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<td>Family planning</td>
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2. TITLE AND SUBTITLE
Research on the safety of contraceptive steroids

3. AUTHOR(S)
Goldzieher, J.W.

4. DOCUMENT DATE | 5. NUMBER OF PAGES | 6. ARC NUMBER | 7. REFERENCE ORGANIZATION NAME AND ADDRESS
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Contraceptives, oral
Drug dose response

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15. TYPE OF DOCUMENT
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AID CONTRACT CSD/2821

APRIL 1974

RESEARCH ON THE SAFETY OF CONTRACEPTIVE STEROIDS

PREPARED BY:
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DIRECTOR OF CLINICAL SCIENCES
AND REPRODUCTIVE BIOLOGY

SUBMITTED TO:
AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C.

SOUTHWEST FOUNDATION
for RESEARCH and EDUCATION
San Antonio, Texas
AID CONTRACT csd/2821

FINAL REPORT

30 March 1974
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A.  
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   II. Antiovulatory Potency

E. The Effect of Testosterone and Other Adrenal Steroids on PMS-Induced Ovulation in the Immature Rat
REPORT SUMMARY

A. 1. Project Title and Contract Number:
Research on the Safety of Contraceptive Steroids
AID/csd 2821

2. Principal Investigator, Contractor and Mailing Address:
Joseph W. Goldzieher, M.D.
Southwest Foundation for Research and Education
P. O. Box 28147
San Antonio, Texas 78284


5. Total A.I.D. funding of contract to date: $988,761.83

6. Total expenditures and obligations through previous contract year:
   Voucher #23 - $602,174.83

7. Total expenditures and obligations for current year:
   Voucher #36 - $390,114.09

8. Estimated expenditures for next contract year: NONE

Information Required by Paragraph F

|                      | VO. #23 Spent through 6-30-72 | VO. #36 Spent & Committed through 9-30-73 | Current Year
|----------------------|--------------------------------|------------------------------------------|----------------
| Salaries and Wages   | $260,186.70                    | $378,092.84                              | $117,906.14    |
| Fringe Benefits      | 25,758.50                      | 49,604.66                                | 23,846.16      |
| Travel and Transportation | 9,345.83                   | 16,444.81                                | 7,098.98       |
| Other Direct Costs   | 30,572.35                      | 63,325.83                                | 32,753.48      |
| Overhead             | 172,503.83                     | 333,904.21                               | 161,400.38     |
| Equipment and Materials | 77,367.59                    | 105,911.77                               | 28,544.18      |
| Research Services by Non-U.S. Personnel | 26,440.03                   | 45,004.80                                | 18,564.77      |

$602,174.83 $992,288.92 $390,114.09
B. Narrative summary of accomplishments and utilization.

At the completion of this three year investigative period, the major outlines of the metabolism of ethynylestradiol have been clarified. The primary form of conjugation and excretion in the urine is as the 3-glucosiduronate, with the 17-glucosiduronate as a secondary conjugate. Further, small amounts of sulfates are also excreted. Di-conjugates are part of the excretion pattern, but their exact nature remains to be established. The major urinary steroid metabolite is ethynylestradiol itself. In addition to this substance, the 2-hydroxy and 2-methoxy ethynylestradiol as well as the 6-oxygenated derivatives have been identified. There is also a significant amount of de-ethynylation, with the production of estrone and estradiol. The D-homo compound, described by others, has been found to be an insignificant metabolite.

Excretion of urinary radioactivity follows a semi-logarithmic pattern, with approximately 50% of the radioactivity being excreted in the first 24 hours. Fecal excretion has not been examined. A similar pattern exists in the baboon.

Plasma radioactivity has been studied in part, but the main effort has been concentrated on the urinary studies. At the termination of the contract, work was in progress to develop a radioimmunoassay method for plasma ethynylestradiol and mestranol.

The clinical studies with various dose levels of ethynylestradiol and mestranol, with and without one of the three selected progestational compounds, have enrolled a total of 219 patients, and a completion of 1,952 cycles. Analyses of the clinical and biochemical effects of this drug therapy are in progress.
Endometrial biopsies obtained during the estrogen phase of the clinical investigations reveal, contrary to current opinion based on animal studies, that ethynylestradiol and mestranol are about equally potent, and if anything, mestranol has slightly greater estrogenic effect on the endometrium under the experimental conditions.

Studies of antiovulatory action confirm the endometrial findings, in that ethynylestradiol and mestranol are about equally potent in inhibiting ovulation. While 50 mcg a day alone of the two estrogens is insufficient to inhibit ovulation to a satisfactory extent, higher doses manage to do so. However, it is of great interest that very small doses of the estrogens, combined with very small doses of the progestational compounds, manifest a synergistic effect on ovulation inhibition. This synergism has not previously been demonstrated.

A cross-sectional study of plasma gonadotropins on a large number of patients confirms our previous study with respect to the influence of combined oral and injectable agents on the suppression of LH and FSH.

Studies of plasma FSH and LH during the experimental cycles of the clinical study have been obtained; the data are completed except for patients still active, who may supply material for an additional 176 cycles. The data are currently undergoing statistical analysis.

Muscle biopsies have been obtained from normal, pregnant, and oral contraceptive subjects who do or do not show changes in carbohydrate metabolism. Electron microscopic studies have been performed on these muscle biopsies, and photographs of capillary basement membranes have been completed. Approximately 35 of the 80 biopsies have had capillary basement membrane diameter calculated, and measurements of the rest are in progress.
The data have been validated by a comparison carried out in Dr. Siperstein's laboratory.

Plasma levels and protein binding of cortisol, testosterone, and androstenedione have been carried out during the clinical trial cycles. The data have been completed except for the pending 176 cycles, and statistical analysis will be undertaken shortly.

Glucose tolerance tests have been performed as scheduled in the clinical trial patients. Since the final cycles, when both estrogen and progesterin are being taken, are most important and, since these last cycles comprise the fewest number, this statistical analysis will be undertaken only when essentially all the test cycles have been completed. In the meantime, relevant studies are in progress from two other research grants: One in baboons and beagles who have received the ethynyl estrogens in various doses, and another in baboons who have received an atherogenic diet, and either ethynylestradiol, stilbestrol, or premarin. Glucose tolerance and lipoprotein studies have been performed in both of these experiments, and their results are currently undergoing statistical analysis. The insights from these studies will be of great value in carrying out the multivariate analysis on the carbohydrate and lipoprotein changes in the present program.

Finally, basic studies on adrenal-ovarian interaction originally initiated in rats, have led to the development of a new class of potentially contraceptive steroids, and an entirely new potential method of antiovulatory action. In the course of these studies, it was found that synthetic corticosteroids inhibited ovulation in the FMS-primed immature rat, while natural corticosteroids did not. Similar studies were carried out in baboons, and it was shown that one or two injections of triamcinolone
acetophenide given at the beginning of the cycle, consistently inhibited ovulation in the baboon. This could be reproduced for periods up to a year or more. Lately, patients scheduled to receive corticosteroids for various therapeutic indications have been fitted into this program, and a similar finding has been observed in human female subjects. Ongoing studies of plasma estrogen, progesterone, and gonadotropins indicate that a novel mechanism of ovulation inhibition occurs in such individuals.
Final Research Report

The general background, project objectives, and relevance of the objectives remain as stated in the original contract proposal. Of particular relevance are the clinical studies, which will provide an insight into ethynyl estrogen metabolism, the relative potency of the two ethynyl estrogens, the demonstrated synergism between estrogens and progestins, the nature and time course of plasma steroid, glucose tolerance and lipoprotein changes. Of particular relevance is the discovery of a new class of synthetic steroids which may provide a novel approach to contraception.

D. ACCOMPLISHMENTS TO DATE

I. Metabolism of synthetic estrogens.

This aspect of the studies, using ethynylestradiol and mestranol with radioactive label synthesized in our laboratories, have been ongoing for three years. Attention is directed to the list of publications (Page 26), wherein a number of reports dealing with this aspect are included. In particular, our metabolic studies have been reported at the Symposium at Sukhumi in the USSR in December 1971, and at the Symposium on Pharmacological Models of WHO at Geneva in 1972 (Appendix A). Further data will be presented at the Endocrine Society meeting in June 1974 and at the International Congress on Steroids in Mexico City in September of 1974. Abstracts of these papers are included in Appendix B.

Methodological aspects have been discussed in some length in previous reports, and these data will not be reiterated. The salient features of this work are:

a). A consistent pattern of urinary conjugates of ethynylestradiol
has been established. The majority of the urinary excretory products are isolated in a single peak on the special chromatographic system which we have developed. These appear to be glucuronide conjugates, primarily the 3-glucosiduronate of ethynylestradiol itself. Some 17-glucosiduronate also appears to be present. The relative amounts of these two conjugates have not been established, and the conjugation form of the subsidiary metabolites of ethynylestradiol has not been elucidated as yet. The subsequent peaks in the conjugate chromatogram indicate the presence of a small amount of sulfates, as well as some diconjugates which are currently under investigation. The feature of importance is the relative consistency of this pattern, and the fact that hepatic function in terms of steroid conjugation can be studied in part by these findings, and in part also by the pattern of free compounds.

b). The free compounds, primarily derived from the glucuronides, consist of ethynylestradiol (the major metabolite) and oxygenated derivatives—chiefly 2-hydroxy and 2-methoxy ethynylestradiol, 6-hydroxy and possibly 6-keto ethynylestradiol. Other minor metabolites have not been investigated. The above compounds have been isolated and definitively identified either by recrystallization to constant specific activity or by mass spectrometry. It is also of considerable interest that removal of the ethynyl group also seems to occur in vivo, with the result that estrone and estradiol appear in the urine. Proof that this is not a technical artifact has been obtained. These data will be ready in time for the various presentations mentioned in Appendix B.

The pattern of conjugates (glucuronides and sulfates) and the nature of the hydroxylated derivatives of the free steroids give an overview of
the manner in which liver function metabolizes this compound. This will provide a useful parameter for future projected studies in which the effect of ethnic background, systemic diseases, nutritional factors, etc. will be studied in populations of the developing world.

In studies of these estrogenic compounds, as well as of other compounds used for contraceptive purposes, a pharmacological model is necessary for those studies which cannot be performed in man. Concurrent with our clinical studies, extensive investigations have been undertaken in the baboon, and these have been detailed in the Sukhumi and Geneva Symposia (See Appendix A). These studies indicate that, in many respects, the sub-human primate serves as a useful model for the necessary pharmacological investigations. Moreover, data have been accumulated from various other sources indicating that certain commonly used test animals such as the beagle dog and very possibly the rat, do not serve as satisfactory models for many aspects of the study of these compounds. Future development of contraceptive hormones will have to take these findings into account.

Our early studies on plasma levels of contraceptive estrogens were summarized in the Sukhumi Symposium (See Appendix A). Work on plasma constituents was stopped at this stage, since it was felt that lengthy technological development was required for successful pursuit of this work. Accordingly, the chromatographic separation techniques used for urine were developed. Lately, attention was devoted to developing a radioimmunoassay for ethynylestradiol and certain of its possible metabolites, as well as for mestranol. Some of the necessary steroids and antigenic materials have been synthesized by Dr. P. N. Rao (see list of publications). At the present time, a radioimmunoassay procedure.
specific for ethynylestradiol or mestranol has been developed, and the final stages of testing are ongoing. Reports of the methodological developments will be presented in Mexico City in September 1974 (See Appendix B).

II. Clinical Study.

This study was intended as a comparative dose-response evaluation of ethynylestradiol and mestranol, and a study of the effect of the superimposition of norethindrone, norgestrel, or megestrol. Each of these regimens was to be continued for six cycles. While random assignment of drug treatments was anticipated, it appeared early on that the contraceptive effect of ethynylestradiol or mestranol at 50 mcg/day was insufficient, and that individuals protected with an IUD would have to be used during this aspect of the study. Consequently, randomization was impossible, but in the other doses it was followed as well as possible. Medications of uniform bioavailability were manufactured by Wyeth Laboratories, who also provided the clinical data assessment forms for this study. A very substantial dropout rate occurred in the course of the study, necessitating a continuing enrollment and a prolongation of the study. By agreement with AID, this study will actually be completed in the course of the new contract number CM/pha-C-73-32. The status report as of the end of February 1974 is as follows:

There have been 219 patients enrolled in the study; no further enrollment is anticipated. Active patients at the present time: 30. Total number of cycles to date: 1,952. The status of patient progress and completed cycles is given in Table 1.
Clinical evaluations are performed at monthly intervals, and data sheets are sent at regular intervals to Wyeth Laboratories, where they will eventually be processed according to the computer program they have developed for analysis of this clinical data format. It is anticipated that analysis will get underway in April, since the uncompleted cycles at that time will form only a very small body of information, and since most of the estrogen-alone cycles will have been completed by then. While these studies are not intended to provide information regarding clinically useful regimens, they will provide an insight into the adverse effects expected by estrogen-alone cycles, and the reversibility of these effects when progestational compounds are added to the regimen. Primarily, however, these cycles were intended to provide laboratory parameters to study the effects of the medications, singly and in combination.
III. Histological Studies.

a). Endometrium.

A comparison of the two estrogens, at different dose levels, was undertaken with the endometrium of the second treatment cycle as the target tissue. A substantial number of endometrial biopsies was obtained, and these were interpreted in blind fashion by Dr. M. Maqueo. The data are now complete, the manuscript has been written (See Appendix C), and will shortly be submitted for publication. It is included herewith for AID approval. The findings indicate that, contrary to the data from animal studies, and contrary to some studies based on vaginal cytology, ethynyl-estradiol and mestranol are approximately equally potent. This is of considerable importance, since various claims have been made regarding potency in humans, solely on the basis of rodent experiments. Further, these studies show that 50 mcg/day of these estrogens given in cyclic fashion already reach a maximal effect, and no additional histological change is seen within two cycles when the dose is increased to 80 or 100 mcg/day. This is not to say that continued high-dose medication is not more likely to produce cystic glandular hyperplasia than low doses. The study was intended to look at normal histological development, not the appearance of pathological changes. We believe there is no other comparative study of this kind in the literature. The manuscript is submitted as Appendix C.

b). Capillary Basement Membrane Study.

In view of the fact that the carbohydrate metabolism changes associated with the use of oral contraceptives have been discussed in the light of the complications of diabetes mellitus, it was decided to undertake
an examination of the capillary basement membrane as a marker of the genetic diabetic predisposition, to study the correlation of this factor with the appearance and degree of carbohydrate alterations on glucose tolerance tests. Normal pregnant women, diabetic women, women on oral contraceptives who develop no change in carbohydrate tolerance, and those who did develop some change in carbohydrate tolerance were included among those who gave permission for a muscle biopsy. Approximately 80 such biopsies were obtained. The clinical data obtained in these patients is shown in Table 2, and these data are already on punch cards for computer analysis. The tissue was fixed for electron microscopy, sectioned and photographs prepared by Dr. Villegas. Analysis of the photographs of 30 cases have been completed, and these photographs were submitted to Dr. Siperstein of the University of California, San Francisco, an authority in the field of capillary basement membrane research. An excellent correlation between the measurements of his technician and those of Dr. Villegas was found, and the remainder of the photographs are now being evaluated. When this set of measurements has been completed, we propose to hold a conference between Dr. Siperstein, Dr. Leonard Madison, a prominent diabetologist, our computer expert Mr. Tazewell Dozier, and the Principal Investigator, in order to design the multivariate analysis which will be necessary to correlate the various clinical and laboratory features with the histological measurements.

IV. Antiovulatory Studies.

Plasma progestin determinations by radioimmunoassay have been carried out in as many of the cycles of our clinical trial subjects as possible. In addition, large numbers of samples from clinical trials with other proposed commercial formulations have been submitted for plasma progestin assay. These data are shown in Table 3.
<table>
<thead>
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<th>Data Field No.</th>
<th>Card Col. (s)</th>
<th>Description</th>
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<td>Register No.</td>
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<td>6 - 7</td>
<td>Biopsy No.</td>
</tr>
<tr>
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<td>8</td>
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<td>4</td>
<td>9</td>
<td>Medication</td>
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<tr>
<td>5</td>
<td>10 - 12</td>
<td>No. of cycles</td>
</tr>
<tr>
<td>6</td>
<td>13 - 14</td>
<td>Day of cycle</td>
</tr>
<tr>
<td>7</td>
<td>15 - 16</td>
<td>Age</td>
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<tr>
<td>8</td>
<td>17 - 19</td>
<td>Height (cms.)</td>
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<tr>
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<td>20 - 21</td>
<td>Weight (Kgs.)</td>
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<td>27</td>
<td>No. of provoked abortions</td>
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<td>2 Hrs. Post-Prandial I (Pre-Biopsy)</td>
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<td>Fasting glucose II (Pre-Biopsy)</td>
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<td>24</td>
<td>38</td>
<td>2 Hrs. Post-Prandial II (Pre-Biopsy)</td>
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<td>39</td>
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<td>Glucose tolerance curve</td>
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<td>History of glucose tests</td>
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<td>DRUG AND DOSE (MG/DAY) FOR 21-DAY CYCLES</td>
<td>NO. CYCLES WITH PLASMA PROGESTIN VALUE (NG/ML) IN A GIVEN RANGE:</td>
<td>TOTAL NO. OF CYCLES</td>
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<td>CONTROL CYCLES</td>
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<td>2. IUD USERS</td>
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<tr>
<td>MESTRANOL</td>
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</tr>
<tr>
<td>A. .05 MG/DAY</td>
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</tr>
<tr>
<td>B. .08</td>
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<td>C. .10</td>
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<tr>
<td>ETHYNYLESTRADIOL (EE)</td>
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<td>D. .05 MG/DAY</td>
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<tr>
<td>E. WITH DIMETHISTERONE (SEQ.) .05 + .25</td>
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<tr>
<td>F. .08</td>
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<td>EXPERIMENTAL COMBINED E + P PREPARATIONS</td>
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<td>G. VARIOUS</td>
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<td>H. .03/0.3</td>
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<tr>
<td>EE + NORGESTREL</td>
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<td>I. .02/0.4</td>
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<tr>
<td>J. .02/1.0</td>
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<tr>
<td>L. .03/0.6</td>
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<td>M. .03/1.5</td>
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<td>N. .04/2.0</td>
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<td>MESTRANOL + CHLORMADINONE ACETATE</td>
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<tr>
<td>P. .10/1.0</td>
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</table>

* Recalculation of series J, with one subset including 14 ovulations in 138 cycles omitted.
From these findings, several important conclusions emerge. Again, contrary to the studies from animal data, ethynylestradiol and mestranol seem to be approximately equal in potency, with mestranol possibly being the more potent substance. Further, the addition of a progestational substance greatly enhances the antiovulatory efficacy, and there appears to be a true synergism between the estrogens and progestins, since as little as 20 mcg of the ethynyl estrogens combined with submilligram quantities of various progestational agents yield an antiovulatory effectiveness comparable to that of much larger doses of estrogen alone. These findings are of course consistent with clinical trials on these low dose formulations, but they supply for the first time an insight into the mechanism of action. A manuscript (Appendix D) is in preparation.

Our studies of FSH and LH levels on long-continued therapy have been continued, and large numbers of samples have been accumulated from the patient material in Mexico City, with the collaboration of Dr. Rice-Wray. These data confirm our previous published studies on the effect of sequentials, combinations, and injectables on the plasma FSH and LH pattern, and extend these findings over a much larger material. The data are displayed in the following figures.
LEVELS OF PLASMA FSH by menstrual cycle day in fertile women using an IUD
LEVELS OF PLASMA LH by menstrual cycle day
in fertile women using an IUD
LEVELS OF PLASMA LH in fertile women using 0.05 mg ethynylestradiol or 0.06 - 0.075 mg mestranol and 0.5 mg norgestrol or 2.5 mg lynestrenol or 4 mg norethindrone acetate or 10 mg norethindrone for 5 to 12 yrs. by menstrual cycle day.

Eugynon & Ovral
Ortho - Novum 10 mg
Anovlar
Lindiol
LEVELS OF PLASMA FSH in fertile women using .05 mg ethynylestradiol or 0.06 - 0.075 mg mestranol and 0.5 mg norgestrel or 2.5 mg lynestrenol or 4 mg norethindrone acetate or 10 mg norethindrone for 5 to 12 yrs. by menstrual cycle day.
LEVELS OF PLASMA FSH in fertile women using 0.1 mg mestranol & 1.0 mg ethynodiol diacetate for 5 to 10 yrs. by menstrual cycle day.
LEVELS OF PLASMA LH in fertile women using 0.1 mg mestranol & 1.0 mg ethynodiol acetate for 5 to 10 yrs. by menstrual cycle day.
V. Plasma Corticosteroid Studies.

Controversy still exists as to whether the elevated total plasma cortisol observed in subjects on oral contraceptives really represents an increase in unbound (biologically active) circulating cortisol. Subjects in the clinical study have been repeatedly sampled for plasma steroids, cortisol determined by radioimmunoassay, and the percentage of free and protein bound cortisol determined by equilibrium dialysis of isotopically labelled material. Mathematical analysis of these data has been withheld, because of the relatively small number of cycles on the estrogen-progestin combinations. However, statistical analysis of the estrogen-only cycles is scheduled in the immediate future.

VI. Plasma Androgens.

In analogy with plasma cortisol, changes in plasma androgens have been described as a consequence of suppressed ovarian function and suppressed adrenal androgen synthesis by oral contraceptive formulations. The relative role of estrogen and progestin have not been elucidated. We have developed relatively specific radioimmunoassays for plasma testosterone and plasma androstenedione, and these have been serially measured in the clinical trial subjects, together with equilibrium dialysis studies, which will permit computation of bound and unbound levels. Statistical analysis of these parameters is planned for the immediate future.

VII. Glucose Tolerance Tests.

Glucose tolerance tests have been performed in the clinical trial subjects, as described in the original protocol. The vast majority of
these planned studies were carried out as scheduled. However, the inherent limitations of clinical trials have made dietary control impossible, which probably introduces an increased variability into glucose tolerance test results.

Glucose tolerance tests are of course influenced by a variety of factors such as change in body weight, age, genetic variables, and so forth. Simplistic analysis, such as has been published in previous studies of combination or injectable contraceptives serves little purpose in analyzing these data. It is contemplated to discuss the analysis of these variables with expert diabetologists, including Drs. Leonard Madison of Dallas and Dr. Siperstein of the University of California, San Francisco. The necessary information from the clinical records, together with data on plasma cortisol, and other significant variables will be stored on tape, and the appropriate form of statistical analysis will be undertaken. Relevant animal experiments which are being carried out at Southwest Foundation at the present time with respect to the effect of estrogen on carbohydrate metabolism in baboons and beagles will be used to determine the significant variables, since there is much greater control in these animal studies.

VIII. Lipoprotein Studies.

Ultracentrifugal analysis of plasma lipoproteins has proceeded according to schedule, but a number of important samples remain to be collected during the terminal portion of this study. The same statistical questions which apply to analysis of glucose tolerance apply even more.
significantly to analysis of lipoproteins. Here too, ongoing animal studies will be highly relevant for comparative purposes. At the present time, a study in baboons is terminating where the effects of normal or high cholesterol feeding, restriction of physical activity, and type of estrogen (natural, synthetic steroidal, non-steroidal) on glucose tolerance and serum lipoprotein patterns has been examined, and will also be investigated extensively on autopsy material which is obtained at the conclusion of the study. This experiment has been in progress over two years, and termination of the test animals is being initiated in March 1974. These studies, together with studies of the ethynyl estrogens in baboons and beagles, which will also supply data on lipoprotein fractions, will serve as models for the statistical analysis of these data. Once the analysis of the animal information has been explored and optimized, the same analytical techniques will be brought to bear on the human data, which of necessity embody a much greater variability. It is not anticipated that analysis of these data will be initiated before the end of 1974.

IX. Studies of the Adrenal-Gonadal Relationship.

These studies, which were of an entirely basic nature, and intended originally to explore the interrelationship of the adrenal and ovarian controlling mechanisms at the hypothalamo-pituitary level, have yielded results which promise to be of significant practical value. The initial studies examined the effect of ACTH administration in an animal model, the PMS-primed immature female rat. These data show that ACTH administration blocks ovulation in this species. Studies in adrenalectomized animals revealed that it was not the ACTH itself which had this action, but that
presumably some adrenal steroid was active. There ensued a series of
studies trying to identify the steroid of consequence. Attention focused
on the androgens, and in addition to papers listed in the bibliography, a
summary paper on the effect of testosterone and certain other compounds is
presented in Appendix E.

In the course of these studies, it was found that while the natural
adrenal corticosteroids had no ovulation inhibiting effect, certain synthetic
corticosteroids such as dexamethasone and triamcinolone were effective in
inhibiting ovulation in this rat model, both when given intraperitoneally
and even intrahypothalamically. On the basis of these findings, Hagino
went on to establish the relevance of this model by experiments in the
cycling female baboon. It was found that two injections, on cycle day 1
and 2, of triamcinolone acetophenide repeatedly blocked ovulation and
yielded short, anovulatory cycles (see list of publications). On the basis
of this information, it was decided to investigate the effect of this
steroid, an accepted therapeutic agent in the treatment of rheumatoid
arthritis and certain other disorders. Women who were scheduled to have
this treatment in any event, were investigated when the injections were
confined to the very early days of the menstrual cycle. In a forthcoming
paper (See Appendix A) data will be presented indicating that the findings
in the baboon are also valid in the adult human female.

As a consequence of these observations, a contract proposal has been
generated with NICHD, to try to develop synthetic steroids which possess
the antiovulatory action of these substances but which have lost the gluco-
corticoid potency of the original compounds. This contract is being undertaken
by Dr. P. N. Rao, a synthetic organic chemist, in consultation with the
Principal Investigator of the AID contract. A variety of synthetic organic chemical approaches will be undertaken, in an effort to develop a new class of steroid compounds which appear to have a different mechanism of antiovulatory activity than steroid substances heretofore described. The possibility, highly theoretical at the moment, exists that a new contraceptive modality will develop from these investigations.
PUBLICATIONS FROM STUDIES SUPPORTED BY

CONTRACT csd/2821

1. A cross-sectional study of plasma FSH and LH levels in women using sequential, combination or injectable steroid contraceptives over long periods of time.

2. The effect of contraceptive steroids on endometrial sinusoids and the failure of these changes to correlate with breakthrough bleeding or systemic vascular effects.

3. A placebo-controlled double-blind crossover investigation of the side effects attributed to oral contraceptives.


5. Separation of free and bound steroids in the competitive protein binding assay for testosterone: Accuracy and reproducibility.

6. A metabolic balance study of the effects of an oral steroid contraceptive on weight and body composition.

7. The metabolism and effects of contraceptive steroids in primates.

8. Ovarian morphology after prolonged use of steroid contraceptive agents.
9. Some perspectives in the development of population control methods.
Goldzieher, J. W.:
Lecture presented at the Benjamin Franklin Award Lecture,

10. Perspectives in population control.
Goldzieher, J. W.:
Presented at the Symposium on Statistical Problems in Population
Research sponsored by the International Association for Statistics
on The Physical Sciences and The East-West Population Institute,
Honolulu, Hawaii, Aug. 2-6, 1971.

11. Practical determination of total plasma cortisol using competitive
protein binding. Presented at The 8th International Congress on
Clinical Chemistry, Copenhagen, Denmark, June 18-23, 1972.
de la Pena, A., and Goldzieher, J. W.:
To be published: Submitted to Clinical Chemistry.

12. Problems in the experimental design of clinical trials with oral
contraceptive agents, IN: Pharmacology and The Future of Man.
Goldzieher, J. W.:
Proc. 5th Intl. Congr. Pharmacol., San Francisco, 1972,

Goldzieher, J. W.:
Presented at the South-East Asia and Oceania Regional Medical

14. Perspectives in population control.
Goldzieher, J. W.:
Presented at the New Zealand Family Planning Association,
Auckland, New Zealand, Aug. 12, 1972.

15. Non-human primates in contraceptive research, IN: Pharmacological
Models in Contraceptive Development.
Goldzieher, J. W., Joshi, S., and Kraemer, D. C.:

16. Synthesis of compounds of potential value in the radioimmunoassay
of 17a-ethynylestradiol and mestranol.
Rao, P. N.:

17. Antisera for radioimmunoassay of mestranol and ethynylestradiol.
Rao, P. N., de la Pena, A., and Goldzieher, J. W.:
(Manuscript in preparation).


