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PD - AAM-788

PROJECT STATEMENT

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April 16, 1973

A. Project Summary

1. Statistical

Project Title: Research on the Safety of Contraceptive Steroids, AID/csd-2821

New or Extension: Extension

Contractor and Address: Southwest Foundation for Research and Education  
P. O. Box 28147  
7480 West Commerce Street  
San Antonio, Texas 78284

Principal Investigator: Joseph W. Goldzieher, M.D.  
Director, Division of Clinical Sciences

Duration: Current RAC authorized period:  
June 30, 1970 - June 30, 1973.  
Requested Extension: June 30, 1973  
June 30, 1976

Total Estimated Cost: \$2,221,405

Proposed Additional Funding: Current Year - \$823,239

Funding by Fiscal Year:

FY 70 -	\$912,792
FY 71 -	0
FY 72 -	0
FY 73 -	82,634 (overhead adjustment prior to proposed additional funding)
	823,239 (project extension)
FY 74 -	0
FY 75 -	402,740

Project Manager: J. J. Spidel/M. I. Perry, PHA/POP/R

2. Narrative

Current studies of the metabolism and side effects of the steroids used in oral contraceptives which are now being carried out in healthy American women will be extended to five less developed countries. These studies will allow evaluation of subjective side effects (e.g. nausea), objective side effects (e.g. high blood pressure, weight gain), and hormonal and metabolic changes. The studies in developing countries must be carried out since baseline data now available from the U.S. cannot be reliably extrapolated to populations of different ethnic makeup, and where malnutrition, anemia, parasitic disease and other chronic diseases are prevalent and may well affect the response to and tolerance of the hormonal agents in oral contraceptives. The study will provide objective data concerning the safety and side effects of oral contraceptives under use conditions and allow appropriate family planning program decisions, such as modification of current oral contraceptive regimens by adding vitamins or other nutritive supplements.

B. Expanded Narrative Statement

1. Project Description, Background and Progress Report

The proposed project is an extension of an AID funded research project to study the metabolism, safety and side effects of steroids used in oral contraceptives. Baseline data among healthy women has been acquired; it is now appropriate to carry out similar studies among women in developing countries where chronic disease and malnutrition are prevalent to determine the adequacy and safety of current oral contraceptives in these patient populations.

The purpose of the currently funded study is to investigate and compare the subjective and metabolic effects of the two ethynyl estrogens (ethynylestradiol and mestranol) in dose-response fashion, and to examine the effects of added progestational steroids of the classes commonly used in oral contraceptives.

The major objectives of the study are three-fold:

- (1) To investigate the metabolism of ethynyl estrogens
- (2) To study adverse endocrine and metabolic effects of contraceptive steroids and
- (3) To study the effects of these steroids on carbohydrate and lipid metabolism.

A summary of current status and achievements of this program is as follows:

I. Clinical investigation of side effects.

As of this time 166 patients have been enrolled in the study, of whom 56 are currently active, and 69 have completed the full 12 cycles. The clinical data are being tabulated and stored for computer analysis; the extensive laboratory investigations are proceeding on schedule and will be subjected to data analysis upon completion of the study.

Histological investigations of the endometrium are in progress; studies of ovarian morphology have been completed and published; biopsies for electron microscopy of capillary blood vessels are nearing completion.

Plasma gonadotropin assays in over 1000 samples from long-term oral contraceptive users have been completed and the data analyzed. This material is being prepared for publication. Other studies on plasma-gonadotropins with different permutations at very low levels of steroid dosage are in progress. A dose-related comparison of the 2 ethynyl estrogens is emerging from the San Antonio clinical trial.

II. Adrenal-gonadal interactions are being investigated in two directions:

- (1) A study of the adrenal product(s) which inhibit ovulation in the PMS-primed immature rat; and
- (2) A study of the ovulation-inhibiting action of certain synthetic corticosteroids. This has now been translated from rats to primates and found to apply in this species as well. The results have been published.

III. Identification of the urinary metabolites of ethynylestradiol has progressed through the development of a variety of analytical techniques. Some of the isolated unconjugated metabolites are currently being tested to see if they are adequate for definitive identification by mass spectrometry.

IV. In parallel studies, the metabolism of these steroids has been investigated in nonhuman primates; these results were published recently in a World Health Organization symposium.

Fifteen publications have resulted from this program to date.

In the extension of the contract, it is proposed to carry out a clinical and laboratory investigation of the acceptance and safety of two AID-supplied, commercially-available oral contraceptives (in double-blind fashion for assessment of subjective side effects and acceptance), with a group of women using IUDs as a comparison population. It is intended to study the pill or IUD-users over a period of one year; subjective assessment and physical findings will be measured according to the Protocol for Systemic Contraceptives developed by the International Fertility Research Program (IFRP) at the University of North Carolina, and will be data-processed by IFRP; laboratory studies will be performed at selected intervals and the samples will be analyzed at Southwest Foundation to avoid interlaboratory variance. These studies will include measures of glucose tolerance; plasma lipoprotein pattern; plasma progestins, FSH and LH as measures of the pituitary-gonadal system; T4 and T3-resin uptake as thyroid studies; various liver function tests including studies of the hepatic conjugation and metabolism of the ethynyl estrogen; serum iron and iron-binding capacity, as well as blood count and hematocrit (these latter to be done locally). In addition, each of the five collaborating centers will be urged and assisted whenever possible to conduct additional onsite investigations relevant to the nutritional and vitamin status and changes occurring therein during the study period.

The baseline studies in the San Antonio population will continue to their completion at the end of the first year of this contract. Analysis of subjective and objective symptoms will be performed by utilization of available computer facilities and programs at Wyeth Laboratories; analysis of the laboratory findings will utilize programs already developed by SFRE for the ongoing San Antonio study.

The major purpose of these studies is an evaluation of the safety, acceptability and tolerance of two oral contraceptives in five LDC populations as compared to populations in developed countries, and an evaluation of the possible adverse influences of concomitant nutritional and health problems on these parameters in LDC populations of women using oral contraceptives, as compared to control subjects electing to use IUDs.

Collaborative arrangements have been worked out with investigators in Nigeria, Pakistan, Singapore, Thailand and Mexico for the conduct of these studies.

## 2. Significance to A.I.D. Objectives

Oral contraception is the preeminent means of contraception in both developed and less developed countries. Excluding the Peoples Republic of China, there are certainly over 30 million women who are current users of oral contraceptives. Millions more use the pill in China, where it is the leading means of fertility control. The tempo of acceptance of orals is accelerating as developing countries move to remove restrictions on availability, making non-clinical distribution a reality. Accordingly, AID purchases of orals have greatly increased in recent months. In the recent period 7-1-72 to 2-17-73 AID obligated \$8.8 million for the purchase of 37 million cycles of oral contraceptives.

Unfortunately the use of the millions of cycles of oral contraceptives has not been trouble free. Side effects, particularly break through bleeding, have been a problem with some of the preparations supplied. Data concerning subjective side effects and metabolism is therefore needed to allow selection of the best preparations for use in AID's program.

In view of the millions of women exposed to these substances over prolonged periods of time for contraceptive purposes, it is essential to know the intermediate metabolism, clinical pharmacology, and mechanism of adverse effects of these substances.

In the developed countries of the world there have been extensive studies which inquired into the side effects of oral contraceptive usage, such as subjective symptoms (nausea, depression), objective changes (weight gain, hypertension) and metabolic changes (glucose tolerance, plasma triglycerides and lipoproteins), as well as alterations in hormonal function (serum thyroxine, steroid, and gonadotropin levels, etc.). In a very few instances, double-blind designs or women using IUDs have been included as controls. Toxicity studies such as liver function tests have also been performed. Southwest Foundation has been conducting an in-depth investigation of these and other parameters in an essentially healthy and well-nourished population of largely Mexican-American women. Studies such as these form an important baseline of information, but the results cannot be extrapolated to populations of the less developed countries, where malnutrition, parasitism or other chronic diseases pose a serious problem which might well affect the response to and tolerance of the hormonal agents in oral contraceptives.

This study will provide essential data for family planning administrators and clinicians who are not yet using oral contraceptives in their programs. Major programs, such as that of India, are held back from including orals because of fear of side effects of oral contraceptives in their own patient populations. Objective data is vitally needed to allow a realistic assessment of risks and benefits to LDC patient populations - at present all too often the decision to exclude the pill has been made on the basis of conjecture and theoretical risks and side effects. It will also allow selection of the best oral contraceptive preparations now available, or modification of current regimens by altering the estrogen-progestin balance and/or ingredients or addition of nutritive supplements such as vitamins.

### 3. Relation to Existing Knowledge

Over the last 15 years, there has arisen an enormous literature on the clinical and laboratory findings associated with the use of oral contraceptives. Much of this is now of historical or pedagogical value, since it relates to compounds or dosages no longer in use, or represents experimental designs or approaches whose inherent flaws or biases have become evident with increasing sophistication on the part of investigators involved in this field.

In the course of these investigations it became clear that the subjective symptomatology elicited was determined (with a few exceptions, such as nausea and vomiting) to a great extent by the approach of the clinical investigator and his staff, by the methods of elicitation of information (probed or unprobed), and particularly, by the nature of the population undergoing study. Thus, carefully coordinated and standardized multi-center trials revealed, more than anything, the diversity of response of different ethnic and socio-economic groups. The demographic implications of these findings were of no interest to the industrial sponsors of the research, and their significance emerged only when oral contraceptives were utilized in minority-group populations within the developed countries and, eventually, in the less developed countries of the world.

Side by side with great variability in the nature and incidence of side effects went an equal variability in acceptability and, hence, in contraceptive effectiveness. In some populations, thousands of cycles of contraceptive use were recorded without an undesired pregnancy, while in other populations as many as 10% of the users had become pregnant within the first year of use, and the rate of discontinuation among the remainder was prohibitively high.

In most of these earlier studies, attention was focused entirely on the target population of pill users, and no comparison group was available. Thus the role of coincidence, as well as the inevitable biases of the investigator-group made the results equivocal. In recent years, studies have emphasized the importance of these factors. Probed studies have been shown to elicit 2-6 times higher incidence of symptoms than unprobed studies. Double-blind investigations with placebo controls have shown that the vast majority of subjective complaints, as well as the majority of objective complaints such as weight gain, occur with almost the same frequency in the placebo controls as in the medication-users. Thus, the vast majority of the earlier, uncontrolled studies have been invalidated to some extent. Initial reports of objective findings such as hypertension, noted with high frequency in some centers devoted to the investigation of such phenomena, have been set in the proper perspective of their relative infrequency by large prospective studies, while other investigations have shown such phenomena to be highly correlated with the ethnic group under study.

Of the many metabolic and biologic effects of oral contraceptives which have been identified, changes in liver function tests, increased plasma lipids, and decreased glucose tolerance are common, and high blood pressure sometimes occurs. The metabolic changes appear to be reversible, but in developing countries at present there is no clear measure of their effects upon health.

Several of these effects might be expected to be of less significance in less developed countries where obesity contributes less to hypertension and abnormal tolerance to glucose. However, other effects are of greater theoretical concern because of prevalent health problems including anemia, (secondary to frequent childbearing, nutritional deficiencies and parasites such as malaria, hookworm, schistosomiasis), other liver diseases, and a generally more precarious

Recent studies have suggested that oral contraceptives influence the metabolism of vitamin B<sub>6</sub>, folic acid and to a lesser extent other vitamins and minerals. Biochemical and clinical findings point to an increased need for vitamin B<sub>6</sub>, (pyridoxine) by women using oral contraceptives. Although gross clinical symptomatology of vitamin B<sub>6</sub> deficiency has not been observed, it has been suggested that depression can result. The absorption of polyglutamic folic acid, the major food form of folic acid, is substantially impaired in women receiving oral contraceptives and folic acid deficiency has been reported in some subjects. The clinical result of folic acid deficiency is anemia with the occurrence of megaloblastic ~~red~~ blood cells. Oral contraceptives also appear to increase the requirement for vitamin C and perhaps for vitamin B<sub>2</sub> (riboflavin) and zinc.

On the other hand, the diminished blood loss and increased iron absorption of women receiving oral contraceptives suggest that their dietary requirement for iron may be slightly reduced relative to other women. Estrogens are used to improve calcium absorption and to reduce bone resorption in women with post-menopausal osteoporosis; oral contraceptives also increase calcium absorption. Niacin, vitamin K, and copper may be needed in lesser amounts by oral contraceptive users. Even less is known of the effect of oral contraceptives and their components on the requirements for other vitamins and minerals. The most important of these nutritionally related problems from a clinical point of view probably relates to anemia.

Although megaloblastic anemia has been reported, the net effect of oral contraceptives on the blood status of the average user in a less developed country is unknown but could be beneficial. The most commonly occurring anemias doubtlessly relate to parasitically caused blood loss and depletion of iron stores from childbirth. The decreased menstrual blood loss and the provision of iron in the seven "spacer" pills of the 28 day regimen commonly used in less developed countries may result in increased hemoglobin levels. The avoidance of childbirth also allows some restitution of iron stores. Alternate means of fertility control, notably abortion or use of IUD's, is associated with increased blood loss.

Another issue of concern to family planning experts relates to the administration of oral contraceptives shown to be satisfactory for U.S. and European women to smaller, lighter and less healthy women in developing countries with differing ethnic, nutritional and health status. In particular there has been concern about metabolism of the estrogenic component of the pill in poorly nourished women who may have liver disease.

Finally, the world wide use of the oral contraceptive preparations, geographically localized phenomena have been observed, such as the relative hepatic intolerance to estrogens of Scandinavian women. In all these investigations, great care has been taken in the clinical protocol to limit the studies chiefly to healthy women and to exclude women with disorders which might complicate the interpretation of the findings. While this approach is entirely valid from the experimental point of view, it fails to come to grips with a fundamental problem: that a very large proportion of the world's potential users are not "healthy," in the sense that they suffer from various degrees of malnutrition, mineral and vitamin deficiency, parasitism, chronic debilitating diseases and so forth. Thus an evaluation of the safety of oral contraceptive drugs in the populations of the developed countries is only a partial answer to the question of their worldwide safety, an answer which necessarily ignores many questions relevant to this much larger target population.

Investigations designed to fill in some of the needed information must be designed to take advantage of the lessons that have been learned in clinical pharmacological investigations of oral contraceptives over the last fifteen years. Moreover, current knowledge should permit some limitation of the investigative field to those areas which have been found to be of greatest concern in the developed countries, taken together with hypotheses as to problems which might occur as a consequence of malnutrition, parasitism and chronic disease. Therefore, studies on the safety and acceptability of oral contraceptives in the less developed countries should focus on a number of basic operational principles: (1) studies should be carried out with at least two preparations which have financial, logistic, or other advantages as well as clinical and pharmacological acceptability; (2) the studies should be simultaneous and double-blind, since investigator bias is known to be a pertinent factor; (3) a non-drug-using comparison group must be studied simultaneously, in the same manner, by the same protocol, even though certain of its features might appear irrelevant. It is ethically unacceptable to have a contraceptively unprotected group. Concurrent use by women of two contraceptive modalities (one being a placebo) is extremely difficult to implement. A practical solution, with well-known limitations, is a control group of subjects who have elected to contracept with intrauterine devices; (4) the trial should be conducted simultaneously in populations from various LDCs; (5) as much objective as well as relevant laboratory investigation should be planned concurrently to maximize the yield of the effort; (6) both clinical and laboratory procedures should be standardized and centralized as much as possible to minimize methodological variance; (7) maximum use should be made of existing facilities and experience both with respect to laboratory technology and computerized data analysis.

Incorporation of these basic principles into a multinational clinical trial will help to improve the quality and interpretability of the emerging data and will hopefully yield insights into the reasons for the acceptability and safety of oral contraceptives in various populations. The relevance of medical problems encountered in the developed countries will hopefully be clarified, and permit the less developed countries to concentrate on questions pertinent to their own situation rather than to those of foreign socio-cultural systems. Initial insight will be gained into the potential role of malnutrition, mineral and vitamin deficiency, parasitism, and chronic intercurrent disease on the acceptability and safety of the oral contraceptives. On the basis of these initial insights, in-depth studies can be designed in the future to explore specific aspects whose importance is surfaced by these preliminary explorations. Moreover, such studies will give public health and demographic officials much-needed information to enable them to make programmatic decisions regarding the incorporation or exclusion of contraceptive modalities in their population-control efforts.

#### 4. Relationship to Other Research

Over the past 2 1/2 years the Southwest Foundation has been engaged in an AID-sponsored investigation of the clinical and laboratory consequences of the administration of the synthetic steroids used in oral contraceptive formulations to a carefully monitored population of women in San Antonio, Texas. Rather than employing commercial formulations, which inevitably include both an estrogen and a progestin, they have elected to examine the individual effects of the two estrogens, ethynyl estradiol and mestranol, in dose-related fashion, and then

to examine the superimposed effects of the various courses of progestin after the changes produced by the exposure to cyclic estrogen treatment have been stabilized. The menstrual pattern and clinical symptomatology resulting from these regimens are of minor importance, since such regimens are not intended for routine use. Instead, they have concentrated on various laboratory parameters. Plasma levels of FSH, LH and progestin have been measured to assess the qualitative effects of these steroids on the pituitary-ovarian axis. Glucose tolerance tests have been performed to measure changes in carbohydrate metabolism. The serum lipoprotein profile has been explored by ultracentrifugal fractionation of the plasma lipoproteins and subsequent examination of the cholesterol, triglyceride, phospholipid and protein moieties. Plasma cortisol and the steroid-binding potential of the plasma with respect to cortisol and other steroids (testosterone, androstenedione, testosterone sulfate) have been monitored. As an investigation of basic nature, yet one which gives important clinical insights into hepatic function, the urinary excretion pattern of the metabolites of the ethynyl estrogens have been under study. This range of investigations was designed to provide insights into those areas of drug effects and side-effects of major importance which could be carried out in relatively small population numbers. Studies of important but extremely infrequent events, such as thromboembolic phenomena, could not be incorporated into such an experimental design.

On the basis of Southwest's experience in this study, and the baseline information being gained in a population of relatively well-fed and disease-free women from a developed country, it now seems most appropriate and important to turn to the populations of less developed countries, and to study the differences in these parameters which might be brought on by the nutritional and other factors outlined above.

Other side effects to oral contraception can probably only be studied with efficiency in developed countries, where accurate medical diagnosis and vital registration of cause of death allow the conduct of meaningful epidemiologic investigation of rare phenomena such as deaths from thromboembolism. Similarly, the interrelationships between cancer and oral contraceptives are being thoroughly explored by U.S. investigators (funded largely by NIH) and a duplication of these studies is not appropriate.

##### 5. Proposed Work Plan and Time Schedule

The aim of this investigation is to compare, in double-blind fashion, the safety and acceptability of two marketed combination-type oral contraceptives and further, to compare these findings with similar data from a group of women using IUDs. The results in five centers in less developed countries will be compared with each other and with relevant information from the ongoing study in San Antonio.

The overall duration of the study will be 3 years, commencing July 1, 1973. Enrollment of subjects should begin on that date and continue for a period of 18 months. An even enrollment rate should be maintained, at the rate of 11 new enrollments per center per month, totalling 200 subjects for each center. Two or 3 of the 11 enrollments should be subjects electing to use IUDs. The remaining 8 or 9 subjects choosing the oral contraceptive (O.C.) should be randomly assigned to one of the two AID-provided oral contraceptives designated simply as preparations "A" and "B". In this manner, a total of 80 subjects will be enrolled for each of the O.C.s and 40 for the IUD, per center. The observation of all subjects will continue for 12 months, with follow-up intervals as described below. Thus, the clinical trial aspect of the study will extend over approximately 30 months. It is hoped that all centers will be able to start simultaneously, thus leaving some months at the end of the contract for detailed data analysis.

## 6. Research Methodology

### A. Qualifications for Enrollment

Women between the ages of 15 and 44 will be enrolled. Every effort should be made to enroll only subjects who can be expected to remain with the program for 1 year and who can be contacted with relative ease if they fail to keep return appointments.

### B. Workscope:

1. Acceptability - All qualifying subjects will be examined according to a standard IFRP admission and physical examination form, once they have agreed to participate in the program (with or without blood studies) and have signed the informed consent form. There will be clinical interviews after 1, 3, 6, 9 and 12 months (cycles) of treatment, and follow-up will be instituted in case the subject fails to keep her appointment. At these visits the follow-up form and the questionnaire "Symptoms during last menstrual cycle" on the physical examination form will be filled out.

2. Safety - In those subjects volunteering for the laboratory tests, a fasting blood sample (approximately 30 ml.) and a 2-hour oral glucose tolerance test will be obtained before beginning contraceptive (O.C. or IUD) use, and after 1, 3, and 12 cycles or months of treatment. The physical examination will be repeated at the 12-month visit. The glucose tolerance test is omitted at the end of cycle 1. This sequence of studies will be performed in only 54 of the 200 subjects (in each center) over the 30-month period. The laboratory tests will be scheduled during the last 5 days of tablet use in the O.C. subjects and during the late luteal phase in the IUD subjects. If subjects are enrolled and scheduled correctly, there will be a maximum of 12 laboratory studies during any one month.

3. Special studies - In a selected group of volunteer subjects (not to exceed 10 per center) more intensive studies of liver function will be performed by examination of the urinary metabolites of ethynyl estradiol. A single oral or intravenous tracer dose of <sup>3</sup>H-ethynyl estradiol will be given and urine will be collected for 2 or 3 consecutive 24-month periods. Staff from Southwest Foundation will assist in carrying out these studies. Selection of the subjects will be made by the Principal Investigator from among the O.C. and IUD users in the project.

### C. Data Handling

Completed admission, follow-up and physical examination forms will be checked locally for accuracy and then forwarded once a month to Southwest

Foundation for processing. They will then go to IFRP in Chapel Hill, North Carolina, for data processing, computer storage and analysis. IFRP will perform computer analysis of clinical data every 6 months or more often if needed, and will communicate the findings to the collaborating centers and to Southwest Foundation.

Blood samples will be shipped in special refrigerated containers to Southwest Foundation once weekly. All supplies (syringes, tubes for blood collection, plastic tubes for shipment by airfreight, etc.) will be provided by Southwest. The collaborating centers will have to have available a centrifuge for spinning down blood samples to obtain the serum or plasma necessary for shipment. Full directions will be provided for the blood collection procedure.

The basic laboratory analyses performed by Southwest Foundation will include FSH, LH and progesterin levels to assay ovarian function and pituitary activity; T<sub>4</sub> and T<sub>3</sub>-uptake tests for thyroid parameters, glucose tolerance; ultracentrifugal analysis of serum lipoproteins; hemoglobin, serum iron and serum iron binding capacity to study iron stores and metabolism; SGPT and alkaline phosphatase (as well as radioactive ethynyl estradiol tracer studies) to assess liver function. Laboratory data will be reported quarterly to the collaborating centers. The centers are free (and encouraged) to perform any additional studies relevant to their particular interests at the same time, provided no change in the protocol is required.

Whenever possible, the collaborating centers will be asked to perform hemoglobin and hematocrit examinations, and to undertake whatever studies of mineral and vitamin status can be performed with the available facilities. In particular, studies of 11 other parameters which have been found to change in nutritional studies in the U.S. will be encouraged. These are: calcium, copper, zinc, ascorbic acid, vitamin A, B<sub>1</sub>, folic acid, carotene, serum albumin and  $\alpha_1$  and  $\alpha_2$  globulins.

The laboratory data will be stored in the computer at SFRE and will be analyzed by the program developed for the ongoing AID-sponsored study of San Antonio women taking various contraceptive steroids. Statistical analysis must take account of the considerable within-patient variation (in parameters such as the glucose tolerance test) and the influence of age and weight (and changes in the latter during the course of the experiment). These factors can be compensated by the use of sophisticated statistical analysis. It is clearly not enough to look at trends in carbohydrate tolerance, or in the various measurements of the ultracentrifugal lipoprotein profile, individual by individual, over the duration of the experiment. With the multiplicity of interrelated factors a variety of computerized analytical approaches will have to be examined.

In cases where the data do not satisfy the assumptions for analysis of variance, the use of nonparametric analyses such as the rank sum test and/or serial correlation will be investigated. If visual inspection of the data reveals apparent trends of interest, these may be investigated and analyzed for their significance utilizing regression techniques.

The clinical data will be checked as they are received weekly at SFRE and corrected and finalized by SFRE staff before being submitted to computer storage at IFRP. The IFRP programs double-check the quality of the data, and provide means for analysis and printout of the clinical results of each center. Further, this collaboration makes possible comparisons of the LDC data with U.S. studies employing the same clinical format.

While Southwest has not as yet definitively elucidated the structure of the various excreted ethynyl estrogen metabolites, sufficient methodology and information is at hand to be able to interpret a urinary pattern to reveal the extent of free, glucuronide-, sulfate-, or diconjugated metabolites. It is thus possible to utilize the pattern of urinary metabolites to gain an insight into various aspects of hepatic function, without becoming involved in the prohibitively laborious task of identifying each metabolite according to criteria which establish structure definitively in the eyes of the steroid biochemist. Changes in these patterns, as they are observed between individuals, and as they vary in the course of oral contraceptive therapy, should provide additional insights into hepatic functional capabilities. These, together with the data from transaminase and phosphatase measurements, can then be correlated with the clinical information on hepatic health or impairment in the individual subjects.

The procedure to be followed will consist of the oral or intravenous administration of a tracer dose (usually 25  $\mu$ Ci) of ethynyl estradiol and the collection of urine for 48-72 hours under carefully controlled conditions. The urine is then flown to San Antonio for processing. The fraction of administered radioactivity which appears in the urine is determined, and the proportion of radioactivity which is non-conjugated, or conjugated to glucuronic acid or sulfuric acid is determined by standard hydrolytic procedures.

D. Development of a Radioimmunoassay Method for Measurement of Plasma Levels of Ethynyl Estradiol

In studies on the pharmacology of any drug, it is important to be able to determine circulating levels of the drug in the blood or plasma, and frequently also the excretory products in the urine. Recently, radioimmunoassay methods have been developed for the measurement in plasma of endogenous estrogen levels, and such determinations have provided much insight into physiological mechanisms. It is clearly important to an

understanding of the pharmacology of ethynyl estrogens to be able to determine plasma levels of the hormone, thus permitting measurement of absorption of the agent from the digestive tract, half-life of the administered material, rapidity of conjugation and inactivation, as well as circulation of metabolites which might act as a reservoir.

It has been postulated that for smaller women in LDCs, who may have liver disease which slows deactivation of contraceptive steroids, too high a dose of such compounds is received in current preparations. A plasma level assay could quickly determine if this was the case and allow appropriate adjustment of dosages.

It is proposed that a radioimmunoassay antigen for ethynyl estradiol be synthesized, providing Southwest is successful in overcoming the major problem of the instability of the 17-ethynyl group during the required maneuvers of organic synthesis. It is anticipated that it will be possible to successfully protect the ethynyl group and thus make an antigen linked at other than the 3-position to albumin. Upon synthesis of this material, it will be injected (combined with appropriate adjuvants) into rabbits and antibody prepared. The development of antibody can be monitored by the fact that ethynyl estradiol of high specific activity, labelled with tritium, has been prepared in Southwest's laboratory, and this material can be used for assessing the titer of the antibody.

All studies, U.S. or foreign will be carried out in accordance with HEW Guidelines for Human Experimentation. The proposed studies have been approved by Southwest's Committee for the Protection of Human Subjects.

#### 7. Researcher Competence

Dr. Goldzieher is the Director, Division of Clinical Sciences; Senior Foundation Scientist of Southwest Foundation for Research and Education, San Antonio, Texas. He serves as visiting professor at Baylor, Tennessee, Hahnemann and the University of California. He is on the editorial board of the journals Contraception, Clinical Chemistry, Journal of Clinical Endocrinology and Metabolism, and Archives of Internal Medicine. He serves as a consultant to WHO and is on the Central Medical Committee of the International Planned Parenthood Federation.

Dr. Goldzieher is the author of 280 scientific papers and is an established and senior scientist in the field of endocrinology and contraceptive steroid research.

His research team at Southwest has demonstrated competence in this work during the previous contract period.

#### 8. Contribution to Institution Building

In addition to further strengthening the population research program at the Southwest Foundation, this program will strengthen LDC competency in clinical research.

The various LDC staffs will gain additional experience in the operation of a highly coordinated multi-center clinical and laboratory study.

The data obtained in the course of this study will be transmitted in continuing fashion to the staffs of the LDCs, for their information as well as for utilization in joint publications and in reports to governmental agencies of the countries involved. It is hoped that such information will enlarge the data base on which national evaluations and decisions regarding the utilization of various contraceptive modalities will be made.

The present proposal is explicit in its encouragement of additional indigenous investigations of interest to the staff of the various LDCs, particularly with respect to studies of vitamin and mineral deficiency effects. Some of the centers may also have ongoing studies which will generate useful and informative comparisons to the proposed investigation.

Although exact sites for the collaborative studies have not been selected, there are more than enough such study groups from which to choose. At present, consideration is being given to:

1. The Department of Obstetrics and Gynecology, University of Ibaden, Nigeria.
2. Hospital de Gineco-Obstetrica No Uno, Mexico City, Mexico.
3. The National Center for Fertility Research, Karachi, Pakistan.
4. Mahidol University, Bangkok, Thailand.
5. Kandang-Kerban Hospital, Singapore.

It may be possible to substitute a facility in Egypt or Guatemala for one of the above five.

#### 9. Utilization Plans

The objective of this contract is to develop heretofore unavailable data concerning safety and side effects of oral contraceptives in developing countries. This data must be provided to physicians and program administrators in LDCs to allow sound judgments concerning the use of oral contraceptives in LDC programs.

One way information of this nature will be disseminated is through the new A.J.D.-sponsored Population Information Program which has a mailing list of over 10,000 key individuals in population in LDCs.

In addition, Dr. Goldzieher has been very prompt in publishing the findings of this project in the scientific literature. In the first 2 1/2 years, 15 publications have been completed or are in press. The completed findings to

date have all been published except for the gonadotropin data on long-term oral contraceptive users. Every effort has been made to make research results promptly available in the scientific literature. In addition, dissemination efforts have included presentation of data at major scientific meetings, in advance of ultimate publication.

Good evidence for wide dissemination of these data is the vary large number of reprint requests Southwest has received for these publications. Notably, many reprint requests have come from Latin America and a number from Southeast Asia, in addition to large numbers of requests from the developed countries.

The involvement of collaborating clinical groups in five developing countries will serve to ensure early availability and utilization of this data within these countries.

#### 10. Budget Analysis

The budget appears high for the proposed scope of work, but should be quite close to what is required for the modified scope of work described under Section 11, Internal and External Reviews, which follows. The cost of collaborative clinical studies is estimated at this time and will require individual negotiation. A budgetary summary is as follows:

<u>PERSONNEL</u>	<u>TIME</u>	<u>YEAR 1</u>	<u>YEAR 2</u>	<u>YEAR 3</u>
J. W. Goldzieher, M.D.	30%	\$ 7,000	\$ 7,385	\$ 7,791
Research Associate, Ph.D.	100%	21,109	22,269	23,493
A. de la Peña, M.S.	75%	12,162	12,830	13,545
LDC Center Monitor	100%	12,000	12,660	13,356
Data Processor	100%	6,500	6,857	7,234
Research Associate, M.S.	100%	9,675	10,302	10,868
Research Assistant	100%	7,000	7,600	8,060
Filing and Logging Clerk	100%	4,500	4,758	4,975
B. Chenault, M.D.	100%	9,866	-----	-----
Research Assistant	50%	4,311	4,548	4,798
Social Worker	100%	7,559	-----	-----
Medical Technician	100%	6,035	-----	-----
Four Research Technicians	100%	30,127	31,784	33,532
Glassware Washer	100%	<u>4,500</u>	<u>4,747</u>	<u>5,008</u>
Total		\$142,344	\$125,740	\$132,660
Fringe Benefits		<u>21,351</u>	<u>18,861</u>	<u>19,899</u>
TOTAL PERSONNEL		\$163,695	\$144,601	\$152,559

<u>PERSONNEL</u>	<u>TIME</u>	<u>YEAR 1</u>	<u>YEAR 2</u>	<u>YEAR 3</u>
<u>Personnel 5 LDC's:(Estimated)</u>				
Five Principal Investigators	50%	\$ 27,500	\$ 27,500	\$ 27,500
Ten Clinic Interviewers and Data Compilers	100%	40,000	40,000	40,000
Five Laboratory Technicians	50%	10,000	10,000	10,000
Five Shipping Clerks	50%	<u>5,000</u>	<u>5,000</u>	<u>5,000</u>
<b>TOTAL LDC PERSONNEL</b>		<b>\$ 82,500</b>	<b>\$ 82,500</b>	<b>\$ 82,500</b>
<u>Equipment</u>				
Ultracentrifuge w/ accessories		14,000	-----	-----
Two Micro-Kjeldahl burners		600	-----	-----
Coleman Jr. Spectrophotometer		<u>800</u>	-----	-----
<b>TOTAL EQUIPMENT</b>		<b>\$ 15,400</b>		
<b>TOTAL MATERIALS, SUPPLIES AND SHIPPING</b>		<b>\$ 48,613</b>	<b>\$ 39,963</b>	<b>\$ 44,963</b>
<b>TOTAL TRAVEL</b>		<b>\$ 13,756</b>	<b>\$ 12,801</b>	<b>\$ 12,801</b>
<b>INDIRECT COSTS</b>		<b><u>\$ 116,414</u></b>	<b><u>\$ 102,997</u></b>	<b><u>\$ 109,916</u></b>
<b>TOTAL COSTS</b>		<b><u>\$ 440,378</u></b>	<b><u>\$ 382,862</u></b>	<b><u>\$ 402,739</u></b>
<b>3 YEAR TOTAL = \$1,225,979</b>				

11. Internal and External Reviews

The current research program received a formal annual review on February 25, 1972 in Washington, D. C. by the Office of Population, Research Division. The proposed extension, described herein, was reviewed favorably by the Office of Population and by the RIGC.

We have requested review by outside experts and have received comments from the following: Dr. D. S. Lane, Professor and Chairman, Department of Biochemistry, University of Ottawa; Dr. W. N. Spellacy, Professor, Department of Obstetrics and Gynecology, School of Medicine, University of Miami; Dr. M. Brin, Associate Director Biochemical Nutrition, Hoffmann-LaRoche, Inc.; and Dr. R. P. Dickey, Chief, Section of Reproduction Physiology and Associate Professor, Department of Obstetrics and Gynecology, Louisiana State University College of Medicine.

In general these reviewers were in agreement with the importance of the proposed study and were favorably disposed to the proposed line of investigation. One reviewer, providing a summary statement, commented "In summary, I am favorably disposed to this proposal, in that I consider the work important, the personnel qualified, and the procedures proposed in the main realistic."

However each of the reviewers felt that specific modifications were needed which would enhance the merit and utility of the proposed work. These comments relate to two areas: 1) Additional studies needed to assess safety of oral contraceptives in LDCs and enhance utility of this work to AID programs, and 2) suggested improvements in methodology.

#### Suggested Additional Study Areas

1. **Nutritional Studies.** Two of the reviewers noted that to leave these studies to the responsibility of the collaborating laboratory is unlikely to yield useful results. The proposing office agrees with this assessment and believes that the study should routinely include the following nutritional studies: a) Folic acid levels as judged by anemia and morphological changes in white blood cells, b) pyridoxine (vitamin B<sub>6</sub>) levels, c) any anemia or improvement in blood status relating to iron deficiency. Note: These iron studies are proposed in the original study.
2. **Endometrial Support.** One reviewer suggested endometrial support and breakthrough bleeding should be studied in more depth. Note: The proposing office feels that this will be adequately covered by use of the oral contraceptive symptom survey questionnaire which will carefully measure bleeding patterns of oral contraceptive users.
3. **Alterations in Blood Clotting.** One reviewer suggested clotting factor alteration should be studied. Note: The proposing office considers this an interesting study, but one of little practical value. There is much data to show changes in clotting factors after use of oral contraceptives, during pregnancy, etc. Since these changes cannot be correlated with thromboembolic disease, studies which might show subtle differences in such changes, depending on ethnic group and other factors, would be of little practical importance.
4. **Electrolyte Changes.** Note: The proposing office agrees that studies of fluid retention seem appropriate because they are a common side effect. The subjective side effects will be well studied, but the protocol should ensure that an accurate assessment of hypertension is made in the follow-up of patients on oral contraceptives.

Suggested Improvements in Methodology

1. Clinical assessment of the varying states of how malnutrition and chronic diseases would be evaluated were not well described. Note: The proposing office believes that a thorough physical examination and appropriate laboratory diagnostic studies should be carried out.
2. The glucose metabolic studies should be more complete including a 3 hour glucose tolerance test and measurements of blood insulin, glucagon, insulin/glucagon ratio and growth hormone. (These parameters change before glucose and also offer an insight into the possible mechanisms of derangement.)
3. The proposed laborious ultracentrifugation studies of lipid metabolism can be simplified. One could suffice with determinations of total cholesterol and triglycerides and LDL cholesterol as determined by the Fredrickson-Levy method of precipitating HDL cholesterol and using the triglyceride /5 as the VLDL cholesterol fraction.

The proposing office agrees that the changes in methodology noted in 1,2, and 3 above should be incorporated into the proposal.

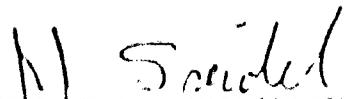
In summary, the proposing office feels that the above changes in work scope (i.e., relating to nutritional studies and electrolytes, and the changes in methodology relating to clinical assessment of physical condition, and glucose and lipid metabolism) can easily be incorporated into the study. Furthermore we believe that these changes can be accomplished within the funds proposed.

12. Proposing Office General Evaluation

Although minor modification is required (as described above), this is a sound and relevant research program. It seeks to provide objective data concerning safety and side effects of the most important contraceptive method now in use in developing countries. This information is likely to reassure those hesitant to use oral contraceptives in their programs, and will provide guidance for the proper use of orals in service programs.

The proposing office has little doubt of the competence of the research workers to carry out this protocol. We will modify the protocol as described above to ensure the careful evaluation of health, nutritional, and blood status prior to pill use so that changes in these parameters following pill use can be evaluated.

In summary, we feel that this program is highly deserving of continued funding.

  
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Signature of Monitor: J.J. Spidel

  
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Signature of Monitor: M.L. Perry

  
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Signature of Office Director  
R. T. Ravenholt