MELLON REPRODUCTIVE BIOLOGY
CENTERS AND CONTRACEPTIVE
DEVELOPMENT & FUNDING AGENCIES

1993

Family Health International, P.O. Box 13950, Research Triangle Park, NC 27709
TABLE OF CONTENTS

Reproductive Biology Centers

Baylor College of Medicine ............................................. 2
Department of Cell Biology

Eastern Virginia Medical School ...................................... 6
The Jones Institute for Reproductive Medicine

The Population Council .................................................. 10
Center for Biomedical Research
(Reproductive Physiology)

University of California - Davis ..................................... 15
California Regional Primate Research Center

University of Connecticut Health Center .......................... 19
Contraceptive Development Research Center

University of North Carolina ......................................... 21
The Laboratories for Reproductive Biology

University of Virginia Health Sciences ............................ 26
Center for Recombinant Gamete Contraceptive Vaccinogens

University of Washington .............................................. 33
Population Center for Research in Reproduction

Contraceptive Development and Funding Agencies

Agency for International Development (AID) ..................... 38
Contraceptive Research and Development Program (CONRAD) ............................................. 43
Family Health International (FHI) .................................. 46
Mellon Foundation ......................................................... 49
National Institute of Child Health and Human Development (NICHD) ...................................... 53
Rockefeller Foundation .................................................. 58
South to South .............................................................. 73
The Population Council - Center for Biomedical Research
(Contraceptive Development) ........................................... 79
World Health Organization (WHO) .................................. 85
The Mellon Training grant project at Baylor College of Medicine is comprised of two components which are directed towards the development of contraceptive methods. First, specific research projects are ongoing which are supporting junior faculty in their programs to develop contraceptive methods which can be tested in animal models. Secondly, the training program for post-doctoral fellows and graduate students will be elaborated to insure the commitment of young scientists in the area of contraceptive development.

A. Research projects. Initially, two research junior faculty carrying out research directed toward contraceptive development have been supported.

Dr. Hanna Beekman is carrying out studies to demonstrate the feasibility of regulating hormone action *in vivo* using cholesterol-derived triplex-forming oligonucleotides which can be taken up by cells to inhibit transcription. It has been found that specific oligonucleotide sequences can bind to nucleotide sequence of cellular genes or RNAs with high affinity and selectivity and subsequently arrest gene expression. The use of a triplex strategy employs single-stranded DNA oligonucleotides that bind to the major groove of a double stranded target DNA to form a triple-helix or "triplex" in a sequence specific manner. When these triplex-forming oligonucleotides are targeted to a vital promoter region, they will selectively repress transcription and will inhibit production of proteins. These studies have demonstrated the potential for this novel approach to regulate not only hormone action for contraceptive development but may have significant implications in other contraceptive strategies.

The specific aims of this project are to carry out studies using these triplex-forming oligonucleotides to determine the efficacy of this approach in animal models and evaluate contraceptive efficiency. Although initial studies will be carried out in small animals under the direction of the investigator at Baylor, the association with the Institute of Primate Research in Nairobi, Kenya will allow us to rapidly move into primate model.

Dr. Joy Mulholland has been examining the effects of progesterone on uterine stromal cell gene expression using subtracted cDNA libraries. A number of cDNA clones have been isolated from the library which may play a determinative role in stromal cell differentiation and embryo implantation. When embryos attach to the luminal epithelium of the uterus, they induce proliferation and differentiation of the stromal cells underlying the attachment site. As the cells differentiate. This "decidualization" of stromal cells is critical to the establishment of pregnancy in women as well as in rodents which provide effective animal models for the study of implantation. The failure of stromal cells to decidualize prevents the subsequent migration of the embryo into the uterus and development of the placenta, resulting in the loss of embryos at a very early stage of development. If stromal cell differentiation is inhibited, pregnancy can be interrupted even before it can be detected biochemically, thus providing an acceptable pregnancy termination method for many cultures.

B. Training of post-doctoral fellows in contraceptive development research.

Although the junior faculty described above will be initiating their own projects in this area of research, there are other faculty who have major projects directly or indirectly related to
contraceptive development. These faculty interact with other faculty which are carrying out projects which are critical for the development of new techniques for developing in proved contraceptive technology. The joint training of post-doctoral fellows in laboratories established in contraceptive development with those in state of the art molecular biology laboratories, will be critical the advancement of techniques in this area.

1. Specific examples of training in contraceptive biology.

(a) The development of molecular approach to contraception vaccine development. It is clear that contraceptive vaccines hold great promise for large scale contraception. The co-director of this program, Dr. B. Dunbar has had 20 years of experience in the area of contraceptive vaccine development and therefore has a clear understanding of the needs for future development of safe, effective, contraceptive vaccines. Other faculty members in the department have established new methods using injectable forms of nucleic acids designed to be incorporated into cells for limited or long term expression of specific proteins. These procedures have been shown to be effective in eliciting antibody titers and are considerable implications in vaccine development in general as well as in contraceptive vaccine development.

(b) The development of molecular approaches to enhance immunogenicity in proteins in vaccine development. It has become increasingly apparent that a limitation in contraceptive vaccine development has been the lack of immunogenicity of specific peptides and recombinant proteins. The development of better expression systems designed for immunogenicity enhancement in contraceptive vaccines, is crucial. Because different investigators in the Reproductive Biology center have established a variety of expression systems, including eukaryotic glycosylation expression systems, the training of fellows working with gamete antigens for contraceptive vaccines will be invaluable.

2. Formal training in reproductive biology. Because the center has regular seminars and workshops in reproductive biology, and offers formal coursework in reproductive biology to graduate students (many post-doctoral fellows audit) the exposure of young investigators in this area is extensive.

3. Twinning collaboration with Reproductive Biology Center at the Institute of Primate Research in Nairobi, Kenya

The Institute of Primate Research (IPR) has had an established program in Reproductive Biology for many years. An alumnus (Dr. Eric Schwoebel) of the Department of Cell Biology at Baylor College of Medicine took a position as a five year fellow with the World Bank at the IPR. During his tenure at Baylor College of Medicine, Dr. Schwoebel was involved in the molecular cloning of cDNAs for zona pellucida proteins, the expression of these proteins in a variety of bacterial expression vectors. He was involved in the purification of these proteins for testing in the primate model (cynomolgus monkeys). Dr. Schwoebel's position at the IPR in collaboration with the existing scientists who have expertise in primate reproduction will be invaluable in future collaborations with investigators of the proposed studies.

Dr. B. Dunbar will visit the IPR on an annual basis (first trip in June, 1993) and will provide supplies for the Primate Inst. which are difficult to obtain in Africa. collaboration with this institution is particularly important in view of the future need for primate models for rapidly
evaluating new contraceptive methods. Dr. Bambra of the Institute of Primate Research is in
the process of identifying a post-doctoral fellow to train at Baylor College of Medicine. The
fellow will work directly with the directors of the program on projects directed toward testing
in the primate model. As contraceptive development projects progress (i.e. the oligonucleotide
triplex and glycosylated ZP expression studies), investigators will travel to the IPR to deliver
samples and design animal studies with Dr. Bambra and Dr. Schwoebel.
Summary of Contraceptive Development Efforts undertaken by Mellon Foundation Supported Investigators at The Jones Institute

The Mellon Contraceptive Research and Training Center at The Jones Institute is taking a three pronged approach to Contraceptive Development with each avenue of study falling under the direction of one of the Mellon supported investigators.

Dr. Mahony is studying factors that effect sperm maturation. So far efforts have been focused on developing the methodology for establishing primary cultures of epididymal epithelial cells and on gaining a better understanding of the normal maturational process that occur within the epididymis. Acquisition of sperm motion is under hormonal control. The relationship between maturational antigens present on epididymal spermatozoa and the androgen-dependent proteins secreted by the epididymal epithelium are being investigated. This study will involve the treatment of male cynomolgus monkeys with agents (5α-reductase inhibitors and GnRH antagonists) to abrogate the production of these androgens. The ultimate goal of these studies is to study, in a controlled manner, the effects of various potential contraceptive agents on sperm maturation.

Dr. Gordon is studying ways to enhance and extend the effectiveness of suckling-induced subfertility for lactating women. Current breastfeeding norms coupled with the demands of modern living lead to a situation where breastfeeding is no longer reliable as the sole source of contraceptive protection beyond four to six months in many women. The physiological mechanisms responsible for the contraceptive effects of breastfeeding are not completely understood. However, current thinking is that the neural stimulus provided by the infant sucking in some way reduces the pulsatile release of GnRH from the hypothalamus, thereby reducing tonic LH/FSH secretion and hence maintaining ovulation. The rhesus and cynomolgus monkeys have been used extensively as models for postpartum physiology. When housed with their infants, gonadally intact monkeys do not constitute an ideal model for monitoring the effectiveness of a GnRH antagonist. Since all of the indices of effectiveness (LH, FSH, E₂, P₄, levels) remain fully suppressed in most lactating mothers until the baby is weaned. Ovariectomy of female monkeys during the menstrual cycle initiates a rapid rise in gonadotropin levels. However, when ovariectomies are performed during the first month postpartum, the rise in serum gonadotropin concentrations is both delayed and less marked suggesting that the proximal signal provided by the sucking infant is able to attenuate the gonadotropin hypersecretion that normally follows ovariectomy. We have recently established that when ovariectomies are performed at five months postpartum, levels of gonadotropins rise rapidly even though suckling continues. We propose to utilize this model to predict the contraceptive effectiveness of the GnRH antagonist regimens to be tested.
Dr. Lanzendorf has been establishing a working non-human primate (cynomolgus) in vitro fertilization system that can be used to directly assess the actions of contraceptive approaches designed to act at the level of oocyte maturation and/or fertilization. To this end, the following have been accomplished:

1. Five male monkeys have been identified which consistently produce good quality semen that fertilizes oocytes in vitro.

2. A successful method for capacitation and hyperactivation of cynomolgus monkey sperm has been identified.

3. Culture mediums which produce high rates of oocyte maturation, fertilization and cleavage have been identified.

4. High rates of fertilization are currently being obtained, although rates may vary depending on an individual monkey's response to hyperstimulation.

5. High rates of blastocyst formation are being achieved and are slightly higher than those of other centers doing similar procedures.

Studies were also performed in the development of an in vitro assay which evaluates human sperm penetration through the human zona pellucida. Referred to as the Zona Penetration Assay (ZPA), this test uses the zona pellucida of nonviable human oocytes. This assay can be used with the Hemizona Assay (HZA) and Sperm Penetration Assay (SPA), tests which assess zona binding and plasma membrane penetration, to allow a sequential evaluation of gamete interaction and fertilization events. In the future, we will be using the ZPA to evaluate various contraceptive agents to determine at what level fertilization is inhibited. In addition, this assay can be used as an indicator of fertilization to study normal physiology.

With this working system, we are planning the evaluation of potential contraceptive agents for the inhibition of early fertilization events and cleavage. Currently, Dr. Sergio Oehninger is currently involved in the production of recombinant human ZP3. ZP3 is the main punitive zona receptor for sperm binding. We will initially perform competitive studies in the monkey IVF system to assess fertilization inhibition in vitro. These studies will parallel those in the human using the ZPA and HZA. Once produced, antibodies to ZP3 will be developed and tested in the monkey IVF system as a contraceptive agent.

Endometriosis, the disease in which the lining of the uterine endometrium grows outside the uterus, often results in infertility in the human. Some investigators believe that the abnormal cells within the peritoneum produce a toxic component which may inhibit fertilization, cleavage or implantation. We are currently collecting intraperitoneal fluid from monkeys with induced endometriosis. These fluids will be added to the
medium used for monkey IVF to determine if some "factor" in the fluid inhibits fertilization or cleavage. The information gained from this study will not only provide information which may assist in the treatment of infertility, but may provide an agent which blocks fertilization or early development.

Studies will also be initiated to evaluate the use of low-dose anti-progestins as a method of contraception without disruption of the ovarian/menstrual cycle. Preliminary studies have demonstrated that low weekly doses of anti-progestins did not prevent ovulation but did prevent conception in monkeys. The established monkey IVF system will be used to evaluate the effects of these compounds on in vitro fertilization and cleavage as well as implantation following embryos transfer. This study will allow us to better understand if the mechanism of action is on the gametes/embryos or on the reproductive environment.
The Population Council
Center for Biomedical Research
(Reproductive Physiology)
A large fraction of the Basic Research Program in Male Reproductive Physiology at the Center for Biomedical Research is focussed on the development and differentiation of the testes and on autocrine and paracrine regulation of testicular functions. This report will summarize research projects that involve: (1) studies of the differentiation of Leydig cell precursors in vitro; (2) identification of Sertoli cell proteins that affect germ cell development; (3) characterization of a germ cell factor that regulates Sertoli cell function; (4) determination of the roles of inhibin and activin in intercellular signalling in the testes; and (5) analysis of the transmission of HIV in genital tract epithelial cells.

The experimental approach to define the mechanisms that control pubertal differentiation of Leydig cells involves characterizing changes in Leydig cell function during their developmental maturation in the testis. Purified Leydig cells at three stages of maturation were obtained from rat testes. These included progenitor cells, immature Leydig cells, and adult Leydig cells that were purified from 21-, 35-, and 90-day-old rats, respectively. The functions of these cells were assessed by measuring steroidogenic enzyme activity and levels of LH and androgen receptors. Despite their morphological dissimilarity from immature and adult Leydig cells, progenitor cells exhibit characteristics that identify them as part of the Leydig cell lineage. For instance, they possess low levels of steroidogenic enzyme activity as well as receptors for LH and androgen. In related studies, an in vitro culture system for purified Leydig cell progenitors will enable testing of the direct effects of various hormones on development of the cells' ability to make steroids. Previous studies showed that treatment with LH and androgen in combination increased the production of testosterone by Leydig cell progenitors. Recent studies of the progenitor cells have characterized the expression of several genes expected to be important in Leydig cell differentiation and the development of steroidogenic capacity. These include the genes
for the receptors for androgen and LH, cholesterol side chain cleavage P_{450} and 5α hydroxysteroid dehydrogenase. Understanding the mechanism by which Leydig cell progenitors achieve their capacity for steroidogenesis will mark a significant advance in the ability to regulate testicular function, and thus fertility, in animals and humans.

A comprehensive study of Sertoli cell protein secretion is being undertaken to determine physiological significance of these proteins in the regulation of germ cell development in the testis. Testins I and II, two structurally and immunologically related Sertoli cell proteins, were shown to be components of the junctional complexes between epithelial cells in the reproductive tract. More importantly, the secretion of testins by Sertoli cells appears to be inversely related to the number of germ cells both in vitro and in vivo. This finding suggests that germ cells can regulate specific Sertoli cell function which were also indicated by the identification of a factor released by germ cells that affects the secretion of testins I and II by Sertoli cells. These observations support the concept that newly identified proteins from Sertoli and germ cells will lead not only to a better understanding of specific cell interactions, but also to more general knowledge of testicular function.

Cytokines and opioids within the seminiferous tubule may act as mediators of cell-cell interactions. Studies indicated that germ cells produce an inhibitory factor that attenuates the response of Sertoli cells to FSH. This factor inhibits the Sertoli cell response by affecting the signal transduction system used by FSH at the level of the guanine nucleotide binding subunits of adenylyl cyclase. In contrast, Sertoli cell proenkephalin gene expression is increased in the presence of this germ cell factor, suggesting that opioids are involved in a regulatory loop between Sertoli and germ cells. Previous studies showed that the opioid-derived peptides are regulators of proliferation and differentiation, a finding consistent with the concept that a local germ cell-dependent opioid regulatory circuit regulates the size of the germ cell population and determines its growth in the tubule. Germ cells may also provide a microenvironment in which their secretory factors modulate the responsiveness of the Sertoli cell to extragonadal stimuli.
Current experiments are aimed at establishing how these germ cell factors are regulated.

Gonadal inhibins and activins modulate physiological functions in the testis, in addition to the well-known feedback regulation of pituitary FSH secretion. These observations suggested that inhibins and activins, and their receptors, are co-expressed in gonadal tissues and play a role in the complex intercellular signaling that controls testicular function. To study the activin receptor and its function in the testis, cDNAs for two species of the Type II receptor (ActRII and ActRIIB) from rat testis were cloned and characterized. The genes for these receptor subtypes are widely expressed in the reproductive tissues of male and female rats. While ActRIIB gene is constitutively expressed in reproductive tissues, the expression of two different-sized ActRII mRNAs (6 and 3 kilobases) is tissue-specific and age-dependent in rat testis. The 6-kilobase mRNA is the predominant form in the immature testis, while the 3-kilobase mRNA increased with age and became the major form in mature testis. Interestingly, germ cells express high levels of the 3-kilobase ActRII mRNA. The structure of the two activin receptor genes and the possible mechanisms involved in the regulation of their expression in Sertoli and germ cells are under investigation.

Previous observations showed that the expression of inhibin β-B-subunit gene is tissuespecific. Two species of inhibin β-B-subunit mRNAs (4.4 and 3.3 kilobases) of equal abundance were identified in the testes of many species. In the ovaries, however, one predominant band of 4.4 kilobase and a minor band of 3.3 were detected. Recent studies of the structure of the two mRNAs suggested that the two heterogeneous-sized inhibin β-B-subunit mRNAs found in the testis may be derived by transcription of the gene from alternate initiation sites. The identification of the transcription start sites and the characterization of the structure of the 4.4-kilobase β-B-subunit mRNA and its protein product are in progress.

Development of a vaginal contraceptive that will also protect women and men against the sexual transmission of AIDS is a long-term goal of our Center. Previous studies revealed that HIV-infected white blood cells, which are present in semen and in the vagina at menses, carry
the virus to the epithelial cells that line the genital tract; HIV-infected blood cells are able to attach to these epithelial cells. The blood cells then shed virus onto the surface of the epithelial cells, which in turn become infected with virus. New virus produced by the epithelial cells can subsequently infect other cells of the body. Recent studies led to the elucidation of molecules involved in attachment of HIV-infected blood cells to genital tract epithelial cells, in shedding of virus from the blood cells, and in uptake of HIV by the epithelial cells.

Because this culture system mimics the sexual transmission of HIV, it can be employed to test compounds for their effectiveness in inhibiting such transmission. Research has concentrated on identifying nontoxic compounds that may be good candidates for a vaginal formulation to inhibit HIV infection. Investigators identified a class of compounds that block attachment of HIV-infected blood cells to epithelial cells and inhibit HIV infection at very low doses. Research to determine which of the compounds in this class is the most effective will pave the way for clinical studies.
The research of the Mellon Foundation Reproductive Biology Center at the California Regional Primate Research Center (CRPRC) is focused on the non-human primate model. The objective of the Center is to train investigators for contraceptive research studies with this animal model and to develop the understanding of primate sperm biology which will be needed for new approaches to immunologic and barrier contraception. Studies with non-human primates are essential elements in the development of most contraceptive technologies, yet little is known of the physiology of primate sperm or the normal biology of sperm interaction with the female reproductive tract. This lack of knowledge limits our ability to interpret results of contraceptive trials in non-human primate species. Our inability to investigate the mechanism of contraceptive action in the female limits the scope of most primate studies to simple tests of safety and efficacy.

One important area of Center research involves basic research on sperm transport and physiology in the primate female reproductive tract. This research area is highly relevant to contraceptive development because the female tract is the site of action for barrier contraceptives and most immunocontraceptives. The anatomy and physiology of the female reproductive tract is highly variable among mammals, and non-human primates are uniquely suitable as animal models for sperm transport studies. Methodology has been developed at CRPRC for studies of sperm transport kinetics including methodology for sperm recovery from the female tract, for evaluation of sperm motility and for testing of acrosomal function. Research is underway to develop in vitro assays for sperm interaction with uterine cells and oviductal cells to complement established tests of primate sperm-cervical mucus interaction and sperm-zona pellucida interaction. Methods for the culture of macaque oviduct cells have been developed and studies have revealed that these cells produce a protein which is similar to an oviduct-specific protein isolated from baboon oviducts.

Although macaques are widely used as primate models in contraceptive research, previous research at CRPRC has identified a number of potentially important differences between the physiology of macaque sperm and human sperm. Unlike human sperm which capacitate spontaneously in vitro, macaque sperm require the addition of "activators" (caffeine and dbcAMP) for capacitation. Our experiments suggest that a second messenger system other than cyclic AMP might also be involved in macaque sperm capacitation. The phorbol ester, PMA, and the synthetic diacylglycerol, DOG, are products of the IP3 second messenger system and stimulate protein kinase C. Both PMA and DOG rapidly elicit the acrosome reaction in macaque sperm and increase sperm-zona pellucida binding and subsequent acrosome reactions of bound sperm. The similarity of results with PMA, DOG and the activators caffeine and dbcAMP suggest that both second messenger pathways are involved in mediating sperm capacitation. How these pathways are involved in the zona-induced acrosome reaction still needs to be clarified and future studies will focus on the development of methods to functionally isolate the two second messenger pathways. Macaque sperm-zona pellucida interaction results in more rapid induction of the acrosome reaction.
than is observed with human gametes, but macaque sperm appear to be less sensitive than human sperm to induction of the acrosome reaction with agents such as follicular fluid or progesterone. The capability to induce acrosome reactions in motile sperm will be needed for experiments on the mechanism of action of immunocontraceptives which interfere with fertilization, and efforts are underway to develop this capability. We are also developing an in vitro fertilization model (IVF) to test the ability of immunocontraceptives to inhibit sperm penetration of the zona pellucida and fertilization. The IVF model will be particularly useful to evaluate contraceptive actions which involve sperm fusion with the oolemma.

In the area of female reproductive endocrinology, the two principal issues for study have been early pregnancy signals and the development of improved assays for reproductive hormones in non-human primates. The endocrine profiles of ovarian steroids and relaxin have been compared in humans and macaques during the menstrual cycle, early pregnancy and spontaneous abortion. Overall, the profiles were similar and support the concept that the endocrinology of early pregnancy in the macaque and human are similar, further validating the macaque as a model for study of early pregnancy signals in humans. Fecal assays for ovarian steroid hormone metabolites have been developed and validated in studies that compared serum, urinary and fecal steroids in paired samples from laboratory macaques. The same assay systems have been applied to studies of South American primate species, thus demonstrating the broad application of this technique. The fecal assays involve a simple solubilization step prior to assay with enzymeimmunoassays, making them appropriate for field studies as well as laboratory studies.

In the area of immunocontraceptive development, cynomolgus macaques have been immunized with purified, cloned rabbit zona pellucida proteins provided by Dr. Bonnie Dunbar of Baylor University and the immunized females have responded with increased serum titers of antibodies to two of the three proteins. Subsequent studies utilizing serum from immunized females indicate that antibodies to one of the zona proteins can prevent sperm-zona pellucida binding and acrosome reactions of bound sperm. Histology on tissue obtained at necropsy from these animals showed normal ovaries with developing follicles in numbers that were consistent with the animals' advancing age. Analysis of estrogen and progesterone profiles after immunization also support the finding that immunization has not disrupted ovarian function.

A peptide containing 15 amino acids from the sequence of the human ZP3 protein has been synthesized and conjugated to two carrier proteins. This peptide is immunogenic in mice and the mouse antibodies bind to human and macaque zona pellucida. Current studies in macaques reveal that the peptide is also immunogenic in primates, although the titers of antibodies produced are lower. Menstrual cycles of the animals are being monitored to determine whether there is evidence of ovarian pathology, and the animals are being bred to ascertain fertility. This model is being developed to support two long-term aims: identification of an epitope with contraceptive properties
without associated effects on ovarian function, and development of a model of the study of premature ovarian failure in women. In further support of the latter goal, the up-regulation of class II major histocompatibility antigens on cultured granulosa cells and the subsequent ability of these cells to present ovarian and non-ovarian antigens to specific T cells are being investigated.

To study the effect of contraceptives on HIV transmission, the rhesus macaque/SIV system has been used to develop a model of the heterosexual transmission of HIV. This system has demonstrated the ability of commercial spermicides containing nonoxynol-9 (N-9) to prevent the vaginal transmission of SIV. Despite the demonstrated antiviral efficacy of N-9 spermicides, it has been proposed that the vaginal irritation that results from chronic use of N-9 in women may increase the efficiency of HIV transmission. This hypothesis is currently being tested in the rhesus macaque/SIV system. Thus, rhesus macaques are given an intravaginal dose of N-9 that produces vaginal irritation and then challenged vaginally with SIV. This model can be used to test the effect of experimental contraceptives on heterosexual transmission of HIV and is also being used to develop vaccines capable of preventing the heterosexual transmission of HIV. It also characterizes the target cells involved in the genital transmission of these viruses. Both of these efforts have led us to define the cellular basis of the mucosal immune system in the lower female genital tract. This work has shown that the immune cell populations in the genital tract of rhesus macaques and women are very similar.
University of Connecticut Health Center
Contraceptive Development Research Center
THE MELLON CENTER
AT THE UNIVERSITY OF CONNECTICUT HEALTH CENTER

The Mellon Center of the University of Connecticut Health Center is a multi-institutional Center whose research mission is to develop new contraceptives. This Center is also an NIH funded Contraceptive Development Center.

The focus of our Mellon Center is immunocontraception in which the target is surface molecules of the sperm cell. Immunocontraception directed against the gamete surface is the most sophisticated barrier method of contraception, where the barrier is at the molecular level. Remarkable advances have occurred recently in immunology, molecular biology and fertilization research. These advances have combined to open possibilities for immunocontraception that were previously imagined but unattainable.

OVERALL SIGNIFICANCE OF CENTER RESEARCH:

Contraception in women: A contraceptive vaccine based on PH-20 is for use by women. PH-20 is a sperm protein shown to give 100% effective contraception in immunized female animals, and it thus has a reasonable chance of being developed into a marketed contraceptive product.

Contraception in men: One of the most significant features of Center research is that it focuses on novel strategies for male contraception. We consider one of the imperatives of contraception research to be development of methods for men because of the serious inadequacies of the few available male methods.

An extensive clinical literature shows that certain men, who are otherwise healthy, are infertile because they make anti-sperm antibodies (Bronson et al., 1984; Bronson, 1988). This "experiment of Nature" establishes that an immunocontraceptive approach for males is feasible. A number of our projects aim to determine the details of immunization methods that will make this approach a reality.

Significance of a variety of projects at different stages of development: The projects in the Center are at different stages of development in terms of a usable contraceptive product. The most advanced is the PH-20 vaccine for women. Other projects in the Center are focused on sperm antigens that might ultimately be used in a second generation, multi-antigen vaccine in which PH-20, peptides from PH-30 and p95 would be used together. Such a second generation vaccine might require fewer injections, give longer-lasting contraception or have other unforeseen advantages.

The Center also has expertise in certain state of the art technologies important for all developments in immunocontraception. One lab provides expertise for any application of sustained release of sperm antigens from bioerodible devices. Having this technology in the Center benefits all Center projects and immunocontraceptive development in general.
University of North Carolina
The Laboratories for Reproductive Biology
RESEARCH SUMMARY

THE LABORATORIES FOR REPRODUCTIVE BIOLOGY

IMMUNOCONTRACEPTION

MICHAEL G. O’RAND, Ph.D.
Cell Biology and Anatomy
University of North Carolina School of Medicine

RICHARD T. RICHARDSON, Ph.D.
Cell Biology and Anatomy
The University of North Carolina School of Medicine

The major focus of this laboratory is to isolate and characterize protein molecules involved in the binding of human sperm to human zona pellucida. The synthetic peptide, P10G from a sperm specific antigen is an autoimmune epitope in humans and when conjugated to a carrier protein inhibits fertility in mice. P10G is not a T-cell epitope, but rather a B-cell epitope. It does not elicit an autoimmune response in the female mouse.

A recombinant rabbit sperm antigen (Sp17) is a testis- and sperm-specific zona binding protein. Sp17 mRNA encodes the 17kD member of the family of rabbit testis/sperm autoantigens and is also found in mouse and human testes. Two interesting features of the deduced amino acid sequence of Sp17 are a potential sulfated carbohydrate binding site and a calmodulin binding site. Recombinant Sp17 has been expressed in COS cells and the expressed protein binds to solubilized rabbit zona pellucida. There is a striking complete identity of the N-terminal 53 amino acid residues of mouse and rabbit Sp17. Interestingly this N-terminal sequence has a 45% identity to the N-terminal region of the human testis cAMP dependent protein kinase type II α-regulatory subunit.

Nuclear autoantigencic sperm protein (NASP) has been characterized by cloning and sequencing the cDNAs of human and rabbit. There is striking sequence identity with a histone binding protein. In the human testis, NASP is localized in primary spermatocytes and round spermatids. Spermatozoa contain NASP in the acrosomal region. This histone-binding protein may play a role in regulating early events of spermatogenesis.

The testicular serine protease, acrosin, is being studied because of its central role in fertilization. Acrosin clones have been obtained from a rabbit testis cDNA library and sequenced to further characterize the role of acrosin in sperm-egg binding.

PATRICIA M. SALING, Ph.D.
Obstetrics and Gynecology and Cell Biology
Duke University Medical Center

The molecular basis of gamete interaction leading to fertilization is under investigation with the present emphasis on sperm interaction with the egg’s extracellular matrix, the zona pellucida (zp). The key regulatory event of gamete interaction, exocytosis of the sperm’s acrosome, occurs as a consequence of sperm-zona interaction, triggered by binding to one of the zp glycoproteins, ZP3. We have identified the sperm’s receptor for ZP3 as a 95 kD sperm plasma membrane protein (p95) with intrinsic tyrosine kinase activity, and shown that the activity of p95’s tyrosine kinase regulates acrosomal exocytosis. All of the features of p95 identified so far relate the receptor to the protein tyrosine kinase receptor family, represented by receptors for growth factors such as EGF and PDGF. Homologs of p95 have been identified in other mammalian sperm, including human. The primary structure of human p95, deduced by cloning and sequencing its cDNA, indicates it contains a typical single pass transmembrane region with a tyrosine kinase catalytic domain located intracellularly. The extracellular domain of the receptor protein appears unique based on a lack of homology with database sequences. In addition to studying the signal transduction cascade initiated by this receptor, we also intend to exploit the extracellular domain of the protein in contraceptive development.
SPERMATOGENESIS AND SPERM MATURATION

EDWARD M. EDDY, Ph.D.
Gamete Biology Section
Laboratory of Reproductive and Developmental Toxicology
National Institute of Environmental Health Sciences

Gene expression during spermatogenesis in mammals is being investigated with the goal of defining intrinsic and extrinsic mechanisms regulating development and function of male gametes. The products of genes expressed uniquely in spermatogenic cells, and only during particular phases of male gamete development are attractive targets for the design and testing of highly specific male contraceptives. Genes currently under investigation are glyceraldehyde 3-phosphate dehydrogenase which is expressed after meiosis in haploid spermatids and is a key enzyme in the glycolytic pathway.

The P70 gene codes for an abundant heatshock protein synthesized only during the development of male gametes and believed to function as a chaperon for other molecules essential for male gamete assembly and function. Homologous recombination is being used to knock-out the p70 gene in order to determine the role of its product in spermatogenic cells.

Other genes expressed at different phases of germ cell development and under investigation are hexo kinase and the high affinity laminin-binding protein. Other genes of interest are those expressed only in spermatogenic cells and encoding structural proteins unique to the sperm flagellum.

SUSAN H. HALL, Ph.D.
Pediatrics
University of North Carolina School of Medicine

Molecular mechanisms by which FSH regulates the functions of the Sertoli cell during the spermatogenic cycle are under investigation. Particular emphasis is placed on immediate responding genes that code for proteins important in the transcriptional regulation of other Sertoli cell genes. Several genes in this category have been cloned from rat Sertoli cell cDNA libraries. The gene for nuclear factor kappa B (NF-κB) is acutely regulated by FSH. This gene has been studied extensively in cells involved in the immune system. Efforts are underway to examine its role in FSH regulation of Sertoli cell function. Other FSH regulated genes are the rat homologue of mouse TGFβ-1 Stimulated Clone-22 (TSC-22). TSC-22 encodes a leucine-zipper containing protein which lacks a classic DNA binding region. Thus this protein could interact with DNA indirectly by binding to other leucine zipper-containing DNA binding proteins such as c-fjun and thereby modulate transcription. Other interesting FSH regulated genes which have been identified are the human B cell translocation gene-1 (BTG1) which codes for an antiproliferative factor and genes for mRNA splicing factors. Analysis of the roles of these gene products in mediating FSH regulation of Sertoli cell function will extend our understanding of the hormonal regulation of Sertoli cell function in spermatogenesis.

DAVID R. JOSEPH, Ph.D.
Pediatrics and The Program in Molecular Biology and Biotechnology
University of North Carolina School of Medicine

Studies are directed to understanding the mechanism of androgen binding protein (ABP) gene regulation and the role of ABP in spermatogenesis and sperm maturation. ABP is an androgen binding protein secreted by the Sertoli cell and transported through the male reproductive tract. Recent studies indicate that ABP interacts with a membrane receptor and thereby initiates a signaling mechanism that regulates cellular functions. Through this mechanism it could control metabolic functions in developing germ cells and in the epididymal epithelium. Determining the sites of action of ABP and the mechanism of cell regulation are research goals of this laboratory. Moreover, further analysis of the steroid binding site in ABP may allow the design of potent antagonists that prevent the binding of androgens to ABP. Such agents might also control fertility by inhibition of spermatogenesis or sperm maturation without interfering with androgen receptor action.
Current research examines the cation-independent (CI) and cation-dependent (CD) mannose 6-phosphate receptors (MPRs) as potential mediators of germ cell-Sertoli cell communication and/or the targeting of hydrolytic enzymes to the acrosome. The CI-MPR, a multifunctional protein with distinct binding sites for mannose 6-phosphate (M6P) and for insulin-like growth factor II, may mediate growth factor effects in addition to the more clearly defined role of MPRs in targeting acid hydrolases to lysosomes. Both pachytene spermatocytes and round spermatids synthesize predominantly the CD-MPR and lower levels of the CI-MPR. Sertoli cells in culture, unlike germ cells or any other cell type to our knowledge, synthesize the CI-MPR almost exclusively.

Spermatogonia and/or early spermatocytes appear to have higher levels of CI-MPR than germ cells at later stages of spermatogenesis. Germ cells and Sertoli cells have surface MPRs that mediate endocytosis. Furthermore, Sertoli cells secrete at least ten M6P-containing glycoproteins. Studies are underway to identify constituents of this glycoprotein family and to determine their effects on germ cell function. By defining the function of M6P receptors in the seminiferous epithelium, this research addresses key questions concerning the regulation of spermatogenesis and the formation of an essential sperm organelle, the acrosome.

To assess the potential role of asparagine-linked oligosaccharides in targeting hydrolytic enzymes to the acrosome, we have produced transgenic mice expressing boar proacrosin with intact N-glycosylation sites or with these sites deleted by site-directed mutagenesis. Expression of this heterologous acrosomal constituent disrupts spermatogenesis causing male infertility.

STEROID CONTRACEPTION

FRANK S. FRENCH, M.D.
Pediatrics
Director, The Laboratories for Reproductive Biology
University of North Carolina School of Medicine

The major research focus has been on androgen regulation of reproductive functions. Current research is on the analysis of androgen receptor response elements regulating gene transcription. The family of simple response elements mediating androgen regulation lacks specificity and also functions to varying degrees with glucocorticoid and progesterone receptors. However, there also exist complex elements specific for androgen receptors. Studies are focusing on the components of these elements that impart androgen specificity. This laboratory has had a long-standing interest in the role of androgens in spermatogenesis and sperm maturation and androgen interactions with peptide hormones regulating these processes. Of particular interest are the interacting roles of FSH and androgens in regulating Sertoli cell functions.

To identify human epididymal proteins that bind spermatozoa, human epididymal cDNA expression libraries are being screened with human sperm antibodies. Positive clones will be sequenced and from the derived amino acid sequence, synthetic peptides will be prepared. Antibodies will be raised against these synthetic peptides for further characterization of the proteins. Additional approaches for isolation of human epididymal secretory proteins that bind spermatozoa will be included in a collaboration with Drs. Joseph, O’Rand and Richardson.

KENNETH S. KORACH, Ph.D.
Laboratory of Reproductive and Developmental Toxicology
National Institute of Environmental Health Sciences

Research centers on understanding the intracellular mechanism of action of estrogenic compounds and estrogen stimulation of reproductive tract tissue. Receptor mediated estrogen stimulation of mouse uterine tissue has been shown to require multiple rather than a single receptor interaction with the genome. Potent estrogens produce both
an early and late peak of estrogen receptor binding to chromatin while weak estrogens produce only the early peak. Nuclear receptor sites analyzed during the second peak show a high degree of salt resistance suggesting a strong receptor-chromatin association and binding to nuclear matrix, a site of active gene transcription.

A series of stilbestrol-based compounds is being investigated for their estrogenic hormonal activity. Most interesting, these compounds have high receptor binding properties, but poor uterine biological activity. The compounds have been shown to produce differential uterine responsiveness. Because of these unique properties the compounds are being investigated for their neuroendocrine activity. Preliminary results from a pituitary culture system indicate one compound is selectively more active in inhibiting FSH release than uterine stimulation. These results suggest the possibility that at certain doses the compound could have contraceptive activity without reproductive tract side effects.

ELIZABETH M. WILSON, Ph.D.
Pediatrics, Biochemistry/Biophysics
University of North Carolina School of Medicine

Research is focused on mechanisms of androgen receptor regulation of reproductive functions. Cloning and sequencing of the androgen receptor complementary DNA has lead to extensive analysis the functional domains of the receptor including those involved with steroid binding, nuclear translocation and transcriptional activation. Current studies include the role of phosphorylation in androgen receptor functions and identification of factors that regulate androgen receptor degradation. Recent studies have also investigated the mechanism of action of antiandrogens and their effects on androgen receptor phosphorylation nuclear uptake and degradation.

OVULATION CONTROL

DAVID W. SCHOMBERG, Ph.D.
Obstetrics and Gynecology and Physiology
Duke University Medical Center

Mechanisms of growth factor and gonadotropin receptor regulation in the developing ovarian follicle has been a major emphasis of this laboratory. Our group has completed its work on the identification of the various transforming growth factor β-sub-types (TGF-β) produced by ovarian follicular cell types. TGF-β 1 and TGF-β 2 mRNA were found to be produced by both human granulosa and cumulus cells. Studies have led to the discovery of a putative small-molecular-weight polypeptide produced by cultured granulosa cells which is not TGF-β and which inhibits tritiated thymidine incorporation/cell proliferation. Isolation and cloning of this factor are being attempted currently. Advances in this area should have application contraceptive development.

Follicle-Stimulating Hormone receptor gene promoter-SV40 cDNA constructs have been prepared for an attempt to elicit ovary-specific oncogenesis in transgenic mice. From any tumors developed we hope to establish immortalized FSH receptor-expressing cell lines which will be of great value in studies on the regulation of granulosa cell function. Susan Hall, David Joseph and Deborah O'Brien will collaborate in characterizing the cell lines derived from Sertoli cell tumors in male offspring.

REGULATION OF GONADOTROPIN SECRETION

WILLIAM L. MILLER, Ph.D.
Biochemistry
North Carolina State University

We are identifying a substance in bovine pituitary culture media that inhibits FSH production. This may be inhibin, follistatin, or a new protein suitable for contraceptive development. Regulatory effects of inhibin, estradiol and progesterone on GnRH-receptor mRNA in ovine pituitaries are also under investigation. 100-Fold regulation of this mRNA has been found and understanding the mechanisms of such changes could lead to development of additional methods of regulating gonadotropin secretion.
University of Virginia Health Sciences
Center for Recombinant Gamete
Contraceptive Vaccinogens
ABOUT THE CENTER

Rapid progress in the fields of reproductive biology, immunology, molecular biology, and chemical engineering has laid a foundation for development of contraceptive vaccines based upon gamete immunogens obtained through recombinant DNA techniques. Monoclonal and polyclonal antibodies have allowed definition of specific sperm and egg molecules playing key roles in the processes of sperm and egg development, sperm capacitation, and fertilization; recombinant DNA technology has made possible the definition and manipulation of the genetic material encoding these molecules; advances in protein synthesis and in bioprocess development and product purification have allowed sufficient quantities of rDNA products to be obtained in relatively pure form. Injections of recombinant vaccine immunogens in primates have shown strong and specific immune responses. In vitro effects of antibodies from inoculated primates on blocking sperm-egg interactions have been observed. Thus, the theoretical pathways for the emergence of a contraceptive vaccine based upon recombinant DNA techniques are at hand and initial results have been promising.

The long term mission of the Center for Recombinant Gamete Contraceptive Vaccinogens is to provide the essential organizational framework to allow convergence and assembly of the disparate technologies and personnel required to identify, formulate and test vaccine candidates and thus maximize the potential development of a contraceptive vaccine for humans. The Center currently emphasizes a vaccine for females, although research germane to a male vaccine is also underway. The vaccine will consist of one or several gamete associated immunogens which elicit immunological responses that function to prevent fertilization. The Center coordinates research and development activities encompassing seven stages of a recombinant contraceptive vaccine product development program: 1) Fundamental discovery and evaluation of gamete specific molecules [vaccinogens] derived from the sperm, egg, egg investments or accessory reproductive organs; 2) Genetic engineering of genes encoding specific vaccinogens into appropriate expression systems; (3) Production and purification of recombinant vaccinogens under good laboratory practices; Formulation of vaccine doses; 4) Small animal and primate testing of vaccine formulations for immunogenicity, safety and efficacy; 5) Evaluation of mechanisms of vaccine action; 6) Human trials in association with industrial sponsorship; 7) Development of diagnostics to monitor infertility status.

Research projects in the Center include: 1) Evaluation of peptides derived from ZP3 [Dr. Kenneth Tung]; 2) Identification and cloning of a new human sperm surface antigen which functions in sperm/zona binding [Dr. Michael O’Rand]; 3) Cloning and characterization of a human sperm antigen identified with infertile sera [Dr. Erwin Goldberg]; 4) Development and testing of an oral sperm vaccine for stimulating secretory immunity in the oviduct [Drs. John Herr, Charles Flickinger, and Roy Curtiss III]; 5) primate efficacy trials of injectable contraceptive vaccines based upon human sperm immunogens LDH-X [Dr. Erwin Goldberg]; 6) SP-10 [Drs. Herr and Flickinger]; and, 7) studies of sperm autoantigens recognized by serum from vasectomized rats [Drs. Stuart Howards, Flickinger, and Herr]. Attention will be placed on development of multideterminant vaccine [combination formulations] if indicated.

Scientific support Cores provide specialized technical assistance to Center members. A Tissue Specificity Core [Dr. Flickinger] provides immuno-cytochemical, ELISA and Northern Analysis at the protein and mRNA levels for determining the gonod specificity of gene products of interest. A
Bioprocess/Product Development Core [Dr. Kirwan] will be developed in 1992-1993 to express recombinant DNA in bioreactors and purify the products according to good laboratory practices. An Immunopathology Core [Dr. Tung] will assess the possible pathological consequences of vaccines on gonadal and non-gonadal tissues. In future years, cores for development of oral contraceptive vaccines [Dr. Curtiss] and primate experimental models [Dr. Goldberg] will be initiated. Existing cores performing services in contemporary cell and molecular biology at several institutions are marshalled in support of the Center.

Postdoctoral training in the manifold technologies required for contraceptive vaccine development are offered within the Center at the participating institutions. Postdoctoral positions are supported by the NIH, the Mellon Foundation, and individual research grants.

The Center assembles a multi-institutional, interdisciplinary group of principal investigators with solid experience in the discovery and evaluation of candidate contraceptive vaccine immunogens. The Center is overseen by an Executive Committee representing industry, the FDA, immunology, and obstetrics and gynecology. The Center is supported by a U54 NIH award, is a designated Mellon Foundation Center for Postdoctoral training, and receives industrial-sponsored grants on specific projects.

JOHN C. HERR
Professor of Anatomy & Cell Biology,
University of Virginia
Ph.D., University of Iowa

Molecular Biology of Human Spermatogenesis

Dr. Herr’s research laboratory examines human sperm proteins which are expressed specifically in the testis. Such molecules contribute to the unique cytoarchitectural features of the sperm cell, which is one of the most highly differentiated cell types evolved. The long term goal of the research is to identify sperm molecules which play key roles in events such as sperm-egg interaction and which may be effective components of a contraceptive vaccine for inoculation into females. The research affords opportunities for training in hybridoma production and characterization of monoclonal antibodies, immunocytochemistry, electron microscopy, immunofluorescent microscopy, gene cloning and expression, scale up of recombinant expression vectors, protein separation and purification, and studies of the kinetics of immune responses in primates. Monoclonal antibody diagnostics and a recombinant vaccine are products which may result from these studies. The laboratory has close ties with the pharmaceutical industry for transfer of contraceptive vaccine technology.

During 1991-1993 one of the principal research objectives of Dr. Herr’s laboratory is to characterize and test in baboons the efficacy of a contraceptive vaccine based upon the sperm specific immunogen, SP-10. This immunogen was identified as a primary vaccine candidate by the WHO Task Force on Vaccines for Fertility Regulation.

The biochemical basis of SP-10 polymorphism is being studied by first purifying SP-10 peptides using HPLC and preparative electrophoresis and then obtaining amino acid sequence and carbohydrate composition and sequence data. An epitope library of SP-10 gene fragments will be screened with the MHS-10 monoclonal antibody to map the precise amino acid sequence of the MHS-10 epitope. The gene for SP-10 will be cloned and sequenced and examined for regulatory elements. Further, regions coding for the SP-10 protein will be inserted into bacterial expression vectors and milligram amounts of immunogen will be purified. In order to better understand the structure of baboon SP-10, cDNAs from baboon testes will be cloned and sequenced to determine the homology between human and baboon SP-10. A fertility trial of a recombinant SP-10 vaccine will be conducted in thirty female baboons. The kinetics and specificity of the baboon humoral response to SP-10 will be examined. The baboon polyclonal response to recombinant SP-10 will be employed to study the immunodominant epitopes on the SP-10 molecule.

In summary, the research program in Dr. Herr’s laboratory is geared to develop fundamental information regarding the molecular architecture of molecules found in association with the membranes of human
sperm, including amino acid and carbohydrate structure. In addition, the capacity of such molecules to elicit an immune response and antifertility effects in primates is investigated, potentially leading to development of a novel contraceptive vaccine.

KENNETH S. K. TUNG  
Professor of Pathology, University of Virginia  
M.D., Melbourne University

Molecular Basis of Autoimmunity of Reproductive Organs

The primary research of my laboratory focuses on the pathogenetic mechanism of autoimmune disease and the physiologic basis of immunologic tolerance to organ-specified self antigens.

We recently identified a 13 mer antigenic peptide in the ZP3 protein of the murine zona pellucida. The ZP peptide has a 7 mer B cell epitope that elicits antibody response and reversible female infertility, and an overlapping 8 mer epitope that elicits T cell response and autoimmune oophoritis. A major effort is to design a contraceptive vaccine for females based on this model peptide that will induce reversible infertility without ovarian injury. An even more basic question addresses tolerance mechanism to ZP proteins, a gender specific antigen. For this, we plan to produce T cell receptor transgenic mice using the αβ T cell receptor of a pathogenic T cell clone.

As a second approach to tolerance mechanism, we have succeeded in eliciting autoimmune disease of the stomach and ovary by transferring lymphoid cells from normal mice to syngeneic athymic nu/nu recipients. We have discovered that neonatal cells, enriched in self reactive pathogenic T cells, could transfer disease, whereas adult cells failed to do so. However, adult cells can prevent neonatal cells from inducing disease thus regulatory cells must exist in the normal adult T cell pool. Moreover, both neonatal and adult thymocytes were capable of disease induction thus clonal deletion is not sufficient to control autoreactive T cells. We now aim to further investigate the self reactive T cells by cloning gastric antigen specific T cells. The antigen of interest is the gastric parietal cell H+K+ATPase, β chain.

CHARLES J. FLICKINGER  
Professor and Chair of Anatomy & Cell Biology, University of Virginia  
M.D., Harvard University

Male Reproductive Cell Biology

One of the research projects in Dr. Flickinger's laboratory involves study of the morphologic, immunologic, and physiologic effects of vasectomy and vasectomy reversal (vasovasostomy) in a rat model system. Although vasectomy is a common means of contraception in men, questions remain about its reversal, because an individual may remain infertile despite successful surgical reconnection of the vas deferens. Aims of this research are to determine the nature of changes after vasectomy and the extent to which they are reversible by vasovasostomy. We have found alterations in the testis and epididymis after vasectomy, correlated these changes with antisperm antibodies, and identified dominant sperm antigens post-vasectomy. Future goals are to determine why some individuals are fertile while others are infertile after vasovasostomy, and to learn more about the characteristics of sperm antigens that are recognized post-vasectomy. Antigens that participate in infertility after vasectomy reversal may then be studied further as potential contraceptive immunogens.

Dr. Flickinger's laboratory is also collaborating with Dr. John Herr in morphological, biochemical, and molecular studies of human sperm antigens. For example, immunocytochemistry is being used to localize antigens in the testis and in spermatozoa, and testicular gene expression in various types of spermatogenic cells is being studied using in situ hybridization methods to detect specific germ cell mRNAs.

Other studies have examined the secretion of proteins in the epididymis and sex accessory glands. Proteins secreted by the epididymal epithelium, for example, are believed to play an important role in the post-testicular maturation of sperm, including acquisition of fertilizing ability. Electron microscope radioautography and immunocytochemistry at histological and ultrastructural levels have been used to define the cells and organelles that participate in protein secretion in the epididymis and sex accessory glands.
DONALD J. KIRWAN  
Professor of Chemical Engineering, University of Virginia  
Ph.D., University of Delaware

Study of Bioreactor and Bioseparation Technology

Dr. Kirwan's laboratory is engaged in fundamental studies that will lead to the improvement of bioreactor and bioseparations technology applicable to the industrial scale processing of pharmaceuticals and other biologicals. Bioreactor projects include the use of immobilized, living microbial cells for the production of secondary metabolites, gas-liquid oxygen transfer in high cell density fermentors, scale-up of recombinant fermentations, and diffusional effects in immobilized biocatalysis. The laboratory also is engaged in obtaining engineering parameters relevant to the scale-up and optimization of monoclonal antibody production by cell culture.

The primary activity in the bioseparations area involves fundamental studies of precipitation and crystallization of biological compounds by the addition of organic non-solvents such as aliphatic alcohols. The program includes thermodynamically-based correlation of the solubility of complex organics such as amino acids, antibiotics and proteins in mixed aqueous-alcohol solutions, measurements of single crystal growth kinetics, and crystal size distributions and purity in suspension crystallizers and precipitators.

ROY CURTISS III  
George William and Irene Koechig Freiberg  
Professor of Biology, Department of Biology, Washington University  
Ph.D., University of Chicago

Microbial Pathogenesis, Vaccine Development and Host Response Research

Dr. Curtiss' research is centered in three areas of investigation. First is the study of the mechanisms by which bacterial pathogens in the genera Salmonella, Bordetella, Mycobacterium, Streptococcus and Escherichia colonize and invade the animal host to cause disease. Second is to design, develop and evaluate antigen delivery systems to use for oral immunization to elicit mucosal, humoral and cellular immune responses. Our third area of investigation is to analyze animal host responses to infection and to immunization with various vaccine compositions.

For several years the means by which orally administered Salmonella typhimurium attaches to and invades cells lining the intestinal tract, including cells in the gut associated lymphoid tissue (GALT or Peyer's patches) have been studied. This has included studies on the genetics, biochemistry and functions of lipopolysaccharide, four adhesins, a collection of eight gene products necessary for cell invasion and several gene products encoded on the chromosome and on the virulence plasmid that cause successful colonization of internal organs. Studies have also been conducted using reporter gene fusion technology to determine how the expression of these virulence traits are regulated either in response to the animal host or to the conditions in the micro environments which Salmonella encounters during infection. The information acquired during these studies has facilitated the isolation and characterization of avirulent Salmonella derivatives that have been used to vaccinate animals to induce high-level protection against infection with virulent Salmonella strains. In this regard, avirulent S. typhi strains have been and are continuing to be evaluated in human volunteers where they have been shown to be safe and immunogenic.

Efforts to develop improved immunization strategies using avirulent Salmonella strains as well as recombinant avirulent Salmonella expressing foreign antigens have been significantly expanded with development of successful vaccines to control Salmonella infections in chickens and humans. More recent efforts have been directed at generating transgenic plants expressing bacterial colonization or virulence antigens so that oral feeding of plant material will induce immune responses. It appears that this method of immunization can be used to induce oral tolerance which thus affords means to understand the events in the immune system that lead to oral tolerance. The ability to compare an avirulent Salmonella antigen delivery system vaccine with the feeding of transgenic plants expressing the same foreign antigen(s) affords the possibility to better understand the mechanisms for inducing or not inducing mucosal immune responses with the production of secretory IgA. Since there is very little known about induction of the mucosal immune responses in the reproductive tract, work has been initiated to express sperm-specific and egg-
specific antigens in recombinant avirulent Salmonella vaccine strains to investigate the potential for inducing specific mucosal immune responses in the reproductive tract with readily qualifiable biological consequences, mainly an inhibition in fertilization.

ERWIN GOLDBERG
Professor of Biochemistry, Molecular Biology & Cell Biology, Northwestern University
Ph.D., University of Iowa

Spermatogenesis/Contraceptive Vaccine Development

Research in this laboratory has been devoted to the biochemical and molecular analyses of the testis specific isozyme of lactate dehydrogenase (LDH-C). Both the mRNA and protein product of the murine Ldh-c gene appear first in the pre-leptotene primary spermatocyte, measured by in situ hybridization with riboprobes (Alcivar et al., 1991) and immunofluorescence (Hintz and Goldberg, 1977). The cDNA for human Ldh-c has been cloned from a testis expression library packaged in λgt11 (Millan et al., 1987), and used to probe genomic libraries. Characterization of four clones that encompass virtually the entire Ldh-c locus reveal that the gene is composed of eight exons. One of these clones contains the first coding exon preceded by a 247 nt intron separating it from the first exon in the 5' untranslated region of the gene. The putative genomic capsite for start of transcription is preceded by about a 120 nt sequence immediately upstream that is GC rich and contains six consensus sequences for SP1 transcription factor binding sites but no apparent TATA or CAAT box sequences.

Analysis of mouse genomic clones revealed an organization of exons and introns similar to the human Ldh-c gene. Intron 1 in the 5' UT region was amplified from DNA by polymerase chain reaction (PCR), sequenced and used as a probe to screen a genomic library. One phage clone that contained approximately 20 kb of the 5' end of the Ldh-c gene was isolated. Primer extension and ribonuclease protection experiments on mouse testis RNA identified two putative transcription start sites, contained within a 31 bp long hairpin structure and 22 bp from each other. This hairpin structure may play a role in Ldh-c gene regulation. In contrast to the human promoter region, sequence analysis revealed a consensus sequence for SP1 binding and a consensus CCAAT sequence for NF1 binding in the mouse. Experiments with constructs designed to demonstrate functional activity in vitro and in transgenic animals are in progress.

The application of the cell specificity of LDH-C to contraception technology and fertility control represents another major effort in this laboratory. Recent work has focused on the use of LDH-C as an antigen to provoke antibodies against sperm. Such antibodies in the female reproductive tract interact with the sperm and effectively block fertilization. A synthetic vaccine based on the peptide sequence of human Ldh-c is currently being used in fertility trials in non-human primates. Also by genetic engineering we are constructing a recombinant virus that will express LDH-C and serve as a mechanism to deliver the contraceptive vaccine.

Molecular cloning technology to screen a human testis cDNA expression library with sera from infertile patients has yielded potentially useful peptides for contraceptive development. One of these, designated Ag X-1, is apparently responsible for one or more of the anti-sperm antibodies that may be involved in naturally occurring immunological infertility. This approach to antigen identification and characterization has received major impetus from the establishment of the CRGCV.

MICHAELE G. O'RAND
Professor of Cell Biology & Anatomy,
University of North Carolina
Ph.D., Temple University

Molecular Biology of Sperm Autoantigens

Dr. O'Rand's research laboratory is interested in the study of sperm-egg interaction and the molecules on the sperm surface which participate in this interaction. The long term goal of our research is to define both chemically and biologically a set of sperm molecules which are necessary for one or more steps in the fertilization process. We are interested in specifically investigating the mechanism by which spermatozoa attach to and penetrate (degrade) the zona pellucida of the egg. An understanding of the mechanism of sperm-zona interaction would allow precise targets for both infertility diagnosis and contraception. Currently, the molecular characterization of several different
sperm molecules which bind to and interact with (degrade or release) the zona pellucida is under study. These sperm molecules include the rabbit sperm membrane autoantigen, RSA, which functions as a "glue" to bind the sperm to the zona, the sperm enzyme acrosin and its zona binding site, the sperm enzyme arylsulfatase A and human sperm zona binding proteins.

A number sperm surface molecules involved in sperm-zona interaction are sperm and testis specific molecules which when exposed to the immune system, result in the formation of autoantibodies in the male. In the female these same antigens elicit antibodies which can inhibit fertilization. Consequently, an important step in the development of a contraceptive vaccine is the determination of specific epitopes which bind antibody and block specific functional steps during fertilization.

Research techniques used in the laboratory include molecular biology procedures (cloning, sequencing, expression systems), protein chemistry and peptide analysis with regard to immunogenicity, antibody production and analysis and fertility testing.
William J. Bremner, Professor  
Department of Medicine, UW and Chief of Medical Service, VA Medical Center

My work is directed at developing further understanding of the control systems for human reproduction. Much of this work concerns hormonal control of sperm production, including the separate roles of LH and FSH in the control of human spermatogenesis. A major practical aim of our work is the development of a new, safe, reversible human male contraceptive. We are actively exploring GnRH analogs and progestational agents, together with testosterone as contraceptive agents in normal men. We are committed to the development of a contraceptive for men that would be used in the developing world as well as in the United States. We also study the effects of hormones from the testes (testosterone, estradiol, dihydrotestosterone) on lipid and bone physiology, on human behavior, and on the expression of the human androgen receptor. For example, we have recently demonstrated the ability of physiological levels of testosterone in men to suppress high density lipoprotein levels which has important implications for both male contraceptive development and for the etiology of the increased incidence of cardiovascular disease in men compared to women.

Bertil Hille, Professor  
Department of Physiology and Biophysics, University of Washington

We are interested in the intracellular signaling pathways that control activities of anterior pituitary gonadotropes, with an emphasis on the short-term signals that couple GnRH to secretion of LH and FSH. Single-cell biophysical approaches (with patch pipettes) applied to identified male rat gonadotropes, allow us to record intracellular $Ca^{2+}$, electrical activity, and plasma membrane area (as a measure of exocytosis) on a millisecond time scale as well as to add to the cytoplasmic compartment inhibitors and mimics of second-messenger pathways. We have shown that GnRH initiates intracellular $Ca^{2+}$ oscillations (3-15 s cycle period) by an inositol-trisphosphate (IP$_3$) dependent cyclic release of $Ca^{2+}$ from intracellular stores. Each cycle of $Ca^{2+}$ rise initiates the exocytosis of about 200 secretory granules (as judged by membrane area increase) within less than 1 s. Exocytosis slows while the $Ca^{2+}$ is still high as if the burst of hormone release exhausts the readily available pool of secretory granules within 1 s. In the few seconds between each $Ca^{2+}$ rise, more granules seem to be mobilized and are ready to be released in the next cycle. We are now exploring the $Ca^{2+}$ homeostasis of the cell and actions on the above events of application of a variety of peptide and steroid neuromodulators. These basic studies are giving clues on the mechanisms by which proposed GnRH analogs and steroid contraceptives would act on control of pituitary function and therefore on reproduction.
Alvin Matsumoto, Associate Professor  
Department of Medicine, UW and Gerontology Research, Education and Clinical Center, VA Medical Center

We have had a long-standing research interest in the hormonal regulation of spermatogenesis and male contraceptive development. We are currently performing studies investigating the suppression of gonadotropin and sperm production by combinations of a progestin (levonorgestrel) plus testosterone and a GnRH antagonist plus testosterone in men. We are also performing studies investigating central nervous system mechanisms of reproductive aging in rats and the long-term effects of a 5 α-reductase inhibitor on benign prostatic hyperplasia (BPH) in men.

G. Stanley McKnight, Professor  
Department of Pharmacology, University of Washington

Protein kinases play an essential role in intracellular regulation by phosphorylating key substrates such as ion channels, enzymes, transcription factors, and cytoskeletal proteins. Phosphorylation can reversibly alter the biological activity of these substrates allowing a cell to rapidly adapt to environmental cues and hormonal signals. Our laboratory is using the techniques of molecular genetics to study the cAMP-dependent family of protein kinases that exist in all animal cells. The cAMP-dependent kinases are formed as an inactive holoenzyme composed of catalytic subunits and regulatory subunits. This holoenzyme dissociates to release active catalytic subunit when cAMP binds to the regulatory subunits. To demonstrate the functional role of the kinase in complex intracellular pathways, genetic mutations have been introduced into the cloned protein kinase genes by mutagenesis. These mutated kinase subunits are then incorporated into animal cell expression vectors and subsequently reintroduced into either cultured cells or transgenic mice. Some of these mutations produce a dominant inhibition of kinase activity or alter the cell's ability to form specific kinase subtypes. Particular interest is focused on the role of the kinase in male germ cell development and the modulation of specific transcription factors involved in cAMP-regulated gene expression.

C. Alvin Paulsen, Professor  
Department of Medicine, UW and Pacific Medical Center

Since 1972 our laboratory has been involved in determining whether steroid hormones might be suitable as male contraceptive agents. We are examining the administration of testosterone for this purpose. We are presently involved in the only true efficacy study of a hormonal male contraceptive. This study is showing very low fertility rates in couples in which the man has his sperm production severely suppressed by testosterone. Dr. Paulsen also serves as a consultant to the other project investigators on biopsy procedures and analyses of testicular biopsies on humans and nonhuman primates.
Robert A. Steiner, Professor  
Departments of Obstetrics and Gynecology, Physiology and Biophysics, and Zoology

An array of some 1500 neurons residing in the basal forebrain and hypothalamus synthesizes and secretes a neurohormone called gonadotropin-releasing hormone (GnRH), which ultimately governs the function of the entire reproductive axis. The activities of our laboratory are focused on understanding the molecular physiology of GnRH neurons. We use a variety of molecular and classical approaches to analyze these cells. Using molecular probes (cDNA and cRNA) with *in situ* hybridization and computerized image analysis, we measure levels of messenger RNAs in individual neurons to determine when, why, how and to what degree the genes coding for certain neuropeptides and neurotransmitter processing enzymes are induced or inhibited. This work is providing basic understanding of the control of the brain peptides that regulate reproduction.

Bruce L. Tempel, Assistant Professor  
Departments of Medicine and Pharmacology, UW and Gerontology Research, Education and Clinical Center, VA Medical Center

Voltage-gated potassium (K⁺) channels are involved in determining the electrical excitability and resting membrane potential of many cell types. In the male reproductive system, specific potassium channel genes are expressed in sperm, Sertoli cells and Leydig cells. These K⁺ channels may play a critical role in spermatogenesis or in various functions of the mature sperm such as motility, which is required for fertility. We have chosen the mouse as a model system to study the structure and function of these K⁺ channels because, in mice, molecular, electrophysiological and genetic techniques can be applied in concert. By using electrophysiological techniques, for example, we can identify the K⁺ channels expressed in normal mouse testes and study the control of their function.
Contraceptive Development and Funding Agencies
SUMMARY OF ACTIVITIES IN CONTRACEPTIVE RESEARCH AND DEVELOPMENT
OFFICE OF POPULATION
AGENCY FOR INTERNATIONAL DEVELOPMENT

Jeff Spieler
Acting Chief, Research Division

I. THE ROLE OF RESEARCH WITHIN THE OFFICE OF POPULATION

Effective family planning programs are necessary to improve the health of women and men, to improve the status of mothers, children, and families and to slow population growth. In this context, research plays a vital role within A.I.D.; it builds the scientific and technological base for family planning programs. By supporting contraceptive development, operations research, and natural family planning, including breastfeeding for birth spacing, A.I.D. can help to provide more people greater access to a wider variety of safe, effective, and acceptable contraceptive methods and efficient family planning services.

II. RESEARCH DIVISION OBJECTIVES

The objectives of the Research Division are to:

- Develop and introduce improved and/or totally new methods of contraception;
- Use operations research to identify and test more acceptable and cost-effective methods of delivering family planning services;
- Improve understanding of both contraceptive technology and family planning service delivery, and provide related technical assistance; and,
- Improve knowledge, availability, and effectiveness of NFP, including breastfeeding for birth spacing.

III. PRIORITIES OF THE RESEARCH DIVISION

A. Biomedical Research in Contraceptive Development and Introduction of New Methods

Activities funded by A.I.D. in biomedical research range from the development of new contraceptive methods to their clinical evaluation and introduction into service delivery programs. Priority is placed on contraceptives that best satisfy the following criteria (not in order of priority), recognizing that no contraceptive will be "ideal" for everyone:

- **Effectiveness** - chances of unplanned pregnancy are minimal;
- **Safety** - no short- or long-term health or safety risks; secondary benefits, such as prevention of cancer or STDs, should be maximized;
• **Side effects** - the product should be safe and any side effects should be only minimal and minor, e.g. "nuisance" side effects such as transient menstrual irregularities;

• **Convenience** - the product should be unobtrusive and not interfere with sexual spontaneity, activity, or pleasure; Some people prefer long-acting products;

• **Programmatic issues** - the product should be of low cost, easy to deliver, amenable to local production, stable, and preferably independent from the medical care system;

• **Reversibility** - non-permanent methods should have no impact, or cause only a minor delay, on achieving future pregnancy when desired; and,

• **Other** - desirable attributes include consumer control, safe for use during lactation, privacy, aesthetic and ethical considerations.

Priorities in Contraceptive Technology

**Long-acting Hormonal Methods**

• NORPLANT®/NORPLANT®II implants, provide five years of highly effective contraception
• Biodegradable implants - one to two years of protection and no need for removal
• ST 1435. (progestin-only) implants - single implant for two years
• Injectables - up to three months of protection
• Vaginal rings - three to six or more months of protection

**New Barrier Methods**

• Improved condoms (including non-latex and loose fitting)
• Spermicides/virucides, and new barrier methods (e.g. Lea's Shield® and Reality® female condom) for women

**Methods Suitable For Lactating Women**

• Appropriate injectables, implants, vaginal rings, IUDs, barrier methods, and transdermals that represent no health risks to the infant or the mother

**Improved Sterilization**

• Especially non-surgical and/or reversible methods for men and women
Vaccines

• Prevent pregnancy with vaccines directed at sperm and ovum antigens in women, and FSH or LHRH in men

B. Operations Research

One of the chief strengths of the A.I.D. population program is its commitment to improve the quality, accessibility, and cost-effectiveness of family planning services through operations research (OR). Since it was initiated in 1974, the OR program has been designed to be the "R&D" of family planning services. It provides family planning program managers with the resources to identify service delivery problems and experiment with new approaches to solve these problems.

The OR program is built upon a philosophy of "try and see if it works." By encouraging program managers to adopt a problem-solving mentality, OR contributes to long-term improvements in program performance. For individual family planning programs, and for the population field as a whole, the license that OR has to be innovative has paid major dividends. OR projects testing community-based distribution (CBD) in Tunisia, Morocco, and Egypt were instrumental in demonstrating that family planning services could be safely and effectively provided outside of clinic settings. These early CBD programs were the basis for subsequent programs which have expanded access to family planning services for large segments of the population of developing countries who lack access to clinic-based services.

A.I.D.'s OR program is continuing to play a key role in technical assistance in applied research to enable family planning organizations to expand and improve service delivery. Under the umbrella OR program, "Strategies for Improving Service Delivery," A.I.D. is supporting OR and technical assistance (TA) in more than 30 developing countries through regional projects in Asia & Near East, Latin America & Caribbean, and sub-Saharan Africa.

Priorities in Operations Research

• Promoting family planning acceptance
• Testing new service delivery strategies
• Identifying ways to improve program performance and the quality of services offered clients
• Reaching underserved and hard-to-reach groups
• Increasing access to underutilized and new methods
• Reducing medical barrier to method access
• Improving quality of care
• Increasing the self-sufficiency of programs
• Improving cost-effectiveness
• Institutionalizing the OR approach
C. Natural Family Planning and Breastfeeding for Birth Spacing

In 1985, this program was created to improve fertility awareness and the acceptability, availability and effectiveness of NFP, which is defined to include all methods of family planning based on periodic abstinence, and to promote and increase the effective practice of breastfeeding for child spacing. Activities supported in this area include biomedical, social science and operations research; information, education, communication and training; technical assistance and policy support; and service delivery.

Priorities in Natural Family Planning and Breastfeeding

- Developing better and simpler methods which permit women to identify the fertile and infertile phases of their menstrual cycle (e.g. developing ovulation prediction and detection kits);
- Providing information about NFP and reproductive physiology (fertility awareness) to policymakers, health/family planning programs, clinics and clients;
- Training NFP trainers and instructors;
- Increasing knowledge about and acceptance of breastfeeding as a birthspacing method among family planning providers and users;
- Assessing the fertility impact of breastfeeding, specifically the effectiveness of the lactational amenorrhea method (LAM);
- Developing breastfeeding guidelines to maximize birth spacing effectiveness of LAM, including guidelines for use in suboptimal and long-term conditions;
- Improving and expanding service delivery of NFP, breastfeeding and LAM;
- Meeting technical assistance needs in NFP and breastfeeding in LDCs;
- Publishing findings in journals and presentations at scientific meetings; and,
- Collaborating with international groups and organizations.

Guiding Principles:

- Multiple method approach (no magic bullet)
- Voluntarism & free choice
- 4 method mix = 4 overall use
- Different methods needed at different stages of menstrual cycle

OVERVIEW.ABV/JSPIELER/6-1-93
Contraceptive Research and Development Program (CONRAD)
OVERVIEW OF THE CONRAD PROGRAM

The Contraceptive Research and Development (CONRAD) Program has as its primary mission supporting and augmenting preclinical research and clinical studies directed at developing improved and/or new methods of family planning for use in less developed countries (LDCs). To accomplish this objective, CONRAD supports research and development projects worldwide at universities, research institutes, and private companies through its Extramural Program. Currently over 45 projects are ongoing. CONRAD also has an Intramural R&D Program within the laboratories and clinics of the Jones Institute for Reproductive Medicine in Norfolk, Virginia. This combination of extramural and intramural research provides for a synergism which can significantly accelerate overall progress.

The primary focus of the CONRAD Program is on the early stages of contraceptive research and development, beginning with targeted or applied research studies and progressing through the first two phases of clinical testing in humans. Examples of current clinical studies include intravaginal barrier devices and films, norethindrone-containing implants for women, progesterone suppositories to prolong lactational amenorrhea, and testosterone formulations and gonadotropin releasing hormone (GnRH) antagonists for male contraception. Because CONRAD financial support comes from A.I.D., research on methods that act after fertilization cannot be supported. A major emphasis of the clinical program in Norfolk in recent years has been the initial testing for safety and efficacy of new barrier methods and spermicidal formulations. Development of standardized conditions for postcoital tests and colposcopic examinations to detect possible vaginal irritation has become of paramount importance.

The CONRAD Program also supports research aimed at developing methods to prevent the heterosexual transmission of sexually transmitted diseases, including AIDS. This work is supported, in part, under Interagency Agreements between A.I.D., NIAID, CDC, and the Center for Population Research at NIH. Current projects are focussed on the development of non-human primate models to study the mechanisms of HIV transmission, the identification of cellular and non-cellular components of reproductive tract secretions involved in HIV transmission in humans, epidemiologic studies looking at the impact of contraceptive methods on transmission and progression of HIV infections and the evaluation of compounds for their spermicidal and virucidal properties.

Areas of research where CONRAD either has active projects or is currently seeking additional proposals include:

- Injectable steroid preparations (including microspheres or microcapsules) that inhibit ovulation and/or render cervical mucus impermeable by sperm, or that inhibit spermatogenesis
- Biodegradable or non-biodegradable implants of contraceptive steroids
- Vaginal delivery systems for steroids including rings and suppositories
- Improved or generic intrauterine or intracervical contraceptive devices

44
CONRAD Overview
Page 2

- Methods of birth spacing that are suitable for use by lactating women
- Contraceptive vaccines using ovum, sperm or hormonal antigens for women and men
- Tubal or vas plugs and other devices/procedures that provide a means of reversible sterilization
- Adhesives, chemicals or other materials that can be delivered non-surgically, either transcervically or transcutaneously for female or male sterilization, respectively
- Vaginally applied chemical preparations that immobilize sperm and/or interfere with sperm penetration of cervical mucus, including contraceptive films and suppositories
- New self-inserted barrier methods, including vaginal sheaths, diaphragms and cervical caps
- Spermicides with virucidal and/or bactericidal properties
- Non-steroidal systemic antifertility agents for men and women

Research proposals submitted to CONRAD are peer reviewed twice a year by its Technical Advisory Committee (TAC) which has expertise in reproductive biology, endocrinology, andrology, obstetrics/gynecology, immunology, pathology, pharmaceutical and chemical sciences, biomedical engineering and social sciences. Proposals may be submitted at any time during the year. Due to the limited funds available for unsolicited proposals, submission of an initial informal proposal is encouraged. In this way, the potential interest on the CONRAD Program can be determined before the effort required for submitting a formal proposal is expended. In addition to the TAC reviews, two Working Groups have been established with reviews new proposals in the areas of immunocontraception and AIDS.
Family Health International (FHI) is an international nonprofit biomedical research and technical assistance organization dedicated to improving reproductive health, contraceptive safety and health service delivery. FHI is committed to improving all aspects of reproductive health, from increasing contraceptive choices to slowing the spread of sexually transmitted diseases. Our strength lies not in our size—a staff of 290—but in the network of colleagues—260 clinical research centers in 55 countries—around the world with whom we are privileged to work.

There is an urgent need for new methods of contraception in the Third World as well as the U.S. Since 1971, FHI has worked to broaden the range of safe, effective and affordable contraceptive methods and make them available to families around the world. Currently, FHI is one of the few organizations worldwide working to develop new contraceptive products.

During much of its history, FHI has used clinical trials methodology to provide local data on the safety and efficacy of existing contraceptive methods in countries or programs where they have not been used previously, or to answer questions about the appropriateness of a specific method for a particular population or delivery system. While continuing to conduct these traditional trials, FHI has focused increasingly on activities to develop and secure approval by the U.S. Food and Drug Administration (FDA) of new contraceptives, with the long-term goal of increasing and improving the contraceptive choices for developing country programs.

FHI has designed, implemented, and managed over 500 multi- and single center studies to evaluate the safety and efficacy of contraceptive methods. Multiple, large, non-contraceptive clinical studies were also conducted by a former subsidiary of FHI. Many trials are conducted to obtain marketing approval of new products by regulatory agencies, including the FDA. FHI has sponsored 11 investigational new drug (IND) applications or investigational device exemptions (IDE) over the past decade, and currently sponsors five active IND/IDEs. A premarket approval (PMA) application was recently prepared and submitted for a European company; FHI will serve as their U.S. representative throughout the PMA review process.

Currently, FHI's activities in this area fall into two main categories:


The primary objective of this component is to carry out the necessary research and development activities to secure approval for new contraceptive products according to the standards set by the FDA.

2. Clinical trials to provide information to family planning programs on available contraceptives.
The objective of this component is to design and conduct clinical trials to provide local data on safety, efficacy, and acceptability of existing and new contraceptives to legitimize their use in developing country service programs.

Activities in the contraceptive development/clinical trials component are designed to provide sufficient data to the FDA and regulatory agencies in other countries to secure marketing approval of new products. Included are Phases I, II, and III and the IND/NDA or the IDE/PMA application processes leading to regulatory approval. Products currently falling in this category include:

- norethindrone (NET) injectable microspheres
- biodegradable NET pellets
- non-latex male condoms
- Filshie Clip for female sterilization
- iodine sclerosing formulation for non-surgical female sterilization
- female plastic condom, and
- the Lea's Shield

During the past fiscal year, FHI filed three applications for marketing approval with the FDA: a 510(k) application for a thermoplastic male condom, the Filshie Clip for female sterilization, and support for Wisconsin Pharmacal's application for the female plastic condom, Reality. FHI continues to respond to FDA requests for additional data that are part of the device approval process for these three products. Priorities for contraceptive development and clinical trials research in the current year continue to emphasize the development of long-acting steroidal contraceptive systems, especially the NET products, and of iodine as a possible method of transcervical female sterilization, and continued improvements in the design of the plastic male condom leading to another 510(k) submission. Development efforts in barrier contraception with the Lea's Shield and male sterilization continue in close cooperation with CONRAD and the Association of Voluntary Surgical Contraception (AVSC), respectively. Also during this year, we expect to work closely with the FDA while they review our PMA for the Filshie Clip, which was submitted during FY'92.
Mellon Foundation
The Mellon Foundation's Population Program

The Mellon Foundation's population program makes grants totalling roughly $8-$10 million a year in the following program areas: reproductive biology, applied contraceptive development, population dynamics of developing countries, family-planning service delivery in developing countries and in New York City, and population policy. General emphases which cut across several program areas are the provision of flexible institutional—rather than project—support, and career-development support for junior investigators. Grants are usually made for a three-year period and are usually made to U.S. institutions.

1. Reproductive Biology

Eight centers receive support in this program:

- Baylor College of Medicine
- University of California, Davis
- University of Connecticut
- Eastern Virginia Medical School
- University of North Carolina, Chapel Hill
- University of Virginia
- University of Washington
- The Population Council

Grants are generally at the level of $600,000 for use over three years. The major purpose of the grants is to provide support for junior investigators (either MDs or PhDs) whose research interests are relevant to the development of new contraceptives. To receive support, investigators must be at the level of postdoctoral fellow or beyond, but may not be tenured faculty members.

Two new elements of the program are (i) seed money for research projects relevant to contraceptive development; and (ii) funds for twinning arrangements with centers of reproductive biology in developing countries.

2. Contraceptive Development

Three institutions receive support in this program:

- The Population Council's International Committee for Contraception Research (ICCR)
- Family Health International
- CONRAD
The grant to the ICCR provides flexible support for contraceptive-development research.

The grant to FHI covers contraceptive-development research, a postdoctoral fellowship for developing-country investigators in contraceptive development, and support for the annual meeting of Mellon-supported centers in reproductive biology and contraceptive development.

The grant to CONRAD provides funds for testing the feasibility of concepts which are at too early a stage to receive USAID funds, and for collaboration with scientists in countries in which USAID funds may not be used—China, for example.

3. Population Dynamics of Developing Countries

Eleven demographic centers receive support in this program:

Brown University
University of California, Berkeley
University of Chicago
Johns Hopkins University
University of Michigan
University of North Carolina, Chapel Hill
University of Pennsylvania
Penn State University
Princeton University
University of Texas, Austin
University of Washington

The major program emphases are: links between demography and anthropology and area-studies programs; support for U.S. investigators to carry out research in developing countries at all stages of their careers, including dissertation research; and support for collaboration with investigators and institutions in developing countries.

The Foundation also runs a program of competitive visiting fellowships to enable demographers to "visit" selected anthropology departments and to enable anthropologists to visit selected demographic centers. The goal of the program is to promote research and training which integrate anthropological and traditional demographic approaches.

Grants for demographic research are also made to the Committee on Population of the National Academy of Sciences; and to the Population Council. And the Population Council receives additional support for its social-science fellowship program.
4. **Service Delivery**

The Foundation provides support to Planned Parenthood of New York City and Columbia University for the provision of family-planning services in New York City.

Grants have also been made to the following agencies for technical assistance to developing countries in the area of family-planning service delivery (the current grants for this purpose emphasize work in Vietnam and post-abortion family planning):

- Association for Voluntary Surgical Contraception
- Center for Population and Family Health, Columbia University
- Pathfinder Fund
- IPAS

Support is also provided to PIACT/PATH for its work in establishing local production of contraceptives in developing countries.

5. **Population Policy**

The following organizations receive support from the Foundation for their work in the area of population policy:

- International Women's Health Coalition
- Population Association of America
- Population Action International
- Population Reference Bureau
- Population Resource Center

6. **Miscellaneous**

The Foundation makes some grants which do not form part of the larger programs described above. The following are relevant to contraceptive research:

- Core support to the Alan Guttmacher Institute;
- Support to the World Health Organization for the development of abortion guidelines;
- Support to the University of Chicago for the survey of adult sexual behavior.

CM 9/24/93
National Institute of Child Health and Human Development (NICHD)

- Center for Population Research
- Center for Developmental Biology
- Reproductive Sciences Center
- Disease/Social Factors Center
- Center for Endocrine Evaluation & Safety
- 3 Research Centers
- 3 infertility Clinics
CONTRACEPTIVE RESEARCH - NEW FUNDING INITIATIVES

Introduction

Family planning is an integral part of social well being. Currently available contraceptives including oral contraceptive pills (OCs), hormone implants and IUDs, are over 95% effective; yet over 50% or 3.5 million pregnancies are "unintended" and of these, 1.6 million end in elective abortion. Because many factors influence contraceptive use including: ethnic, cultural, and religious values, availability of services, changing needs with age, occurrence of adverse reactions, and concerns about safety and expense, different methods are necessary. In fact, as each new contraceptive becomes available, overall contraceptive use increases. The Contraceptive Development Branch (CDB) of NICHD acknowledges these diverse needs and the importance of new contraceptive options and has as its mission funding the development of new products.

However, this year the Contraceptive Development Branch is spending about 12.5 cents for each man and woman of reproductive age on contraceptive research initiatives. The budget represents 0.07% of the total NIH budget of about $9.3 billion and is less than 2% of the $420 million allocated to NICHD extramural research. To meet the urgent, diverse needs for new contraceptive agents, contraceptive development research must be expanded and given greater priority. The breadth and scope of the Branch's proposed new initiatives as well as its programs to assess the clinical potential of contraceptive drugs in ongoing research and development are described below.

Female-controlled methods to reduce sexual transmission of HIV in women $2 Million/year/5 years

Barrier contraceptives prevent both conception and STD infection. Barrier methods can be mechanical such as condoms or chemical such as spermicides. The available products are not sufficiently effective in protecting women from the epidemic of STDs including HIV nor are they optimal for prevention of pregnancy. Spermicidal agents such as nonoxynol-9 may be virucidal in the laboratory but their use has not been shown conclusively to prevent the spread of STDs. Consequently there is an urgent need to develop new methods that are controlled by women and are independent of men's action. New chemical entities that rapidly inactivate STD pathogens should be explored as well as improving formulations for their effective delivery. Clinical evaluation of new products must be quickly implemented.
Postcoital contraception $1 million/year/3 years

No postcoital contraceptive methods are currently approved by the FDA for use in the US. Studies abroad have shown high efficacy and safety of antiprogestins (chemical substances related to RU486) as postcoital agents. Clinical testing of antiprogestins must be conducted in the US to establish their effectiveness. The availability of such products in clinical practice must be assured in order to prevent unwanted pregnancies.

Clinical testing facility for barrier contraceptives $750,000/year/5 years

To expedite the development of new methods of barrier contraception, a clinical research facility that could design and conduct Phase I and II studies should be sponsored. This facility could rapidly assess the clinical potential of new barrier devices and spermicidal/virucidal formulations. It could also function as an integral part of the contraceptive evaluation and STD prevention programs for NIH.

Clinical testing facility for contraceptive drugs $750,000/year/5 years

The rapid evaluation of new drugs as potential contraceptive agents requires the ability to quickly initiate clinical studies in suitable volunteers. For this purpose, a clinical testing facility should be established. Such a facility would complement the Branch's preclinical service facilities for chemical synthesis and biological evaluation.

Clinical trials of a new long-acting progestin $400,000/year/5 years

The CDB has been developing a new long-acting progestin (levonorgestrel butanoate) which may have fewer side effects than Depo-Provera, a progestin recently approved by the FDA as a contraceptive agent. The preclinical testing of levonorgestrel butanoate should continue and, if the results are favorable, clinical studies of this new progestin should be initiated.

Preclinical and clinical trials of a new long-acting androgen $400,000/year/5 years

Various male contraceptive approaches currently being explored require the administration of supplemental androgen to maintain male libido. For this purpose, the CDB has been developing a long-acting injectable androgen (testosterone buciclate) which is now undergoing toxicology studies in rats and monkeys. Preclinical carcinogenicity and genotoxicity studies should be initiated, and, if the results are favorable, clinical evaluation of this new androgen must be conducted.
Evaluation of new estrogens $500,000/year/3 years

The CDB has been developing new orally active estrogens which could have fewer side effects than those currently being used in oral contraceptives. Demonstration of the superiority of these new estrogens requires toxicological evaluation and subsequently clinical evaluation. Development of safer estrogens would be an important improvement in oral contraceptives as well as for hormone replacement therapy in menopausal women.

Basic/clinical studies on the causes of breakthrough bleeding $1 million/year/5 years

Breakthrough bleeding is a common complaint among oral contraceptive users and the most frequent reason for discontinuation of the use of long-acting progestin-only injectables. Basic research is needed on factors which contribute to dysfunctional bleeding in order to offer possible solutions to women who encounter these problems with the Ocs, injectables and implants.

New sterilization methods for men $1 million/year/3 years

The availability of a safe, reversible, effective nonsurgical method of vas occlusion may increase utilization of vasectomy for family planning purposes. Blocking of the vas has been accomplished with polymers that solidify in the vas and block the passage of spermatozoa during ejaculation. Preclinical safety studies and then clinical studies should be undertaken.

LHRH analogs as male contraceptive agents: phase I and II clinical trials $750,000/year/5 years

LHRH is the brain hormone that regulates reproductive processes in both the male and the female. Male volunteers injected with synthetic analogs of this hormone become infertile. We must expand the clinical testing of this group of drugs on a larger population of volunteers in order to better assess the potential of these drugs for male contraception.

Developing new contraceptive leads $2 million/year/5 years

Development of new drugs for fertility regulation has been hampered by a lack of new leads. Pharmaceutical industry and university laboratories have synthesized many new chemicals that have never been tested for antifertility activity. A plan should be developed to evaluate new chemicals similar to the program of the National Cancer Institute's in vitro screening for new anticancer agents.
Dysfunctional uterine bleeding is one of the primary reasons for discontinuation of long-acting progestin-only contraceptives. Research into the cause of bleeding and methods for its elimination or control is virtually nonexistent. The CDB should evaluate the clinical potential of estrogens administered both continuously and at periodic intervals to reduce the uterine bleeding associated with progestin-only contraceptive methods.

Synthesis of new antiprogestins $1.6$ million/year/5 years

Antiprogestins of the RU486 class of steroids have broad therapeutic and contraceptive potential. In order to expand women's options in this potentially exciting contraceptive and reproductive health area, a program of synthesis and evaluation of new antiprogestins must be initiated. This could lead to the development of safer and more potent antiprogestins.

Contraceptive Development Branch
Center for Population Research
National Institute for Child Health and Human Development
Rockefeller Foundation
MOBILIZATION OF RESOURCES TO LAUNCH A SECOND
CONTRACEPTIVE TECHNOLOGY REVOLUTION

A Concept Paper

A new strategy of the Population Sciences division, focused on mobilization of resources to satisfy unmet demand for contraception and complete the demographic transition, was presented to the Board last December. This paper outlines the rationale and basic concepts underlying one of the components of that strategy, namely, Mobilization of Resources to Launch a Second Contraceptive Technology Revolution. A grouped appropriation, initiating support for some of the activities described here, is presented following the paper.

The central aim of this component is to lift the whole field of contraceptive research and development rather than run a Foundation-sponsored research program as a member of that field.

The Contraceptive Technology Revolution

The past few decades have witnessed major developments in contraceptive technology that can only be described as a revolution. Before these developments, contraceptive choice was limited to either coitally-related methods which lacked in effectiveness or permanent methods. Contraceptive choices have been broadened. Contraception was moved outside the bedroom by the development of methods such as the pill. People no longer had to make the choice between either a method to be used at every coitus or a permanent method; long-acting reversible methods now offer protection ranging from one month to several years. Highly effective but reversible methods became an available option. Technical developments have allowed sterilization to be performed as an out-patient procedure and without the need for general anaesthesia.

The fruits of the contraceptive technology revolution have been enjoyed by hundreds of millions of people all around the world: people living in the most varied circumstances, in the skyscrapers of Manhattan, in peri-urban slums in Latin America, in rural communities of the Indian subcontinent; people in all socioeconomic strata; people with different cultures, religious beliefs, and value systems; and people postponing a first pregnancy, spacing children, or putting a limit on childbearing.

Contraceptive users in developing countries increased from an estimated 31 million in 1960-65 to 381 million in 1985-90. Whatever the factors underlying their adoption of this reproductive behavior might be, the modern contraceptive technology has given them the means to implement their decisions. The new and improved technologies account for the great preponderance of contraceptive use in both developed and developing countries. In fact, the prevalence of use of the more modern clinic and supply methods, as a whole, does not differ much between developed and developing regions, with an estimated average of 46 and 41 percent, respectively.
The higher contraceptive prevalence in more developed regions is largely accounted for by the use of traditional methods (24 percent, compared with 6 percent in developing regions). These methods were in use for a long time in developed regions before the new technologies were introduced; they require a sustained level of motivation and a back-up of safe abortion services.

While in Western societies the adoption of a small family norm was largely achieved before the new technologies were available, it should be realized that it took fertility a longer time to decline in the West than has been the case in many developing countries. The decline of the total fertility rate from 6.5 to 3.5, which took 58 years in the United States, was achieved in Indonesia in 27 years, in Colombia in 15 years, in Thailand in 8 years, and in China in only 7 years. It was only in postwar Japan that a relatively rapid decline in fertility was achieved without resort to new technologies. This feat, however, was achieved with heavy reliance on induced abortion.

The development of new contraceptive technologies has provided women for the first time with reliable methods, independent of male cooperation, to regulate and control their fertility and better control their lives. For the first time, a woman could pursue education and employment and enjoy both a productive and a reproductive career. They could plan their births to take place at optimal times for childbearing, ensuring more safety for the mother and better chances for child survival and healthy growth and development.

Stalling of the Contraceptive Technology Revolution

The contraceptive technology revolution lost its momentum after the first wave of significant achievements. The high expectations of continued progress were not met. In 1970, Dr. Alan Guttmacher, in a statement to a United States Senate hearing, said, "We are still in the horse and buggy days of effective contraception. I am optimistic in feeling that in five years we shall have methods that are infinitely superior and safer [than the pill and intrauterine device]." An optimistic U.S. Congress report predicted that ten new methods of contraception would become available before 1990 and 20 methods before the year 2000. Apart from improvements in some existing methods, however, only NORPLANT has been added during this period. In fact, it is highly unlikely under the present circumstances that contraceptive choice will be dramatically changed during the remainder of this century.

Current contraceptive options are usable, but the range of choice is inadequate to meet the present and the rapidly expanding needs. One verdict is given by the estimated 100 million couples and individuals whose contraceptive needs are not currently met and who would start practicing contraception if their desire for spacing and limiting births could be fully satisfied by services and appropriate methods. Another verdict is given by the estimated 36-53 million women who resort to induced abortion each year - more than a quarter of them risking their life and health in the process. On the qualitative side, the inadequacy of the present state of the art is being increasingly articulated by women. Not only is an undue burden currently put on the woman, but important needs are left unmet.

Contraceptives should not be looked upon as a temporary measure to ease the world population problem. Contraception will be a permanent feature of the way of life of all succeeding generations on this planet. Our reproductive function is being voluntarily adapted to dramatic
new realities. What we are witnessing is a major evolutionary jump that is science-mediated, rather than brutally imposed by Nature.

**Launching a Second Contraceptive Technology Revolution**

The first contraceptive technology revolution was made possible by the convergence of three factors. First, there was the sense of need and the clarity of a mission, dictated by demographic concerns. Second, science was ripe with new advances in reproductive biology and particularly endocrinology. Third, industry, seeing the rapidly expanding markets and the opportunities provided by science, positioned itself for an active role.

The stalling of the revolution can be traced to the same three factors. The mission lost its clarity, science has been drying up, and industry has retrenched. The mission has been confused by the population debate. As to science, the field of reproductive endocrinology, which has provided exciting leads in the past, holds less promise for the future. New developments in contraception are mostly modifications or variants of existing methods rather than new contraceptive approaches. Expanding the output of any science requires a regularly replenished and expanding scientific pool of trained scientists. There is a genuine concern about the aging of scientists active in contraceptive development. The new advances in cell and molecular biology and biotechnology have not yet been exploited for contraceptive research and development. Industry, for a number of reasons, has retrenched. Of the 12 major pharmaceutical companies that were active in this field in the 1960s, the number remaining by the end of the 1980s was just four - Ortho Pharmaceutical Corp. (a subsidiary of Johnson and Johnson), Organon International, Schering AG, and Roussel-Uclaf (a subsidiary of Hoechst Pharmaceuticals).*

Were it not for the public-sector programs committed to contraceptive research and development, and for donor support, the field might have fallen altogether into scientific oblivion.

If the field is to be revived and the second contraceptive technology revolution launched, the field must again be driven by a mission, science must be reinvigorated, and an effort must be made to bring industry back.

**A Note on Current Financial Resources for Contraceptive Research and Development**

Financial resources for the field of contraceptive research and development are provided by private industry, governments, and foundations. Government support is provided directly or indirectly through UN agencies. Apart from the traditional role of universities and other national research institutions, the 1970s witnessed the emergence of international public-sector research programs that focus on contraceptive research and development. These include the WHO Special Programme of Research, Development, and Research Training in Human Reproduction, the Population Council's International Committee for Contraception Research, and the

* No longer active in contraceptive research are: Syntex Laboratories; G. D. Searle & Co.; Parke-Davis & Co.; Merck, Sharp & Dohme Co.; Upjohn Company; Mead Johnson; Wyeth-Ayerst Laboratories; and Eli Lilly and Company.
largely USAID-supported agencies: Family Health International and the Contraceptive Research and Development Program (CONRAD). Organizational structure and resource flows are shown in Figure 1.

It is difficult to get good estimates of the global budget for contraceptive research and development. The exact amount of money spent by industry is generally considered proprietary information. Within the research budget of public-sector and national research programs, some arbitrary line has to be drawn between what is contraceptive research and development and the reproductive biology research pool preceding it (which provides leads for other fields as well), and also between contraceptive research and development and the research that follows it in terms of service-related, long-term safety, and social research.

A recent rough assessment puts the worldwide funding for contraceptive research and development at about $57 million (Fig. 2), with the U.S. government providing 43 percent, the non-profit sector 18 percent and private industry 39 percent.

It may be noted that the global expenditure on contraceptive research and development, from all sources, is less than 3 percent of annual global contraceptive sales, estimated to be between $2.6 billion and $2.9 billion, and is well below the pharmaceutical industry norm of investing 16 to 19 percent of sales revenues in research and development. The funding of public-sector programs involved in contraceptive research and development represents about 3 to 4 percent of the international assistance for population and family planning, estimated in 1990 to be $802 million. The funding for the contraceptive Research and Development branch of NIH represents only 0.07 percent of the NIH budget. Another figure to note in these budgetary considerations is that about $230 million is generally estimated to be required to bring a new chemical entity from the basic science research pool, through applied basic research, phased animal and clinical testing, up to final development, registration, manufacturing, and finally marketing (Fig. 3).

Three foundations have played an important role in the field of contraceptive research and development. The Ford Foundation made a major investment in launching the first contraceptive technology revolution. Currently, the Rockefeller and Andrew W. Mellon foundations continue to support the field.

A Strategy for the Rockefeller Foundation

Developing technology for fertility regulation is only one part of the broad response of the Foundation to the population concerns and to challenges in reproductive health. It is, however, an area where a relatively small investment can make a difference if it succeeds in mobilizing resources for the field.

In preparation for this strategy, the officers took note of a major 1990 study undertaken by the National Research Council and the Institute of Medicine entitled "Developing New Contraceptives: Obstacles and Opportunities." They also took note of reports of recent consultations between women's groups from developed and developing countries and the contraceptive research and development community, organized by the Population Council and by the World Health Organization. A document on the "Potential for increasing commercial interest in contraceptive research and development" was prepared for the Foundation by McKinsey and Company, Inc., in
1990. In addition, the Foundation, in collaboration with WHO, provided partial support to the Program for Applied Technology in Health (PATH) to conduct a study on enhancing the private sector's role in contraceptive research and development. During the course of the study, PATH conducted 14 in-depth interviews with key representatives of industry to ascertain what factors influence industry's decision to enter into or remain active in contraceptive research and development. Over the past few months, the officers have had extensive informal consultations with the public-sector contraceptive research and development programs, including participation in an interagency consultation in Mexico last March.

Rather than simply adding limited resources to the poorly funded field of contraceptive research and development, it is proposed that the Foundation play a leadership role in addressing the constraints of the field, and that it invest in activities that draw in more resources, and particularly the potentially vast resources of private industry, to launch a second contraceptive technology revolution.

The Foundation is not a newcomer to the field. It has a long track record in supporting the development of contraceptive technology. Flexibility, maintaining a long-term focus, and willingness, if needed, to take risks are assets which the Foundation can bring to bear.

For the successful mobilization of resources, the field needs a clear and appealing mission, a strong science base, and private industry ready to seize the opportunity.

A three-track strategy is proposed: A woman-centered agenda for contraceptive research and development to provide the field with a mission and a focus; a Contraception-21 initiative to reinvigorate the science to provide the leads for new contraceptive approaches in the 21st century; and a new program thrust to promote private/public-sector collaboration.

1. A Woman-Centered Agenda for Contraceptive Research and Development

For a strategy to mobilize resources, the field of contraceptive research and development needs a clear mission and a focus. The first contraceptive technology revolution was goal-driven, with emphasis on methods that could have a demographic impact. When the revolution stalled, the poorly-funded field became opportunity-driven. For a second contraceptive technology revolution, the field must again be goal-driven. It is proposed that the field should focus on contraceptive approaches where the needs of women are still unmet by the existing methods. The strategy of the Population Sciences division emphasizes that responding to unmet demand will go a long way toward achieving the desired demographic objectives. The mission should be clear: What women themselves want is what the world needs for survival.

Meetings with women's health advocacy groups over the last two to three years have identified the three subjects listed below as high priority for women. The officers are designing advisory structures to include frequent meetings with women's groups, particularly groups in developing countries, so that the "women's perspective" helps set directions for Foundation-supported research.

A recent survey of the field has come up with a list of 94 product leads that are currently being pursued. In the face of financial constraints, if for no other reason, a strong case can be
made for the field to focus its efforts, and to focus them on areas where needs are currently not met by existing approaches. Promising new approaches that respond to unmet consumer needs would also be more likely to attract the interest of industry.

Apart from the general need to expand contraceptive choice by more user-controlled and safer methods, three specific needs unmet by currently available methods are being increasingly articulated by women's groups in developed and developing countries: expanding male contraceptive choice and responsibility, a menses-inducer, and protection against sexually-transmitted infections.

Expanding male contraceptive choice and responsibility: There is a need for more participation by men in fertility regulation. Women, for biological reasons, carry all the burden and risks of pregnancy and childbirth. But, biology does not dictate that women should also carry most of the burden of fertility regulation, though as a matter of fact the burden of contraception is unequally shared - with three times more women than men using contraception. This burden of inequality is aggravated by the side effects associated with many female contraceptive technologies. The male has not been the focus of the contraceptive technology revolution. A sustained research effort is needed if men are to have broader contraceptive choices and thus share equally in the responsibility for fertility regulation.

A once-a-month women's pill: Carl Djerassi, a father of the oral contraceptive pill, has recently remarked that if he were restricted to choosing a single new contraceptive to develop, it would be a once-a-month-pill effective as a menses-inducer. Instead of currently used oral contraceptives, which are taken daily for most of the month, a menses-inducer would be ingested by a woman only during those months when she had unprotected coitus. Instead of waiting to see whether she had missed her period, a woman would take a single pill (containing a short-lived and rapidly metabolized drug) to induce menstrual flow at the expected time. Although not acceptable or suitable for every woman, for many such a regimen would be an enormous improvement. At most, a woman would be taking 12 pills annually, rather than the present 250 or more. With such a novel pill, women would not know whether they carried a fertilized ovum. A most important feature of such a method is that the decision to contracept is made post-coitally.

For some women, the menses-inducer would be an attractive back-up to barrier contraceptive methods, which are less effective in preventing pregnancy, but afford protection against sexually-transmitted infections and are not associated with undesirable side-effects. For adolescents, the availability of a menses-inducer could be especially useful.

Unsafe abortion practices have reached alarming levels. The World Health Organization estimates that about 500 women die each day because of complications of unsafe abortion. A menses-inducer that is completely user-controlled could provide a technological response to the abortion controversy. It could transfer the issue from the public domain to the privacy of the individual's moral code. To have a real impact, the menses-inducer must be safe enough to be used outside the health care system.

Protection against sexually-transmitted infections: The need for a method which a woman can use to protect herself against sexually-transmitted infections, including HIV, has become urgent. It is not sufficiently realized that the risk of transmission is generally greater from man to woman than from woman to man, and that the consequences of the infections are often much
Fig. 1
CONTRACEPTIVE RESEARCH AND DEVELOPMENT
Structural Organization/Resource Flows (→)
Fig. 2
Estimated Current Global Funding for Contraceptive Research and Development (PATH 1993)

- NIH ($10 million) $25 million (43%)
- USAID ($15 million)
- US Government
- Non-Profit Agencies $9 million (18%)
- For-Profit Sector $23 million (39%)
Fig. 3
Steps in Contraceptive Research and Development

Basic Research Pool

Pre-Clinical Toxicology

Phase I Clinical Testing

6-12 months

Phase II Clinical Testing

6-24 months

Phase III Clinical Testing

12-48 months

Continued Toxicology

Registration
Manufacture
Marketing

Post-marketing
Surveillance

--- leads dropped
more serious for women. In addition, there is the risk of transmitting infection to the fetus from an infected mother. In spite of this, the available female barrier methods either lack in effectiveness or acceptability. There is no method which can protect women from infection while still allowing them to become pregnant. It is quite common, at least in developing countries, for women to get the infection from their husbands who have multiple sexual partners. The need is for an effective method which women can use and control without the necessity for partner cooperation. It is possible that if such a method becomes available, women will do better than men in compliance, providing more hope for the control of the pandemic of sexually-transmitted infections.

The Foundation will utilize its resources to try to shape the agenda of contraceptive research and development to focus on contraceptive approaches which women need and which are not currently met by existing methods.

The Foundation will support research institutions active in pioneering these new approaches. It will also provide support to international public-sector programs (such as the WHO special program on human reproduction, the Population Council, the Contraceptive Research and Development Program, Family Health International) if they allocate increasing resources to these areas.

South-to-South programs will continue to be supported as an instrument for the mobilization of scientific developing-country resources for the development and clinical testing of contraceptives which women need.

An additional line will be support to voluntary nongovernmental organizations, including women's groups, to articulate the needs and perceptions of consumers about new contraceptives that can be introduced in their countries.

2. Contraception-21

The agenda to develop the contraceptives of the 21st century must be set now. It should be based on the application of new advances in cell and molecular biology and biotechnology to fertility regulation. For this, there is a need to make a significant and sustained investment in human capital, to bring new talent into the field, and to realize the potential of existing human resources. Furthermore, the agenda must be guided by the priorities identified immediately above, reflecting the women's perspective. Contraceptive research supported by the Foundation will be guided by these important social and cultural objectives and concerns, even as it is shaped by developments in molecular biology.

Reinvigorating the science will be a basic step in the mobilization of resources for contraceptive research and development. It is the prospect of major breakthroughs that will capture public imagination, attract scientific talent, and stimulate industry's interest. While advances in cell and molecular biology and in biotechnology have opened new frontiers for medical and biological sciences, contraceptive research and development has yet to benefit from these advances.

There are four main events in the reproductive process that can be targeted in contraceptive approaches: ovulation, production and maturation of sperm, meeting of the ovum and sperm (fertilization), and implantation of the fertilized ovum. So far, only the process of ovulation has
been successfully targeted by modern science. New frontiers now opening up in science can provide novel ways to target the other events in the process, with the possibility of significant breakthroughs in contraceptive technology responsive to the currently unmet needs of women.

Few scientists working in the fields of cell and molecular biology and biotechnology are focusing on leads for contraceptive research and development, and few scientists in the field of contraceptive research and development are fully aware of the potential of these new advances in biology. Compounding factors are the complexity of equipment and methodology required by this research, and the rapid pace of scientific developments.

There is a need for a major and sustained investment in human capital to build a critical mass of scientists active in the field. It was the major investment of the Ford Foundation which stimulated the field of reproductive endocrinology to give the leads for the first contraceptive technology revolution. Now, the field is ripe for another major initiative. The Rockefeller Foundation is well-positioned to play the leadership role in this initiative, with its emphasis on investing in human capital, and its long-standing support for new technology in fertility regulation.

The Foundation will reorient its institutional support to a human capital strategy for the application of new advances in cell and molecular biology and biotechnology to the field of fertility regulation. The Foundation will support a network of research and training centers in developed and developing countries to prepare a new generation of scientists to enter this field.

The Foundation will encourage the few scientists who have already been trained in this field in developing countries to stay in the field and to increase their productivity through a program of South-North linkages, modeled on the Biotechnology Career Fellowship program.

The Foundation will encourage communication between basic scientists, scientists in the field of contraceptive research and development, and private industry, by supporting a series of biennial symposia on new frontiers in contraceptive technology.

3. Private/Public-Sector Collaboration

Any major infusion of resources in the contraceptive research and development field will have to come from industry.

The potential of industry, in terms of finance and expertise, is great compared with other resources. The pharmaceutical industry in the developed world invests a substantial amount of money (about 16 to 19 percent of revenues) in research and development of new products. With U.S. and European companies reporting total revenues of over $90 billion per year and a projected annual growth of 9 to 10 percent over the next five years, there are significant resources available for research activities.

In the literature, the two most frequently cited reasons for industry's decreased involvement in contraceptive R&D during the past few decades are product liability, coupled with a lack of insurance availability to cover contraceptive development and introduction (particularly in the U.S.), and stringent regulatory requirements for product approval, resulting in a long, expensive
registration process and a concomitant decrease in patent protection. Another reason commonly put forth is a hostile political climate, again particularly in the U.S.

The PATH study mentioned above has shown that while smaller companies do view these factors as barriers, large companies actively involved in all phases of contraceptive work do not view them as significant constraints but rather as the "cost" of doing business.

The larger firms will only devote significantly more resources to contraceptive research and development when they can be assured that their efforts will result in a reasonable return on investment compared with other markets. The predominant factor influencing the decision to develop a new product is the size of the projected market.

There is a need to re-examine the market, if industry is to be persuaded to collaborate actively with the public sector and to invest in the development of new products. The contraceptive market in developed and developing countries is such that the majority of the consumers are in developing countries while most of the revenues come from developed countries (Fig. 4). Industry currently perceives the market in developed countries as "mature" and that in developing countries as poor. The perception of the market in developed countries - that it offers little profit potential for new products - may not be well-grounded. High rates of unwanted pregnancy and induced abortion, as well as a heavy reliance on sterilization among the younger population, suggest a "latent demand" for new contraceptives. This is confirmed by the unexpected success of NORPLANT in the U.S. The assumption that the large, rapidly growing market in developing countries is unprofitable is also questionable. Contraceptive commodities in developing countries are now mostly purchased by governments and donors and provided to the public free or at subsidized prices. The private-sector share currently stands at about 17 percent. However, public-sector provision of contraception in many developing countries is giving way to a more significant role for the private sector, and as market economies in developing countries evolve, there will be a progressive increase in the share of the private commercial sector, brightening future profit prospects.

Another major factor lessening private industry's involvement in contraceptive research and development is a perceived dearth of dramatically new product ideas. Unless there is a prospect for new products that are clearly superior in some aspects over existing products, it will be difficult to re-engage industry on any large scale. There is a need to canvass the field of science and to highlight opportunities for private/public-sector collaboration.

Getting the larger pharmaceutical companies engaged will require evidence that the market justifies significant new investment in contraceptive R&D. This issue is addressed in the proposed action described below. At the same time, there is a long list of small supply/device and biotech companies which are interested in and do research and development on contraceptives. They see contraceptives as an attractive niche opportunity for growth. Small and medium-sized companies, however, have constraints in the contraceptive research and development process. In addition to these small companies in developed countries, the pharmaceutical industry in developing countries is also showing increased interest in local production and marketing of contraceptives, and where a capacity exists, in research and development as well. There are opportunities for promoting new partnerships between small and medium-sized industry and public-sector programs of contraceptive research and development which could ease these constraints. These programs can take up promising leads and develop them to the stage where they become attrac-
tive to industry as a more secure investment. They can also collaborate in the clinical testing of products developed by industry.

The Foundation will support efforts to promote private/public-sector collaboration. Initially, two studies will be commissioned to whet the appetite of industry: a study of market prospects for new contraceptives in developed and developing countries, and a scientific study of the prospects for new products based on advances in cell and molecular biology and biotechnology. Industry experts will be involved in these studies. A third study will explore ways in which the Foundation can promote new partnerships between the public sector and small and medium-sized industry, including the feasibility of a program-related investment.

Implementation of this new strategic direction will coincide with the termination phase-out of the following current appropriations: vaccine initiatives, gossypol studies, regional training centers, training for Africans and Latin Americans at the University of Pennsylvania, and contraceptive introduction. Support to the South-to-South program, after a period of transition, will be included in the package.

Implementation of activities under this component will coincide with phase-out of the following current appropriations: vaccine initiatives, gossypol studies, regional training centers, training for Africans and Latin Americans at the University of Pennsylvania, and contraceptive introduction. Support to the South-to-South program, after a period of transition, will be included in the package.
Fig. 4
CONTRACEPTIVE MARKET

Consumers

30%

Revenues

84%

- Developed Countries
- Developing Countries
South to South
Rapid population growth presents a serious problem for developing countries as they try to implement policies to improve the lives of their people. Many governments have, therefore, attempted to introduce family planning programs in order to provide couples with effective means of fertility regulation on a voluntary basis.

Improved contraceptive technology would make family planning efforts more successful, particularly in countries where methods need to be better suited to prevailing social and cultural patterns. Yet, product development in contraception, as in other fields of therapeutics, is principally the domain and responsibility of Western-based pharmaceutical companies. Paradoxically, therefore, the world's major contraceptive and reproductive health needs are in the countries of the third world, while product development decisions are made by corporate leaders in industrialized countries interested, primarily, in hard currency markets. Consequently, developing country scientists who wish to do research in this field often find themselves dependent on foreign companies for research protocols and funding, and have little opportunity to work on their own ideas and innovations.
addition to providing administrative and logistical support for the overall program.

2. Objectives

a. General Objective

The general objective is mutual assistance and collaboration in reproductive health research and, building in the process, a network of experts who can advise the policy makers of developing countries about how reproductive health issues can be addressed through the application of research results to national policy decisions and health program design. The ultimate objective is the overall improvement of the reproductive health status in developing countries.

b. Specific Objectives

* To build a network of research scientists in developing countries who will collaborate in applied, biochemical, clinical and social science research.

* To initiate, design, implement and analyze research projects in population and reproductive health.
* To develop new male and female contraceptive products that are acceptable worldwide with proven contraceptive effectiveness, safety and cost effectiveness.

* To develop specific contraceptives that are under the control of the woman and have the additional properties of being virucidal and bactericidal.

* To collaborate in product development with private industry.

* To ensure a critical mass of research activities in developing countries through training activities for young scientists in reproductive medicine. To ensure institutional strengthening by encouraging a STS exchange program with the optimism that this interactive process would ensure sustained growth in research capability among the developing countries.

* To collaborate with other agencies and Foundations interested in Reproductive Health Research.

* To influence national policies on population and human sexuality.
3. Current Research Interest

The research activities of the STS group are initiated and carried out by developing country scientists. They are based on scientific work initiated in the South and designed for use in the South. For example:

* Development of a one capsule contraceptive implant (UNIPLANT).

* Development of Vaginal Pill Contraception.

* Development of Gossypol as a medical alternative to surgical vasectomy.

* Development of new contraceptives suitable for breast feeding mothers: Progesterone depot injectable and Anti-LHRH vaccine.

* Development of an inexpensive vaccine for prostatic enlargement (benign or malignant).

* Effect of counselling at the time of abortion on contraceptive use and repeat abortion.

* Development of Vaginal Cream or Gel with contraceptive, bactericidal and virucidal properties.
4. Workshop and Training

As there is need to democratize knowledge the STS group periodically organize and participate in workshops conducted by governmental and non-governmental agencies. It is also envisaged that regional training programs, for young faculty staff, on current trends in reproductive health research will be a priority in future activities.

* Gossypol treatment of men who are positive for human immunodeficiency virus in semen.
1. Justification of Contraceptive Development Programs

In spite of a variety of contraceptive methods available in many countries, contraceptive failure leads annually to 25 million unintended pregnancies worldwide. In addition, more than 15 million women who don't use contraceptives have unplanned pregnancies. There are also approximately 55 million legal and illegal abortions per year. Additional methods of birth control are essential to improve the safety, efficacy and acceptability of contraceptive options. We must increase the number of contraceptive choices for men and women as well as facilitate the introduction of existing methods.

Unmet needs encompass:

a. methods that protect against sexually transmitted diseases including AIDS,

b. methods that are under the user's control,

c. methods reducing user failures, side effects, discontinuation rates and cost,

d. methods for special user groups, such as men, nursing women, teenagers and women over 40 years of age,

e. methods that can be used without altering a woman's menstrual cycle,

f. methods for medical abortions.
2. Description of The Population Council’s Contraceptive Research and Development Program

The Population Council program is *unique in several ways*:

a. Research and Development of Contraceptives: From clinical testing to introduction of new contraceptive methods

b. Past achievements: Several Copper T IUDs and the first contraceptive subdermal implant system (NORPLANT®) were developed and are registered today for distribution in 26 countries including the U.S. A new progestin releasing intrauterine device (IUD), the levonorgestrel IUD, was approved in Finland.

c. Scope: Long-acting methods for men and women, methods under the user’s control, barrier methods providing protection against sexually transmitted diseases and abortifacients are being developed.

d. Scale: Activities range from idea generation, basic research, testing of pre-clinical effects in animal models and animal toxicology, conducted in our Center for Biomedical Research located in New York City, early testing of feasibility and formulation to large scale clinical trials, transfer of technology to industrial manufacturers and licensing of commercial distributors and introduction of the approved product after it has been shown to be clinically safe.

d. *Highest ethical standards*: Only methods approved by local and usually also U.S. regulatory agencies are tested clinically and greatest emphasis is put on safety standards, counseling, written informed consent in the user’s native language and local as well as U.S. approval by human ethical committees and investigational review boards. All clinical studies are also conducted in industrial countries.
e. Cost of product: whenever possible, low *public sector price* is assured.

3. Description of Projects under Development and Their Special Advantages to the User

a. *Methods protecting against pregnancy and/or STDs* that are under the woman’s control.

i) Spermicides/Microbicides

Protection against STDs (including HIV infection) for both women desiring pregnancy and women wanting protection against pregnancy. Development of various formulations and preclinical safety testing are ongoing.

ii) Contraceptive Vaginal Rings

A method under the user’s control for nursing and ovulating women, that assures regular menstrual cycles.

*Progesterone ring:* Six month method for nursing women, providing the natural steroid progesterone.

*Nestorone™ progestin ring:* Six to 12-month ring for nursing women. Use of this ring can be continued after weaning.

*Combination ring:* Four to 12 month ring. Provides a progestin and an estrogen, similar to oral contraceptives, but needs attention only twice a month, thereby reducing user failure. Maintains very regular uterine bleeding patterns.
b. Long-acting contraceptive methods for women

i) Implant systems

_NORPLANT® II implant_: Expected to be as effective as NORPLANT® implants. Since it consists of only two implants instead of six, insertion and removal will be easier and the price may be lower.

_Nestorone™ implant_: This single implant, effective for two years, is intended for women who want to space their pregnancies. Nestorone™ steroid is not active when taken orally, thus it is safe for nursing women and their babies. It does not affect lipid levels.

ii) Intra Uterine Devices (IUDs)

_Levonorgestrel IUD_: Highly effective, reduces abdominal pain and uterine bleeding and will be useful for women who cannot tolerate other IUDs. Possible therapeutic use for women with anemia and other pathological conditions.

iii) Vaccines

__hCG vaccine for women__: Up to six months of effectiveness between injections. Prevents establishment of pregnancy, while maintaining regular menstrual cycles and ovulation. (Status: Phase 1 trial completed.)

c. Long-Acting Contraceptives for Men

i) Two-implant system for men

_Peptide implant and MENT implant_: One year method, suppressing sperm production and assuring normal libido through supply of a special health-promoting testosterone substitute. This method is expected to be highly acceptable to this under-provided user group.
ii) LHRH vaccine for men: Up to six months of effectiveness between injections. To be used in conjunction with the MENT implant, that enhances the antifertility effect and maintains libido (Status: Phase 1 trial in the U.S.).

d. Abortifacients:

Intended to correct contraceptive failures. Should not be promoted as a family planning method. Induced abortions number 150,000 every day and the maternal mortality rate from illegally induced abortions is estimated to be 500 every day. Medical versus surgical procedure must be evaluated. The Population Council has been mandated to conduct Phase 3 clinical trials of the antiprogestin RU 486 in the U.S. and other countries. RU 486 is highly effective if combined with a prostaglandin (95%) and is very safe.
World Health Organization
(WHO)
OBJECTIVES AND STRATEGIES

From 1800 to 1990 the world's population grew from 1 billion to 5 billion people, and between 1990 and 2020 it is projected to grow further by another 3 billion, to reach a total of 8 billion. Each day sees the birth of 220,000 new fellow citizens. But the growth in population has not been even in all parts of the world. In fact, it is estimated that in the years ahead, 90% of the increase will take place in developing countries (World population prospects, New York, United Nations, 1988). This imbalance in population growth contributes greatly to the economic inequality between the developed and developing regions of the world. To cite a recent United Nations Population Fund (UNFPA) report:

"...it is safe to say that population growth and change but there are large regional differences in contraceptive use. While the decline in fertility has occurred in conjunction with an increase in contraceptive use in the last decade. In 1990 it was estimated that among married women of reproductive age in developing countries, 380 million (or 51%) were contraceptive users. The corresponding figure in 1980 was 220 million (or 38-40%). As with fertility there are large regional differences in contraceptive use. While in East Asia 70% and in Latin America 60% of married women of reproductive age use contraceptives, only 36% do so in North Africa and the Middle East. In sub-Saharan Africa the rate is a mere 9% (Ross et al. Family planning and child survival programs, New York, Population Council, 1992).

The seemingly favourable trends in contraceptive use and fertility rates in the 1980s give, however, no reason for complacency. In developing countries, the high fertility rates in the 1960s and 1970s, combined with the declining infant and child mortality rates, have led to large cohorts of young people that are now entering the childbearing age expecting to have access to contraception and family planning services. In addition, as further increases in contraceptive prevalence become more difficult to achieve, maintaining a decline in the fertility rate will depend on even greater use of methods with high efficacy.

The unmet need for contraception—defined sometimes as the discrepancy between not wanting to become pregnant but not using a contraceptive method—continues to be large, although it is difficult to quantify accurately. The estimates that 15 million clandestine abortions are carried out each year (Henshaw, Family planning perspectives, 1990, 22: 76-89) and that one-quarter to one-third of the 500,000 maternal deaths each year may be attributable to complications of unsafe abortion procedures (Abortion: a tabulation of available data on the frequency and mortality of unsafe abortion. Unpublished WHO document No. WHO/MCH 90.14, 1990), are two of the many testimonies of unmet needs. Apart from the "hard" demographic data on fertility and contraceptive prevalence, reproductive morbidity continues to cause human suffering and lost opportunities (Fathalla, In: Khanna et al. ed., Reproductive health: a key to a brighter future, Geneva, WHO, 1992: 3-31).

The Programme continues to meet the challenges briefly outlined above within its mandate, which is "to promote, coordinate, support, conduct and evaluate research on human reproduction, with particular reference to the needs of developing countries". In 1992 the Programme maintained its focus on development and assessment of fertility regulating methods and sought to intensify its efforts in human reproduction research through collaboration with other units and programmes within and outside WHO as recommended by its governing and advisory committees. The activities of the four units within the Research and Development component of the Programme, namely Technology Development and Assessment, Epidemiological Research, Social Science Research, and Technology Introduction and Transfer, rely heavily on interaction with the international community of scientists and researchers in reproduction, and on collaboration with the Programme's worldwide network of collaborating institutions. This network has continued to expand and now consists of 105 institutions, of which approximately 75% are in developing countries. The strengthening of the collaborating institutions in developing countries and of their mutual cooperation is one of the main objectives of the Resources for Research component of the Programme. Resources for Research and Research and Development together work towards the common goal of enrolling developing countries not only to participate in global research efforts but also, and perhaps more importantly, to address the reproductive health needs of their populations.
Both Research and Development and Resources for Research rely to a significant extent on the technical support of the Programme’s Statistics and Data Processing Unit. To Research and Development this Unit provides statistical and data processing support for all multicentre and some single-centre research projects undertaken by the Task Forces, as well as technical advice on the design, management, analysis, and interpretation of Task Force projects. To Resources for Research the Unit provides support in the formulation, execution, and review of institution strengthening policies in the areas of biostatistics and data processing as well as training of statisticians and data managers of collaborating institutions.

Finally, as the main instrument within the United Nations for human reproduction research, and as a WHO-executed activity, the Programme has a number of special roles relating to the coordination and promotion of research, the setting of standards and the development of guidelines, and the provision of advice to Member States on matters relating to reproductive health. An important, indeed crucial, element in the execution of these special roles is the dissemination of information through the production of appropriate information materials of both technical and general natures and their wide but accurately focused distribution.

A detailed description of the wide range of activities undertaken during 1992 by the Research and Development and Resources for Research components and by the Statistics and Data Processing Unit of the Programme can be found in the chapters that follow. Some of the highlights in Research and Development as well as a brief description of work done in relation to the Programme’s special roles are given below.

**HIGHLIGHTS OF 1992**

**Development and assessment of reproductive health technologies**

The Unit of Technology Development and Assessment comprises six Task Forces:
- Task Force on Long-acting Systemic Agents for Fertility Regulation;
- Task Force on Post-ovulatory Methods for Fertility Regulation;
- Task Force on Methods for the Regulation of Male Fertility;
- Task Force on Vaccines for Fertility Regulation;
- Task Force on Methods for the Natural Regulation of Fertility; and
- Task Force on Prevention and Management of Infertility.

The Unit also includes the Research Group on Intrauterine Devices (IUDs).

**Task Force on Long-acting Systemic Agents for Fertility Regulation**

In 1992 the Task Force on Long-acting Systemic Agents for Fertility Regulation completed two major Phase III clinical trials on two once-a-month injectable preparations, Mesigyna and Cyclofem, developed by the Programme. One of the trials conducted in 12 centres in Egypt evaluated Mesigyna and Cyclofem, while the second compared the same preparations and the Chinese Injectable No. 1. Results of the preliminary analyses confirmed the high efficacy of Mesigyna and Cyclofem and indicated that ethnicity may influence certain side-effects such as bleeding disturbances. The clinical performance of the two new injectables was superior to that of the Chinese Injectable No. 1, and it may be that the results of this trial lead to a shift, in China, from Injectable No. 1 to Mesigyna or Cyclofem. The Egyptian trial will serve as an important background for the possible approval of the two injectables for the national family planning programme in that country.

The particle size of long-acting injectables is an important determinant of the pharmacokinetic properties of the preparations. The Task Force completed studies on the effect of particle size of depot-medroxyprogesterone acetate (DMPA) while a study of different particle sizes of levonorgestrel butanoate (HRP002) is in progress. A clinical trial in the United Kingdom on the levonorgestrel-releasing vaginal ring revealed, surprisingly, that use of the ring was associated with vaginal lesions in some women. Such lesions have not been observed in previous studies of vaginal rings and their importance is not clear. The ring has now been modified and studies are under way to evaluate factors associated with the occurrence of vaginal lesions, such as stiffness of the ring and also whether the lesions are caused by the released levonorgestrel. Various treatment schemes have been used for bleeding disturbances during the use of progestogen-only methods. The Task Force is engaged in a trial to determine effective therapies for irregular vaginal bleeding occurring during the use of DMPA and Norplant.

**Task Force on Post-ovulatory Methods for Fertility Regulation**

The various possible clinical applications of antiprogestogens in fertility regulation have constituted the main activities of the Task Force on Post-ovulatory Methods of Fertility Regulation. Combination regimens of the antiprogestogen, mifepristone, and prostaglandin analogues have proved to be effective alternatives to conventional surgical methods for the termination of early pregnancy. Such regimens for non-invasive termination of pregnancy are now routinely used in France, Great Britain, and, most recently, in Sweden. There are still, however, some unresolved issues, such as the minimally effective
dose of mifepristone, the most appropriate type and dose of prostaglandin, and the maximum duration of pregnancy for which the treatment remains effective and safe. Apart from these biomedical issues, there is a need to address questions relating to user acceptability and the service facilities that should be available to women choosing this non-surgical method of pregnancy termination. The Task Force is addressing these issues and several studies in these areas were completed and/or published in 1992.

Clinical trials have been completed on the use of mifepristone plus prostaglandin for menses induction and on the use of mifepristone alone for emergency contraception. With regard to the latter it was shown that the antiprogestogen was more effective, and caused significantly less nausea, vomiting, and other side-effects, than the currently most often used method, the so-called Yuzpe regimen (Glasier et al., *New England journal of medicine*, 1992, 327: 1041–1047; Webb et al., *British medical journal*, 1992, 305: 917–931). The publication of these two studies received substantial attention in the public media and the Task Force intends to continue research in this area.

Other potential uses of mifepristone include suppression of ovulation when the drug is given in low continuous doses during the cycle and this line of research, although not strictly post-ovulatory, is also being supported by the Task Force. The observation reported in the 1991 Annual Technical Report suggesting an effect of mifepristone on motility and acrosome formation of spermatozoa in the bonnet monkey has, however, not been confirmed in subsequent animal studies, and the initial optimism about using antiprogestogens for contraception in men has largely disappeared. The Task Force’s ongoing research into the hormonal requirements for implantation in the primate seems to be heading towards the conclusion that luteal estrogen may not be an essential element in the successful establishment of pregnancy.

**Task Force on Methods for the Regulation of Male Fertility**

The main areas of work of the Task Force on Methods for the Regulation of Male Fertility continue to be the evaluation of hormonal contraceptive options for men, the assessment of safety and efficacy of various methods for vas occlusion, and the study of a potential antifertility drug from the plant *Tripterygium wilfordii*. The third edition of the Programme’s acclaimed *WHO manual for the examination of human semen and sperm-cervical mucus interaction* was published in 1992.

During the course of 1992, a five-centre study in Indonesia on sperm suppression induced by combined androgen–progestogen administration was completed and the findings submitted for publication. The multicentre study on the contraceptive reliability of testosterone-induced severe oligozoospermia is progressing in 15 centres in nine countries. Previous studies supported by the Task Force revealed variations in responsiveness of men of different ethnic origins to contraceptive steroids. The underlying mechanisms are being further explored in studies supported by the Task Force and collaborating agencies. Following encouraging results of a study in baboons on the gonadotrophin-suppressing ability of the long-acting levonorgestrel ester HRP 002, a clinical study to test a combination of this progestogen plus androgen in men is planned to start in 1993.

The safety and efficacy of three different methods of vas occlusion, the “no scalpel method”, percutaneous injection of a sclerosing agent, and injection into the lumen of the vas deferens of a polyurethane plug are being assessed in a study in China. Initially, the “cured-in-place” silicone plug method was going to be included in this study but there is a need for further explorative studies on the efficacy of this method and this is being pursued by the Task Force. A consultation to review progress in *Tripterygium wilfordii* research, held in September 1992, proposed a time-limited programme to establish if an antifertility drug could be developed from the active compounds identified.

**Task Force on Vaccines for Fertility Regulation**

The Task Force on Vaccines for Fertility Regulation was established to investigate the feasibility of inhibiting reproduction by immunological means with the objective of developing birth control vaccines that will elicit an antifertility effect which is safe, effective, non-permanent, and free of endocrine and metabolic side-effects. The Task Force is aiming at the development of a vaccine which will be effective for a period of up to 18 months since this is perceived to be a useful interval for users at practically all stages in their reproductive lives. The Task Force’s prototype anti-hCG vaccine was developed solely to demonstrate the safety and feasibility of the approach and not as a final product. It was envisaged, therefore, that this prototype vaccine would not be used beyond the Phase I clinical trial stage and that an improved and, eventually, optimized anti-hCG vaccine formulation would be developed for further clinical testing and product development. However, the results of the Phase I clinical trial with the prototype vaccine were considered sufficiently encouraging for the Task Force to propose carrying out a Phase II trial with this preparation. The primary objective of this Phase II trial is to determine if the level of anti-hCG antibodies estimated to be effective does, in fact, provide protection against pregnancy in fertile women. Most of the activities supported by the Task Force during 1992 have been concerned with the administrative arrangements for the Phase II clinical evaluation of this prototype anti-hCG vaccine, with the preparation of materials for the preclinical and clinical testing of its advanced prototype anti-hCG vaccine, and with the continued design and development.
of an optimized anti-hCG vaccine suitable for large-scale production and use.

The Task Force has continued also with its studies to develop an anti-trophoblast vaccine. This research has concentrated on the use of monoclonal antibodies and molecular genetics techniques in order to identify and characterize tissue-specific antigens expressed on the surface of the human trophoblast and which might represent candidates for vaccine development. Emphasis is being placed on the identification of antigens that are expressed only on the trophoderm of pre-implantation blastocysts in order to develop a vaccine which will have an effect prior to the completion of implantation. As part of its broader-based collaborative efforts in the area of immunocontraception, the Task Force has continued to collect and collate data on anti-sperm and anti-trophoblast monoclonal and polyclonal antibodies with a view to identifying relevant molecules for vaccine development and to establishing an internationally recognized system of standardization of the reagents and nomenclature being used in this area of immunobiology. The data on a number of new antibodies, generated by or submitted to the Task Force, were reviewed at the Task Force’s Third Sperm and Trophoblast Antigen Workshop which was held in Rome in August 1992.

**Task Force on Methods for the Natural Regulation of Fertility**

The top research priority of the Task Force on Methods for the Natural Regulation of Fertility continues to be lactation and its role in the suppression of ovulation. In addition, the Task Force conducts research on indicators of the fertile period, including new possibilities for the measurement of urinary steroid glucuronides, and on natural family planning. The main activity of the Task Force during 1992 has continued to be the prospective, multicentre study of the relationship of breast-feeding practices to the duration of lactational amenorrhea. This study, involving 3850 mother–infant pairs, has been designed to improve understanding of the factors that determine lactational infertility. The recruitment target was reached at the end of 1992 and follow-up is planned to continue throughout 1993. Other ongoing research includes studies on the effects of supplementary nutrition to nursing mothers on the return of ovulation, studies on the immunoactivity and bioactivity of LH and prolactin, and studies on the interface between breast-feeding and the adoption of other methods of contraception.

The accurate estimation of the fertile interval in women is central to the efficacy of family planning methods based on periodic abstinence. Task Force research in this area is directed at the evaluation of inexpensive and simple methods or devices that can be used in the home to measure biochemical or biophysical markers of the fertile period.

The measurement of cervico-vaginal fluid (CVF) volume using a simple device has been tested in a recently completed multicentre trial, as has the measurement of guaiacol peroxidase, an enzyme with a concentration in cervical mucus that is inversely related to blood estrogen levels during the follicular phase of the cycle. The Task Force has supported also the development of assays of urinary steroid glucuronides that require only the collection of urine onto filter paper, thus removing the problems of storage and transport of liquid urine.

**Task Force on the Prevention and Management of Infertility**

The prevention of infertility caused by sexually transmitted diseases (STDs) and the management of infertility, especially in developing countries, are the main foci of the Task Force on the Prevention and Management of Infertility. To pursue its mandate the Task Force’s work is focused on standardizing the investigation of infertile couples, evaluating certain treatments of infertility in the male and the female, developing and evaluating kits for simplified diagnosis of STDs, and estimating the prevalence of STDs through seroprevalence studies in developing countries. The Task Force is also engaged in the development of a vaccine against genital infection with *Chlamydia trachomatis* and in the evaluation of barrier methods for STD prevention. In 1992 the Task Force was instrumental in introducing polymerase chain reaction (PCR) methodology for chlamydial antigen detection in tertiary health care centres in six developing countries. Steady progress continues to be made in the research and development of an anti-chlamydia vaccine.

**Research Group on Intrauterine Devices**

The research objectives of the Research Group on Intrauterine Devices (IUDs) are to study the long-term safety and efficacy of currently available copper IUDs and of interval insertion of new IUDs, and the development and evaluation of implantable, post-placental IUDs. The long-term studies of the copper IUDs initiated in the late 1970s and early 1980s continue to provide unique and important data. In the course of 1992 the results of the ninth and tenth years of use of the TCu220C and TC380A devices have become available and these data have been submitted by the Population Council, which was responsible for the original development and early testing of these devices, to the United States Food and Drug Administration (USFDA) in support of an extension of the USFDA’s approval of up to eight years of use to nine years of use. The ten-year cumulative (net) pregnancy rate for the TCu380A is at the astonishingly low level of 2.1 per 100 woman-years. The 19-centre randomized comparative trial of the TCu380A and Multiload 375 devices has recruited 3627 subjects and is proceeding as planned.
Studies on the safety and efficacy of the frameless IUD—the FlexiGard—continue with 1500 TCu380A and 1471 FlexiGard devices having been inserted. The one-year data show higher pregnancy and expulsion rates with the FlexiGard device but the latter's pregnancy rate of 1.1 per 100 woman-years remains within acceptable limits. A new IUD that is inserted immediately following a normal vaginal delivery of infant and placenta will be pilot tested in up to six centres in 1993. This device, which is similar in design to the FlexiGard but has a polymer cone which biodegrades during uterine involution, hopefully will have a low expulsion rate.

The Research Group has participated in a study sponsored by UNFPA on the clinical, demographic, and economic impact of the adoption of copper IUDs for all new insertions in the Chinese national family planning programme. The report of this study indicated that if China began conversion to copper devices in 1993, by 2002, ten years later, the following events would be prevented: 55.6 million unwanted pregnancies (including 35.6 million induced abortions and 18.4 million live births); 16 300 maternal deaths; 365 000 infant deaths; and 28 800 child deaths. As a result of this study, the Government of China has decided that, from 1993, only copper IUDs will be inserted in its family planning programme.

Research on introduction and transfer of technologies for fertility regulation

The Task Force for Research on the Introduction and Transfer of Technologies for Fertility Regulation constitutes the Programme's second main Unit. It was established in January 1991 and is a joint undertaking of the Programme and WHO's Division of Family Health. This collaboration facilitates coordination of activities and the implementation of research findings in the area of family planning services. The role of this Task Force is to assist governments and other agencies in the optimization of the use of technologies for fertility regulation and the broadening of contraceptive options available to users, based on informed choice between different types of family planning product that have been adequately tested for safety and efficacy, are of the highest quality, and can be provided at an acceptable cost. An important component of the Task Force's work is the conduct of introductory trials.

In 1992 the Task Force developed a strategy for the introduction of new and underutilized methods of fertility regulation. The strategy shifts the focus of the introductory process from being product-driven to addressing user needs and programme capabilities and it provides information for decision-making within a national family planning programme for the expansion of choice of methods of fertility regulation. The strategy is ready for implementation in selected countries in Latin America and sub-Saharan Africa should funds become available in 1993.

With regard to the introduction of the once-a-month injectable contraceptive, Cyclofen, into national family planning programmes, assessments of the results of studies in the first five countries, Indonesia, Jamaica, Mexico, Thailand, and Tunisia have been completed or are in progress. Case studies on the use-effectiveness, reasons for discontinuation, role of the method, and service delivery constraints in each of these countries are being written up based on the experience of some 7700 women. A study in Chile completed its pilot phase in 1992 and was expanded into additional clinics at the end of the year. Arrangements have been completed for introductory studies to begin in Brazil, Colombia, and Peru. The work in Latin America has been assisted by the involvement of the regional centre based at the Research Centre for the Control of Maternal and Childhood Diseases (CEMICAMP) in Campinas, Brazil. The centre is providing the managerial, training, monitoring, and support activities necessary for introductory trials in Brazil, Chile, Colombia, and Peru as well as statistical and data processing facilities and support. CEMICAMP is the first regional centre to be developed by the Task Force.

Research on contraceptive product management is incorporated into the new strategy for introduction and plans have been drawn up for the activities required in the three stages of the new strategy. During 1992 work has been continued by the Concept Foundation of Thailand and the Program for Appropriate Technology in Health (PATH) to make Cyclofen available in the countries in which introductory trials are ongoing or planned. Manufacturing facilities have been established and validated in Indonesia and Mexico and planned in Thailand, and arrangements made for registration of the product in each of these three countries. A licence agreement was concluded with Schering AG for the production and distribution of a second once-a-month injectable contraceptive, Mesigyna, and registration of this product has been obtained in Argentina and applied for in Brazil and Mexico.

Documents on requirements for the production and quality assurance of hormonal contraceptives have been developed. The document on the requirements for production of hormonal contraceptives has been used to ensure that the highest standards are adopted for the manufacture of Cyclofen, it is also being used by the Government of Kenya in researching the viability of local production of oral contraceptives. The document on requirements for quality assurance of hormonal contraceptives is being finalized and will be field tested in 1993.

Epidemiological research

Epidemiological research constitutes the third Unit within the Programme's Research and Development component and is coordinated by the Task Force for Epidemiological Research on Reproductive Health which was estab-
lished in 1990. This Task Force embraces the mandate of the previous Task Force on Safety and Efficacy of Fertility Regulating Methods, namely: to identify the major side-effects, both beneficial and adverse, associated with the different methods of fertility regulation currently available; and to determine the efficacy of currently used methods when this is not known. The main emphasis of the Task Force’s work is to study potential side-effects of contraceptive use which are of public health relevance in developing-country settings. While these objectives remain the most important, the Task Force, in collaboration with other Units of the Programme and WHO, has also involved itself in epidemiological research that is not strictly confined to fertility regulation, but which addresses other related issues, including health service delivery in family planning and reproductive health care. Examples of such research activities are studies on abortion and women’s health, the impact of infection with the human immunodeficiency virus (HIV) on the course of pregnancy and pregnancy outcome, and the evaluation of antenatal care.

In 1992 the multicentre case-control study of cardiovascular disease and steroidal contraceptive use was in its final stage of data collection for the diseases of stroke and venous thromboembolism. The part of the study concerned with acute myocardial infarction will continue for another two years in selected centres. By the end of 1992, a total of 3503 cases and 10 357 controls had been admitted to the study. Analyses of data on stroke and venous thromboembolic disease are expected to be completed in 1994. The Post-marketing Surveillance of Norplant is in the midst of data collection. By the end of the third quarter of 1992 study data computerized in the Programme contained 41% of the target observations of 80 000 woman-years. The publication of results of the Collaborative Study of Neoplasia and Steroid Hormonal Contraception has continued. So far 35 papers have been published from this study and five more have been submitted for publication. Findings from the study relating to DMPA and cancer will be reviewed and summarized at a meeting in the second quarter of 1993.

To highlight the health problems and cost of unsafe abortion, studies on abortion-related morbidity and mortality have been undertaken in countries where safe abortion is not readily available. Such studies have been completed in Bangladesh, Chile, and Ethiopia, and are under way in Benin, Brazil, Guatemala, Senegal, and Uganda. In the two-centre study, undertaken jointly with the WHO Global Programme on AIDS (GPA), on vertical transmission of HIV-1 and on the health of HIV-1 seropositive mothers, enrolment has been completed in Harare, Zimbabwe. In Kampala, Uganda, the follow-up through pregnancy is completed; the follow-up of mothers and infants will continue for another two years. The fieldwork of the study in Bangkok, Thailand, on the risk of HIV acquisition and its association with contraceptive use has ended and the data are now being analysed.

The possible association between vasectomy and increased risk of prostate cancer is being addressed by the Task Force following two recent reports which point towards a doubling of the risk some 20 years after the procedure. A multicentre, hospital-based, case-control study of prostate cancer and vasectomy, to be conducted in China, India, Nepal, and the Republic of Korea is in preparation. In Denmark, the ongoing historical cohort study on vasectomy and testicular cancer has been extended to include not only vasectomies done in hospitals but also those done as an out-patient procedure. This study should finish in 1993.

Analysis of data from the study on the effects on infants of progestogen-only contraceptives used during lactation is nearly complete while the analysis of the study on iron-deficiency anaemia and various contraceptives has started. The two-centre study on the well-being of women using steroidal contraception is completing data collection and the analysis is being prepared. In collaboration with the Safe Motherhood Programme of the Division of Family Health (FHE) and the Resources for Research component of the Programme, the Task Force has developed a research proposal to evaluate a less resource-demanding antenatal care programme.

Social science research

The Task Force for Social Science Research on Reproductive Health, which started to work under its expanded mandate in 1991, is the operating arm of the Programme's Social Science Research Unit. The objectives of the new Task Force continue to emphasize research on the determinants of fertility regulation, which was the primary focus of the former Task Force on Behavioural and Social Determinants of Fertility Regulation which the new Task Force replaces. The scope of research has been expanded, however, to include other components of reproductive health. New priority themes have been added to the main research agenda: (a) sexual behaviour and reproductive health; (b) social dimensions of maternal health; (c) breastfeeding and birth spacing; and (d) causes of induced abortion.

Of the three new research lines initiated in response to the recommendations made in the Report of the External Impact Evaluation in 1989 (breast-feeding and birth spacing, social dimensions of maternal health, and sexual behaviour and reproductive health) it was decided to place major emphasis on sexual behaviour research. Announcement of this research initiative in 1991 generated global interest of unexpected magnitude and confirmed that knowledge in this key area of reproductive health was limited and much in demand. A large number of sexual
behave proposes was generated and, by 1992, 65 of the most promising preproposals had been developed into full proposals. Of these, 45 were selected to be either funded or further developed during the course of three regional research training workshops held in Brazil, Cameroon, and Thailand. Thus far, 25 new projects in sexual behaviour have been approved. They join nine new projects in ongoing research areas, including factors affecting contraceptive use, gender roles, and abortion which were also approved during 1992. A small number of projects in areas of social dimensions of maternal health and of breastfeeding and birth spacing were begun in 1992, but a full-scale effort in these areas is not anticipated before 1994.

Continuing in 1992 was a major research initiative on the determinants of induced abortion. Of 27 projects supported by the Task Force, seven were fully completed by the end of the year. In the area of male fertility and contraception, studies supported thus far under the condom acceptability initiative were fully completed by the end of the year, and new studies continue to be accepted for review. Research was begun on the role of the man in determining family size and in contraceptive decision-making. Acceptability studies of contraceptive methods including vasectomy, female sterilization, the diaphragm, IUD, and monthly injectable methods are under way. Several studies in the area of gender roles continue, and factors affecting contraceptive use remains a major interest of researchers in the developing world and one of the central research lines being supported by the Social Science Research Unit.

Collaborative activities have continued on breast-feeding and birth spacing with the Task Force on Methods for the Natural Regulation of Fertility, on once-a-month and emergency methods with the Task Force on Post-ovulatory Methods, on the acceptability of vas occlusion and male hormones with the Task Force on Methods for the Regulation of Male Fertility, and on injectable contraceptives with the Task Force on Long-acting Systemic Agents. Collaboration with the Resources for Research component included a major workshop for the Arab region in Cairo and an assessment of social science research capacity in institutions of Africa and Latin America.

The Programme's special roles

Apart from the research and other activities that are undertaken by the different Task Forces, as described in the following chapters, the Programme has several tasks that result from its position as the main instrument within the United Nations for human reproduction research. These tasks relate to the coordination and promotion of reproductive health research, the setting of standards and development of guidelines, and the provision of advice to WHO Member States on matters relating to human reproduction and reproductive health. Linked to these special roles is the dissemination of information through publication and distribution of appropriate materials of both technical and general natures. Activities in this last area are described separately in one of the following chapters.

Coordination

The Programme employs a variety of mechanisms—both formal and informal—to coordinate human reproduction research undertaken within WHO, in the United Nations system, and in collaboration with national and international research programmes, non-governmental organizations and industry. One important formal mechanism is the participation of scientists from collaborating agencies in Steering Committee and other meetings of the Programme, and the reciprocal representation of the Programme at meetings of these agencies. This ensures effective exchange of information about ongoing work and future plans, thus avoiding unnecessary duplication of effort. It also permits the identification of opportunities for more intensive collaboration in areas of common interest through mutual technical assistance, joint monitoring and funding of projects, and the co-organization of scientific and other meetings. The projects “Post-marketing Surveillance of Norplant” and “Multicentre study on cardiovascular disease and steroid hormonal contraception” are results of such coordinated efforts. Where warranted and desired, joint committees or working groups may be established to enhance further coordination and collaboration in specific areas of research. The Inter-Agency Working Group on Long-acting Methods of Fertility Regulation, for which the Programme acts as the Secretariat, and the STD Diagnostics Network are examples of such multi-agency groups. Details about the agencies and organizations with which the Programme coordinates its activities, the main areas of research interest of these agencies, and the various joint activities that are currently under way can be found in the individual reports from the Task Forces and from the Resources for Research component. In addition, the Programme maintains close contact with a wide variety of industrial companies.

Access to financial resources is a pre-requisite to almost all research. Over the past decade the private sector has played a reduced role in contraceptive research and development for a variety of reasons, while public sector agencies have endeavoured to take on some of the work that would otherwise have been done by private sector groups. To assess the experience and current thinking of pharmaceutical companies regarding contraceptive research and development, and to propose strategies to enhance their role in this important area, the Programme, together with the Rockefeller Foundation and the Program for Appropriate Technology in Health (PATH) has initiated and co-funded a study on the role of industry in contraceptive research and development.
In order to obtain, *inter alia*, an increased commitment from governments and the international donor community for greater support of contraceptive research and development, the Mexican Government, in collaboration with the Programme, is organizing an international symposium on "Contraceptive Research and Development for the Year 2000 and Beyond" to be held in Mexico City on 8–10 March 1993. Apart from harnessing commitment and support for contraceptive research and development, the objectives of the symposium are: to highlight progress in contraceptive research in the past decade; to identify unmet research needs and existing challenges; and to make recommendations for future research directions. In addition to Mexican scientists, participants in the meeting will include experts sponsored by public sector agencies committed to and involved in contraceptive research. It is planned that a set of recommendations will be developed by the participants which the Mexican and other Governments may transmit to the 1994 United Nations International Conference on Population and Development.

**Standards and guidelines**

The standardization of procedures related to research in human reproduction is an important function of the Programme. Indeed, the WHO Constitution, Article 2, gives the Organization a special mandate in the area of standardization. The setting of standards and the development of research guidelines will promote high-quality research and improve the coordination of research activities. Particular attention in this respect is given to the promotion of ethical practices in human reproduction research, a field where the Programme has an important role to play (World Health Assembly Resolution WHA 41.9).

The development and formulation of standards and guidelines is a function in which all committees that provide scientific guidance to the Programme participate in one way or other. Of particular importance in this respect, however, are the Toxicology Panel (Annex 1), the Scientific and Ethical Review Group (Annex 2), and the Laboratory Methods Group. Activities of this last Group include the supervision of the Programme’s Matched Reagents Programme which is described in detail in one of the chapters that follow. In addition to the regular committees, the Programme convenes special meetings and consultations in order to develop guidelines on specific subjects, and it works closely with the Council for International Organizations of Medical Sciences (CIOMS) on ethical issues.

In 1992 the Programme published *Guidelines for the use of androgens in men* (WHO/HRP/MALE/92). The document provides guidance to clinical investigators and drug regulatory authorities on the clinical use of androgens and the evaluation of new compounds for the treatment of hypogonadism and for male contraception. Although the clinical safety of androgen therapy in general is well-established, the risks and benefits of androgens for fertility regulation in men need to be kept under constant review, as is the case with steroid hormones for female contraception. Also related to male reproductive health is the WHO Laboratory manual for the examination of human semen and sperm-cervical mucus interaction, the third edition of which was published in 1992. The first edition of this manual was published in 1980; the second published in 1987 has been translated into seven languages (Arabic, Bahasa Indonesia, Chinese, German, Italian, Russian, and Spanish). The manual gives comprehensive and detailed guidance on the collection and examination of human semen and of cervical mucus and on the interaction between the two. The manual also deals with sperm preparation techniques and quality control of semen analysis.

As reported in the 1991 Annual Technical Report, the Programme, together with other units and programmes of WHO, collaborated with CIOMS in the development of *International guidelines for ethical review of epidemiological studies* which was subsequently published by CIOMS. The collaboration with CIOMS continues, with plans for regional workshops to prepare the ground for the eventual issue of guidelines for ethical review of social science research with emphasis on reproductive health issues.

**Advice to WHO Member States**

Advice to WHO Member States, to help them make sound policy decisions on technical issues, is provided by the Programme in a variety of ways. Apart from responding to specific requests which are part of the daily work of the staff, the Programme makes information available to Member States through its publications, through its formal and informal contacts with WHO Regional Offices and WHO Country Representatives, and through its collaborating scientists, many of whom serve on governmental advisory committees in their home countries. The Programme also convenes special consultations and expert group meetings to discuss and report on subjects of concern to Member States.

During 1992 reports of two Scientific Group meetings were published in the WHO Technical Report Series: *Oral contraceptives and neoplasia*, WHO Technical Report Series No. 817, 1992; and *Recent advances in medically assisted conception*, WHO Technical Report Series No. 820, 1992. Three meetings for review and summary of accumulated data are planned for 1993. The first of these meetings, on DMPA and cancer, is to take place in Geneva on 27–28 May 1993, during which, *inter alia*, data concerning DMPA from the Programme’s multicentre study on steroid contraception and neoplasia will be reviewed. In October 1992, as a result of the Programme’s work in this area, particularly the research on DMPA and breast cancer,
USFDA finally approved this product for use in the USA, thus widening the contraceptive options available to women in that country. The second meeting will take place on 1–3 June 1993, also in Geneva, and will be concerned with the review of data on combined once-a-month injectable contraceptives, specifically Cyclofem and Mesigyna, and other estrogen/progestogen injectable preparations. The third meeting, scheduled for 30 August–2 September 1993 in Geneva, will address current knowledge and research needs in the area of female sterilization.

As in previous years, Programme staff have provided technical information and briefing papers to Member States on several aspects of the Programme’s work. Special efforts are also being made to keep WHO Regional Offices and WHO Representatives as fully informed as possible about the Programme’s activities in their region/country through the issuing of Country Profiles and Project Summary Sheets which give details about ongoing and completed activities by region and by country.

Promotion of research

Because of its limited funding the Programme largely plays a catalytic role in the promotion of reproductive health research. It does this primarily through the sponsorship or co-sponsorship of scientific meetings on topics of relevance to reproductive health and fertility regulation.

As will be seen in the chapters that follow, the Programme co-organized or participated in a significant number of major international scientific meetings during 1992. Among these, two are worthy of special note. A meeting on “Exogenous Hormones and Dysfunctional Bleeding” was convened by the National Institute of Child Health and Human Development (NICHD), in collaboration with the Programme. The meeting took place in Bethesda, MD, USA on 2–7 May 1992 and the proceedings have been published (Alexander and d’Arcangues, eds. Steroid hormones and uterine bleeding, American Association for the Advancement of Science, 1992). In collaboration with the Indian Society for the Study of Reproduction and Fertility and with the Indian Council of Medical Research, the Task Force on Post-ovulatory Methods of Fertility Regulation organized an International Conference on Fertility Regulation on 5–8 November 1992 in Bombay, India. The conference was one of the final events organized in 1992 on the occasion of the Programme’s 20th anniversary. Some 50 papers were presented in seven symposia sessions and more than 220 abstracts submitted for poster presentation. The conference attracted more than 200 participants from all continents and the proceedings will be published in 1993.

A total of 13 events took place in 12 countries in 1992 on the occasion of the 20th anniversary of the Programme, as detailed in Annex 3. These symposia, seminars and meetings were mostly organized by the collaborating centres of the Programme and were, for financial reasons, most often held in conjunction with other Programme-supported activities. Reports on the Programme’s work on the occasion of the 20th anniversary were published, inter alia, in Geburtshilfe und Frauenheilkunde (1991, 512: 9–14), Acta obstetrica et gynecologica scandinavica (1991, 70: 259–262), and the Nederlands tijdschrift voor geneeskunde (1992, 136: 2184–2186).

CONCLUSION

In 1992 the Programme maintained its focus on research and development and, as can be seen from the following chapters, continued to strengthen research capabilities in developing countries through its role of promoter and coordinator of research in human reproduction, specifically fertility regulation, worldwide. Several major multicentre studies coordinated by the Programme are currently under way in the collaborating centres. The extended mandate recommended by the advisory and governing committees has been pursued vigorously but as indicated in several of the following reports from the Task Forces, this is stretching the human and financial resources. Unless funding increases substantially in coming years, the Programme will be obliged to reduce the scope of its activities and concentrate on a smaller number of priority research areas in order to ensure that its work continues to be of high quality and commands the attention, respect, and esteem that have been extended to its work during these first 20 years of its existence.
Summary of Activities in Contraceptive Development

Centre for Reproductive Biology
University of Edinburgh
Scotland

Mellon Foundation Meeting,
November 7-9 1993
Programme of research activities
Centre for Reproductive Biology, Edinburgh, UK

Introduction

The Centre for Reproductive Biology of the University of Edinburgh forms the major focus within the UK for basic and applied research in the field of contraception and associated aspects of reproductive health. The Centre comprises the Medical Research Council Reproductive Biology Unit (Director: Professor Dennis W. Lincoln) and the University Department of Obstetrics and Gynaecology (Chairman Professor Andrew Calder). Core funding from the Medical Research Council and the University Funding Council supports 88 staff, of which 11 hold full professorial level appointments. An additional 60 research staff, clinical fellows and graduate students are supported from other sources.

As part of the recently-agreed Concordat between the UK MRC and the UK Overseas Development Administration (ODA), there have recently been meetings between the Centre for Reproductive Biology and ODA to establish research priorities for contraceptive development and to initiate collaborative ventures.

(1) MRC Reproductive Biology Unit

Neuroendocrinology
Dr Gerald Lincoln, Dr Nigel Brooks


Peptide receptors (pituitary gland)
Dr Karin Eidne

Study of the molecular structure of the membrane bound receptors of the mammalian pituitary gland, ligand coupling and second messenger activation. Three dimensional evaluation of structure and designer analogues. Expression of these receptors in the gonads, breast, brain and prostate. Recently cloned and sequenced the receptors for human GnRH and TRH.
Pituitary-ovarian axis
Prof Alan McNeilly, Dr Hamish Fraser, Dr Peter Illingworth,

The study of the pituitary ovarian axis, with respect to the regulation of the ovarian follicle (selection) and corpus luteum. Current focus centres on the regulation of gonadotrophin sub-unit gene expression (transgenic knockouts), regulation of the packaging and secretion of gonadotropins from the pituitary, the application of third generation GnRH antagonists, and the role of factors regulating the lifespan of the human corpus luteum. Continuing study into mechanisms of lactational infertility post-partum fertility control.

Testis
Dr Richard Sharpe, Dr Philippa Saunders, Dr Tony West

This work centres on the paracrine mechanisms regulating spermatogenesis. Novel models, based on the use of selective testicular toxicants, are being used to tease apart the mechanisms involved, using both peptide and molecular techniques to characterise the factors involved. Links between exposure to environmental oestrogens during fetal life and the fall in the human sperm count. Molecular mechanisms of interaction between Sertoli cell and Leydig cell.

Gamete biology
Prof John Aitken, Dr Stewart Irvine

Research activities. Cell biology of human spermatozoa and sperm-oocyte interactions, with respect to molecular mechanisms which may be targeted for contraception. The application of these principles for the production of a contraceptive vaccine. Novel molecular mechanisms regulating human infertility have been discovered. Conversely, various zona and sperm antigens have recently been cloned and sequenced (and patented), and constructs are currently being evaluated for contraceptive efficacy in animal models. Involves the organisation of the largest semen donor panel in the country. Major industrial sponsorship.

Endometrium (prostaglandins)
Dr Rodney Kelly Dr Hilary Critchley (Department of OBGYN)

Research activities. Cell biology of antigestagens, the role of interleukin-8 in cervical ripening, the role of seminal immunosuppressive agents in the transmission of HIV. Steroid receptors in the endometrium and the relationship with menstrual symptomatology with particular regard to LNG containing IUDs. Important role in technology transfer.

Human sexuality
Dr John Baneroft Dr Cindy Graham

Research activities. Evaluation of the role of hormones and neurotransmitter mechanisms in the regulation of the sexuality and wellbeing of women, with particular reference at this time to the menstrual cycle and the action of oral contraceptives. Important links with WHO and Industry.
(2) University Department of Obstetrics and Gynaecology

Antigestogens
Professor D.T. Baird

Research activities. This work involves clinical and basic studies into the use of antigestogens. These include work on dose-finding studies with antigestogens in therapeutic termination of pregnancy. Use and mechanisms of prostaglandin analogues in medical termination. The mechanism of action of antigestogens and prostaglandins in the endometrium. Antigestogens in the inhibition of ovulation.

Ovarian paracrinology and endocrinology
Professor D.T. Baird, Dr S. G. Hillier (MRC programme grant)

Research activities. This work includes both clinical and basic studies in women into the mechanism of selection of the dominant follicle. Gonadotrophin requirements for ovulation. Inhibin secretion by the ovary. Paracrine role of growth factors and inhibin/activin peptides. Molecular control of follicular development.

(3) Lothian Family Planning Service

Applications of novel contraceptive development
Dr A.F. Glasier

1. The ODA places particular emphasis on the following areas of research relevant to the health of developing societies:

- Development and field testing of the techniques and systems for the improvement of reproductive health that enable women and men to control their fertility. This includes access to effective contraception; antenatal, natal and postnatal care; the promotion of breast-feeding; reduction in infertility; and the adverse consequences of genital mutilation and abortion.

- Social and epidemiological aspects of HIV transmission, STDs and AIDS; the development of cost-effective methods to enable people to reduce their risk of HIV infection (including effective vaccines), the implementation of cost-effective techniques to mitigate the consequences of HIV infection;

- Development and field testing of effective means that enable people to prevent exposure to, or infection by, malarial parasites, techniques to mitigate the consequences of malaria infection (with emphasis on the development of sustainable means).

2. ODA's Health and Population Research Strategy calls for the development and application of scientific responses to health problems of greatest social and economic importance within developing societies. Multidisciplinary research methods are preferred, combining epidemiological, clinical, social science, laboratory and data analytical methods as appropriate. Such multidisciplinary work requires the establishment of field research capacity within developing countries that can focus, in particular, on malaria, HIV and reproductive health problems. Scientists from Britain and developing countries need to have access to facilities which enable them to undertake research ranging from molecular biology through to community-based studies.