that any programs on contraceptive vaccine development in India will very definitely be channeled through Talwar, particularly in his role of Director of the NII.

We asked Talwar where foreign currency would be useful since apparently there already is adequate funding from the Indian government for a long term attempt to develop a contraceptive vaccine. Foreign currency would pay for costly reagents and supplies, e.g. isotopes, antibodies, etc. One plan would have a U.S. collaborator, e.g. PARFR, provided with funds to purchase these supplies. When Talwar's laboratory needed supplies he would contact PARFR; they would be purchased and sent to Talwar and this would expedite receipt of such materials from months to weeks. A second area where foreign currency would be most useful, would be to pay for traffic between laboratories. For example, in a collaborative project that will be discussed elsewhere in this report between Goldberg's laboratory and Talwar's group, it will be desirable and, in fact, necessary for Goldberg to send at least two of his people for two to three week stays in Talwar's laboratory. Similarly it may be desirable for one or more of Talwar's people to spend some time in Goldberg's laboratory.

One of the more obvious needs of Talwar's program is for more expertise in the area of protein chemistry, particularly as far as antigen isolation, purification and identification are concerned.

Projects for Future

A National Institute of Immunology (NII) has been established recently under the auspices of the Department of Science and Technology. Dr. G.P. Talwar has been

appointed Director of the Institute. The goals of the National Institute of Immunology are:

a) to undertake and promote high quality basic immunologic research

b) to promote linkages between scientific agencies and organizations within the country

c) to organize postgraduate courses, workshops, seminars, symposia and training programs in the field of vaccine development

d) to organize training programs for technicians in immunologic methods

e) to service international welfare centers for immunology

f) to carry on research for the development of new vaccines; immunological agents for communicable diseases

g) to develop immunological approaches for the control of male and female fertility

h) to interact with industry for the manufacture of immunological reagents

i) to collaborate with foreign institutions

j) to publish and disseminate information; to institute professorships and faculty positions; to affiliate with recognized institutions; to receive grants and aids and assistance from foreign sources.

Recent estimates call for a staff of 10-15 senior scientists within two years, with additions at the rate of 5-10 per year to a total scientific staff numbering 50-75. Building plans have been prepared and a site has been chosen consisting of 15 acres on the eastern side of the campus of Jawaharlah Nehru University. A guest residence on campus will house international visitors. An advisory committee of international scientists will visit for at least a week annually. Research will be focused in four areas: reproductive immunology, mycobacteria and leprosy, gastrointestinal infections and disease vectors.

It is clear that reproductive immunology is a very important component of NII

and as such this facility may well assume world leadership in this area of research. Clearly, it would be difficult to identify such a resource elsewhere in the world.

The Institute for Research and Reproduction (IRR) in Bombay

Dr. T.C. Anand Kumar has recently become Director of IRR. The IRR receives funding from ICMR, the Pop Council, Ford Foundation and WHO. Total staff of the Institute is about 320 people with about 30 scientists. Training toward advanced academic degrees is provided through the affiliation with the University in Bombay. In the past, active on-going collaboration has involved several U.S. investigators. Research at the IRR has been particularly focused in drugs that affect fertility. Studies have involved effectiveness of various hormonal contraceptives, tests of the antifertility properties of LHRH, basic research and reproductive physiology with a view of evolving more effective and safer contraceptives. Other research focuses have been on the control of human fertility and on biology of reproduction and immunology. The IRR is the national quality control center for hormone assay. Currently, the group exhibits expertise in biochemistry, pharmacology, genetics and immunology. Projects at IRR include:

1. Suppression of FSH: The group has described several inhibin-like substances similar to folliculostatin which they call "gonadopeptides". They hope to use these substances as regulators of gonadotrophin levels in order to reduce FSH or inhibit FSH binding. They have developed an RIA to their gonadopeptide and this ability, coupled with expertise in in vitro culture of hypothalamus tissue, and bioassay procedures for LH and FSH should allow them to proceed in their endeavors.

2. In Vitro Fertilization: In vitro fertilization in women and the hamster egg penetration tests are both being performed at IRR. Although they have cultured human eggs to the 8-cell stage, they have had no conceptions. We had no time to talk with the investigator in charge of this work. Such procedures in animals could be most useful for evaluation of the anti-fertility effect of various antibody preparations.

3. Vitamin Carrier Protein: These studies are a continuation of work carried on at Bangalore by one of Dr. P.R. Adiga's students who has recently joined IRR.

4. Hospital Services Associated with Reproduction: These areas concern work with patients who have unexplained infertility and genetic approaches such as prenatal diagnosis and studies of inborn errors of metabolism.

5. Clinical Trials of Steroid Nasal Spray: Initial studies suggested that progesterone as a nasal spray might allow a lower pulsatile use of steroids. Device development seems to be the hold-up. Currently, one can't be sure of the same amount of spray being delivered each time.

CRITIQUE

The IRR involves a group of enthusiastic investigators. Equipment, library, and laboratory facilities seem adequate. Several investigators have interests in subjects relating to possible sperm vaccines. For example, Dr. U.G. Hegde is developing some monoclonal antibodies against sperm and Dr. S.K. Shahare measures sperm antibodies. There was inadequate time to determine whether any mutually beneficial projects could result. Because of the high degree of interest, further communication between these investigators and the consultants would be useful. The first impression is that IRR will be supportive and interested but not innovative in the field of immunoreproduction.

The Post Graduate Institute (PGI) in Chandigarh

We were unable to visit PGI but Dr. Shoba Sehgal came to New Delhi to speak with us.

The Post Graduate Institute of Medical Education and Research in Chandigarh is one of four institutes that are under the Government of India (GOI), Ministry of Health. The PGI is located 150 miles north of New Delhi and is charged with medical service training and research responsibilities. Postgraduate training of physicians is done here.

1. Immunopathology and Atherosclerosis: Dr. Chakravarti heads an area of experimental pathology. Dr. Chakravarti has done work on atherosclerosis in rhesus monkeys and he has studied whether vasectomy results in any untoward effects. He is interested in pursuing studies of possible immune complex deposition as a result of the interaction between sperm antigen and antisperm antibodies. We read that the principal U.S. collaborator on this project could be Dr. Kenneth Tung, Professor of Pathology at the University of New Mexico, Albuquerque. Dr. Tung would supply anti-human sperm monoclonal antibodies to test for localization in the arterial wall. Whether such monoclonal's would cross react with monkey tissues, remains to be tested.

2. Immunopathology: Dr. Shoba Sehgal heads this unit. She is a pathologist by training and is familiar with evaluation of tissue immune complex localization, general histology, autoantibodies, and various assays for circulating immune complexes. Physicians have a 5 month training rotation in the department of pathology.

Dr. Sehgal has been intimately involved in testing for Dr. Talwar's group. For example, she has tested the specificity of his monoclonal antibodies to zona pellucida. She is also planning studies with Dr. Moudgal concerning the histopathological evaluation of immunization to FSH and the vitamin binding protein of Dr. Adiga.

Indian Institute of Sciences, Bangalore

The IIS, Indian Institute of Sciences, was established in the early 1900's by Tata and presently has a faculty numbering around 150. It's mission encompasses graduate education and research. The main focuses are engineering and science and a Ph.D. in both can be obtained. The annual budget approximates 5 million.

The investigations involving immunoreproduction are being conducted in the department of biochemistry and include:

1. Vitamin Carrier protein: If female rats are immunized with a vitamin extract from chicken eggs, early abortion results. These riboflavin-carrier proteins are estrogen-induced. They are produced in the liver apparently only during pregnancy.

With passive immunization, abortion occurs 6-16 days later. With active immunization, abortion occurs by day 9. Recently studies have been initiated in monkeys.

2. Anti-FSH: Dr. Moudgal's studies have focused on anti-FSH to control male fertility. Passive immunization causes severe oligospermia but not azoospermia. In females, anti-ovine-LH produces abortion. Since the remaining sperm may also be impaired by the treatment, fertility studies of immunized males are planned.

Funding sources at IIS include:

External

1. Grant from IDRC ($310,000) for the next 3 years with extension contemplated
2. Rockefeller Foundation ($200,000) for 1980-84

Internal

3. Family Planning Foundation of India - Phase I vaccine studies
4. ICMR - Studies on antigonadotrophins

CRITIQUE

The facilities at Bangalore seem good although the laboratory space seems very limited. The investigators feel that if they are going to be involved in immunization studies, that they should have a better perception of immunopathology. They have requested a 1-2 week workshop with 6 teachers. Although such a workshop would no doubt be useful, it would not allow these investigators to become proficient in this field. A close collaborative relationship with an investigator active in this field would allow valid data to result. Dr. Alexander would be willing to work with the group at Bangalore to promote such a collaborative effort. Studies would be jointly conceived. Some of the pathological evaluation could be completed at IIS and other material could be prepared and shipped to Dr. Alexander's laboratory in Oregon. This type of approach would be a useful collaboration and one that would be relatively simple to institute.

The monkey facility at Bangalore seems excellent although limited in size. Breeding success is at the 85% level which is much above that of many facilities. The possibility that Bangalore may become the regional primate center of India makes it even more enticing.

Drs. Alexander and Goldberg felt that a strong collaboration could be developed between them and IIS. Such a study would involve the local immune response. The human cervix has its own secretory immune defense system, and cervical secretions contain relatively high levels of IgA antibodies at certain stages of the menstrual cycle. Ever since this discovery, reproductive immunologists have proposed eliciting a local secretory immune response to sperm membrane antigens; the antibodies would bind to sperm as they swam through this strategic "Straits of Gibraltar" and would impede their motility and fertilizing capacity.

Local secretory immune responses may be stimulated in the bronchioles or gastrointestinal tract. Uncommitted B cells are apparently instructed to respond to specific antigens in Peyer's patches in the gut or in the lungs, and the IgA antibodies produced by plasma cells that subsequently home to various mucosal tissues are generally directed against enteric organisms. Gut activation of an immune response gives rise to the possibility of orally administering sperm antigens to achieve local secretory antisperm antibody production in the cervix. In one recent study, female mice administered spermatozoa via their gastrointestinal tract, had reduced fertility. The approach of oral administration of vaccines is attractive because of the convenience and the elicitation of a local and not systemic response (with possible side effects); however, not enough is known about oral administration of antigens. Research has revealed that the oral administration of antigens can: (a) elicit a secretory immune response, (b) elicit humoral immune responses, including allergic responses, and (c) induce supressor cells that inhibit any immune responses to the antigen. Use of the bronchial (BALT) or gut (GALT) system for development of high titers of IgA antisperm antibodies could

be tested. Bonnet monkeys, native to Bangalore, would be the most appropriate species to use because (a) they have copious cervical mucus so that antibody titers can be evaluated and (b) their high breeding success means that antifertility effects could be easily evaluated.

Central Drug Research Institute at Lucknow

We were unable to visit CDRI but include a description for the sake of completeness.

There seem to be five areas of research associated with the CDRI facility. They include: development of chemotherapeutic and immuno-therapeutic agents for parasitic diseases and microbial infections; and search for anti-fertility agents. We will discuss only the latter.

The CDRI research on reproduction has focused on chemical and local devices. Their studies include: (1) management of the IUD; (2) development of the non-steroidal, postcoital contraceptives, both with synthetic and natural products; (3) development of new oral abortive agents. The group has recently evaluated, with experiments on rats, potential abortive agents from 28 plants. They have also evaluated various synthetics, both non-steroidal and steroidal; (4) development of chemical tubal and vasal occlusives - these studies involve research on both; (5) spermicides of various types; these studies have involved both general female physiology as well as epididymal function. For example, research on the effect of zinc on sperm motility, has been conducted.

An important aspect of the research at CDRI would be the possibility for testing immunoreproductive products. Potential agents could be tested at this facility in association with work done at other facilities in India. Based on the expertise available at Lucknow, it would seem useful to consider a collaborative approach possibly with someone like Larry Zaneveld from Chicago, in which antibodies might be applied vaginally as an immuno-barrier method of contraception. Other more systemic approaches

might well be possible for study in association with researchers from this facility. Research on adjuvants has been conducted here and in New Delhi.

National Institute of Health and Family Planning, New Delhi

The National Institute of Health and Family Planning came into existence in 1977 following an amalgamation of the National Institute of Health Administration and Education and the National Institute of Family Planning. The objectives of the Institute are:

1. Postgraduate training including a two-year course in community health, administration and a Ph.D. program in various disciplines.
2. Short-term in-service training courses (about 25-30 per year).
3. Seminars and workshops in current problems and issues in health and family welfare administration.
4. Extramural training courses.
5. Sponsored training courses.
6. WHO fellowships in national visitor training programs on sociological problems including hospital administration and medical care.

One aspect of their biomedical research is a focus on human reproduction and fertility regulation. There are several active programs:

1. Intra-testicular injections of BCG: Injections of BCG result in infertility in male animals. This may be an important veterinary approach. The fact that such an injection results in inflammation but nonetheless is reversible is a problem for use in man. Multiple bouts of painful testicular inflammation make this less than attractive for use.

2. FSH: Studies are being conducted to determine whether FSH can permanently be used to suppress spermatogenesis.

3. Hormonal localization in Spermatozoa: Dr. Allag uses immunofluoresecence to locate hormone binding on spermatozoa.

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CRITIQUE

The use of antibody to FSH may not be possible for contraception. Other investigators in Germany indicate that FSH may not suppress spermatogenesis on a long-term basis. In one similar study spermatogenesis was initially suppressed but even with active immunization, it returned to normal levels during the third year of immunization. Further basic studies are necessary to determine the precise role of FSH in the maintenance of human spermatogenesis and whether suppression by FSH through the use of antibodies would have any detrimental immunologically-mediated effects. Antibodies to LHRH have also been considered, however in males, such antibodies result in decreasing testicular size, cessation of spermatogenesis and a severe reduction in gonadotrophin levels. Such an approach could be important in veterinary work because it could be used to increase meat production, but its use in man appears to be remote.

Dr. Allag appears to have no colleagues at NIHFP, at least ones who have a similar field of interest. Since there is a great deal of laboratory space, perhaps it would be useful to increase the number of researchers in immunology of reproduction at NIHFP.

It would seem likely and appropriate that NIHFP be involved in human trials at some later date.

National Coordination of Projects

The Department of Science and Technology and the Indian Council for Medical Research have formed a task force to develop internal collaboration between Indian Institutes. Such a task force would allow inclusion of all scientific individuals interested in immunology of reproduction. Dr. Talwar is the leader of this task force. This collaboration involves a communication and spreading of information to various interested investigators as well as opportunities to acquire grant money to do specific projects.

INDIAN SUPPORT FOR RESEARCH ON CONTRACEPTIVE VACCINES

At all the places we visited, we found adequate equipment available. The Indian government is very supportive of research efforts in this area. Dr. Saxena at ICMR states that the government plans to spend 40-50 million dollars in the next 15 years. About 8 million dollars is expected to support research proposals during the next 2 1/2 years.

NEED FOR FOREIGN MONEY

Internal Indian research is well supported and the need for foreign support is limited to importation of specific items on a regular basis. The best approach would be for an agent or laboratory in the U.S. to be provided with funds to purchase tissue culture supplies and radioisotopes to be shipped to Indian investigators. The figure that has been suggested to us is $100,000/year.

SPECIFIC APPROACHES

The purpose of our mission is to determine the most rapid and likely mechanisms for development of a contraceptive vaccine. Because of the strong Indian interest and expertise in this particular research area, it seems very appropriate for there to be strong Indo-American collaboration. Three main approaches could be taken:

1) Already identified projects could be implemented immediately on a collaborative basis.

2) A grants program to support targeted investigator-initiated research could be developed.

3) A task force approach could be used.

We endorse approaches #1 and #3. The plans for each are detailed below.

Identified targeted research projects: Research projects between Indian and U.S. investigators include work in the following areas:

a) LDH-C_4, a defined sperm-specific antigen.

b) Sperm antigens (as revealed by monoclonal antibodies) that have been demonstrated to play a role in fertilization.

c) Antigens specific to the egg or it's coverings.

d) Safety studies associated with vaccine testing.

e) Cryopreservation of gametes.

f) Studies on sperm specific IgA antibodies.

a) Collaborative project on LDH-C_4 Development as a Contraceptive Vaccine: The most viable sperm specific antigen at present for evaluation as a contraceptive vaccine is the LDH-C_4 isozyme that has been studied in Goldberg's laboratory for several years. Immunization of mice, rabbits and baboons with LDH-C_4 does suppress pregnancy by blocking fertilization. It would be useful to demonstrate antigenicity of LDH-C_4 in non-human primates without Freund's adjuvant (FCA). In previous work, hyperimmunization with LDH-C_4 in FCA provoked high levels of antibody titer. In order that these studies progress toward the ultimate goal of human application of the vaccine, it is necessary to immunize effectively without FCA.

We suggest that studies be done in which LDH-C_4 prepared by Goldberg would be tested in monkeys by Talwar for antigenicity with synthetic adjuvants. A minimum of 10 animals per group at 2 dosage levels would be necessary. Animals would be monitored for antibodies to LDH-C_4 in sera by RIA by both laboratories. The experimental work would require that both a chemist and immunologist from Goldberg's laboratory spend 2-3 weeks with Talwar for the initial immunization and serum assay.

Goldberg has three monoclonal antibodies to LDH-C_4. Experiments involving passive immunization might be done to determine the minimum antibody titer necessary to block fertilization. Large quantities of a monoclonal antibody with a high affinity for human LDH-C_4 will be prepared by Goldberg and provided to Talwar to test in monkeys.

Since the natural product antigen must ultimately be replaced by a synthetic

vaccine, these studies will provide an immune response baseline for subsequent experiments with antigenic peptides. Peptide fragments of LDH-C_4 will be synthesized by Goldberg. Talwar will provide conjugation technology and synthetic adjuvants. Antigenicity will be assessed in rodents to select the most likely vaccine candidates that will be tested in the monkey.

These studies, over approximately a two year period, should assess the immunocontraceptive potential of LDH-C_4 with synthetic adjuvants. Annual direct costs for this project are estimated to be $135,000.

b) Sperm antigens: Recent studies have shown that some antigens of sperm are specific i.e. found only on spermatozoa. Because antibodies to some of these antigens result in infertility, Dr. Alexander's group has focused efforts on defining these antigens through the use of monoclonal antibodies. Passive immunization studies should be conducted first in rodents and subsequently in primates in order to determine the usefulness of the approach and the titer necessary to prevent fertility. Subsequently, definition of the specific antigens should be completed. Polyacrylamide gel electrophoresis evaluations are ongoing in Alexander's laboratory. A readily available antigen source is important. This can be accomplished either through synthesis or by finding a ready source of material. Some of the antigens seem to be available in specific cell types in Dr. Anderson's laboratory at Harvard Medical School. Such biochemical approaches in antigen determination can be jointly accomplished with Dr. Talwar's group. Dr. Alexander will send a chemist to New Delhi to provide antigen and monoclonal antibodies and to learn techniques of conjugation and adjuvant preparation. Conjugation increases immunogenicity and thus is important for production of the best immune responses. Based on preliminary passive immunization studies, active immunization experiments can be conducted. Some of these experiments would be conducted in Alexander's laboratory and some in Talwar's.

Total annual direct costs are estimated to be $125,000.

c) Egg specific antigens: The zona pellucida has proved to be an important possible site to prevent fertility. Two approaches are feasible 1) permanent sterilization by destruction of oocytes or 2) causing each oocyte to become incapable of being fertilized. Recent studies strongly suggest that the zona pellucida, a non-cellular gelatin layer surrounding the egg, may be an excellent target. Dr. Talwar's group has 6 putative clones. Further studies are necessary to biochemically define the antigenic sites which will prevent fertilization. U.S. investigators suggest that it is difficult to get zona antigen that does not cross-react with follicular material, presumably because cortical granules are present within the zona. The presence of a sperm specific receptor seems to be species specific thus it is difficult to acquire sufficient material for study. Studies on purification and synthesis will be necessary.

d) Safety studies: Careful immunopathology studies are an important developmental part of any vaccine. It is appropriate for studies of safety to be done at a different institution than where the initial studies were undertaken. Currently, specific guidelines must be instituted. A workshop has been suggested, under the auspices of Science and Technology to provide background for such studies. Based on further communications with Dr. Shoba Sehgal, it would be determined whether a collaborative long-term effort for immunopathological studies would be necessary. It seems likely that short term efficacy studies could be conducted in India but that some long-term studies should be done in U.S. laboratories where parasites and T.B. are more rigorously controlled. Such tests would allow greater acceptance of a vaccine.

e) Cryopreservation: Technology transfer of the techniques in cryopreservation could easily be accomplished. Such techniques would be useful for antibody evaluation and could also have an important veterinary component. Cryopreservation is routinely done at Dr. Alexander's laboratory. Two investigators from her laboratory could bring the appropriate equipment and demonstrate techniques of sperm and egg cryopreservation. Total cost (equipment and travel) is $10,000.

f) Studies on sperm specific IgA antibodies: As explained under the critique of the Bangalore IIS, a special focus should be placed on the development of secretory IgA antibodies. The bonnet monkey is the most appropriate model for such studies because of copious cervical mucus production and high breeding rate, particularly at Bangalore. Development of monoclonal IgA antibodies would be an excellent adjunctive approach which would allow quick evaluation of specific antibodies. Personnel, supplies, travel would approach $70,000/year.

g) Small grants program: A small grants program of investigator-initiated collaborative research could be instituted. The AID/Washington supported Program for Applied Research on Fertility Regulation (PARFR) would be an ongoing organization that could provide an excellent review mechanism. The problems with this sort of program is that it is a shotgun approach. We suggest that short term support of this type will not provide a vaccine. Furthermore, collaboration between investigators of the two countries would be minimal. Contraceptive research requires long-term funding without the constraints of small grants funding.

Task Force Approach: We recommend that a task force of experts from the United States be convened. Individuals with expertise in the area of contraceptive immunology (i.e. adjuvants, vaccination schedules, egg or sperm specific antigens, etc.) hybridoma technology and genetic engineering will be identified. These experts should be concerned with the distinct mission of developing the most appropriate and rational approach to vaccine development. A position paper would be developed. A subset of the task force who are familiar with the areas of Indian expertise in immunoreproduction (Goldberg & Alexander) would meet with the Indian counterparts so that collaborative projects could be arranged.

Depending on limitations of funding, it may be possible to support up to 5 projects. We estimate the approximate cost of each project at $150,000 direct costs therefore $225,000 total costs. Studies on antigen isolation as well as safety testing are expensive. No project should be awarded for less than two years. For 5 pro-

projects this equals $1,125,000/year total costs and a minimum two year commitment. Five to seven years are the minimum necessary for meaningful research results to occur.

Task Force Budget:

Travel for 20 people to attend U.S. Task Force on Contraceptive Vaccine development [15 members + 5 observers](20 x $500)	$10,000
Per Diem for 3 days	9,000
Consultant fee @ $200/day x 15 members	9,000
Miscellaneous	2,000
SUB-TOTAL	$30,000

Three members of task force travel to India to consult with Indian task force and develop collaborative projects:

Airfare 3 x 3,000	$ 9,000
Per diem ($100 x 10 days x 3 people)	3,000
Consultants fee (10 days x 3 people x $200)	6,000
Miscellaneous	2,000
SUB-TOTAL	$20,000
TOTAL	$50,000

Administration and funding should be handled through PARFR.

Suggested Members for Task Force:

Nancy Alexander, Chairman
Erwin Goldberg
B. Dunbar, Baylor Medical School
A. Sacco, Wayne State University
D. Anderson, Harvard
E. Schmell, Johns Hopkins
K. Tung, New Mexico
J. Trotter, University of Virginia
Robert Lerner, Scripps
C. Millette, Harvard
S. Witkin, Cornell Medical School
W. Bardin, Population Council
P. McDermott, London, Ontario
L. Zaneveld, University of Illinois Medical School
V. Stevens, Ohio State
P. Calarco, University of California

ALTERNATES:
A. Menge, University of Michigan
S. Heyner, Philadelphia College Pharmacy
M. O'Rand, University of North Carolina

CLOSING COMMENTS

With vaccines against infectious organisms, the level of immunity achieved can be affected by a variety of factors including age, sex, immunogenetic factors, prior exposure to the antigen, illness, and immunosuppressive agents. It has not been necessary to work out the conditions for a 100% immunization rate with vaccines in the past; however, in the case of contraceptive vaccines it will be essential to achieve and sustain immunity in all vaccinated individuals. New techniques to accomplish absolutely effective immunization must be explored, and follow-up serological studies should be conducted in the initial clinical trials to insure that persistent immunity has been obtained.

Reproductive tissues, gametes, and early embryos all express foreign antigens, but natural protective mechanisms have evolved to prevent sensitization to these antigens and to suppress immune responses should sensitization occur. Such natural protective mechanisms are not fully understood and will probably interfere with the antifertility effects of certain of the contraceptive vaccines.

It will be absolutely essential to test for short-term and long-term pathological side effects of contraceptive vaccines. It will be necessary to conclusively establish that the immune response generated to a reproductive antigen does not cross-react with antigens of any other tissue.

With a vaccine directed against reproductive antigens, there will be a risk of immune complex disease, which can produce extensive or subtle arterial and glomerular damage. With vaccines against embryonic or other pregnancy-associated antigens, there obviously also exists the risk of teratogenesis. A less obvious potential risk is the possibility of enhanced tumor growth. Malignant cells often express embryonic antigens which serve as targets for immunosurveillance. In animals that have been immunized with embryonic tissue, tumors often grow better than in nonimmunized animals because the embryonic antigens are masked by "blocking" noncomplement fixing anti-

bodies, or natural anti-tumor immune responses are inhibited by specific suppressor cells which were generated by the immunization procedure. The possibility that these very serious pathological side effects could develop in healthy people as a result of a contraceptive vaccination program absolutely demands that a cautious solid scientific approach be taken. Vaccine candidates must be well characterized and well tested in animal models before use in clinical trials.

SUMMARY

Immunologic approaches to human fertility regulation are now feasible owing to recent biochemical advances and the new technologies of human monoclonal antibody production and genetic engineering. It remains to be decided which reproductive antigens will elicit the best immunologic protection with the least risk of side effects. Since most reproductive antigens are weak immunogens, suitable adjuvants must also be developed and tested with each antigen system. Unlike vaccination against infectious diseases, contraceptive vaccines must achieve a near 100% efficacy level to be acceptable.

The major effort devoted to development of contraceptive vaccines in India makes it exceedingly likely that breakthroughs will occur. These will lead directly to development of innovative technology for contraception. The strong and viable program that is being put into action in India will not only be accelerated by collaborative efforts, but also will contribute much to our own basic and applied research in immunoreproduction.

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