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COMPARATIVE STUDIES OF THE ETHYNYL ESTROGENS USED IN ORAL CONTRACEPTIVES
I. Endometrial response

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The ethynyl estrogens, ethynylestradiol and mestranol, are incorporated in almost all of the oral contraceptives currently used by about 50 million women the world over. Surprisingly little is known about their relative potency in man; most published estimates are based on animal assays whose parallelism to human response has not been established.

Moreover, the metabolism of many steroid compounds by rodents or primates is often quite different; the effectiveness of various routes of administration may not be the same; the bioavailability of drugs given to animals may have little similarity to that of therapeutic preparations. Uncertainties exist even within the body of published animal experiments: Depending on the assay system used, mestranol may show a potency anywhere from 5 to 50% of that of ethynylestradiol.[1]

The effects of ethynyl estrogens on <u>vaginal cytology</u> have been established in primates as well as in rodents. The vaginal cytology index developed by Stupnicki et al[2] in amenorrheic women demonstrated a dose-response relationship for mestranol over the 25-100 µg/day range. Subsequent investigations showed approximate equipotency for mestranol and ethynylestradiol over the range of 20-150 µg/day (for a 10-day administration period). Henzl et al[6], using a slightly different index, found a detectable vaginal cytological response to mestranol at 20 µg/day but not at 5 µg/day; however, the difference between 20 and 80 µg/day was not as evident as between the 5 and 20 µg dosage, suggesting a plateau effect.

Uterine withdrawal bleeding has also been used as a test parameter. Schane et al tested ethynyl estrogens at levels up to 400 µg/day in castrate rhesus monkeys (7). Although ethynylestradiol produced withdrawal bleeding at 25 µg/day whereas mestranol did not, their overall assessment was that these two compounds were equipotent. Interestingly, mestranol appeared to be more effective than ethynylestradiol in producing sex skin changes. Henzl et al demonstrated a dose-response in postmenopausal women over the range of 5-80 µg/day which appeared to be linearly related to the log of the mestranol dosage. Delforge and Ferin (8) performed a histoplanimetric study on endometrial biopsy tissue from groups of 4-6 reproductive-age women (and two elderly castrate women on replacement therapy) who had received 100 µg/day of mestranol or ethynylestradiol for 13-16 days at the time of biopsy. Glandular surface (perpendicular orientation) was 14.5±10.2 units for ethynylestradiol compared to 4.0±2.8 for mestranol and 26.1±21.9 units versus 15.0±11.2 in the parallel orientation. Average gland diameter was 135.6 versus 71.5 (ethynylestradiol versus mestranol, perpendicular orientation) and 170.3 versus 209.5 (ethynylestradiol versus mestranol, parallel orientation. On the basis of these findings, they claimed that the endometrial effect of mestranol was 50% weaker than that of ethynylestradiol.

These studies on subhuman primates and human subjects have been understandably confined to very small numbers of individuals. Regardless of the exactness of any given measurement, the precision of bioassays in general is limited by individual variability. Thus, the interpretation of the findings must be undertaken with great caution, as Henzl et al

and Schane et al clearly recognize. In order to minimize this factor at least in part, we have studied a substantial number of human endometrial biopsies, over a range of dosage, in reproductive-age women who received cyclic treatment with mestranol or ethynylestradiol alone. Furthermore, to obviate problems of bioavailability, great care was taken to have all the test dosages of the two estrogens prepared in an identical manner under careful quality control.

MATERIALS AND METHODS

Bulk mestranol was kindly provided by Syntex Laboratories, ethynylestradiol by Wyeth Laboratories. Tablets containing 50, 80 or 100 µg of mestranol and 50 or 80 µg of ethynylestradiol were prepared by Wyeth Laboratories using identical formulas for each tablet strength except for adjustment of the amount of diluent due to differences in the amount of active ingredient. The tablet formula and method of manufacture were the same as those used for a currently-marketed oral contraceptive using ethynylestradiol. Complete quality control checks were performed, including tablet-to-tablet content uniformity and dissolution, thereby assuring appropriate control of dose content and bioavailability.

The subjects who participated in this study were menstrually regular, fertile women of reproductive age who gave fully informed, written consent. None had used steroidal contraceptives previously. A numer of women using intrauterine devices for conception control also provided endometrial biopsy material which was randomly interspersed

with the biopsy slides from the women receiving cyclic estrogens. Because of the uncertainty of contraceptive effectiveness at the lowest estrogen dosages, these regimens were tested chiefly in women who also consented to use an IUD during the course of the study. For this reason, random assignment of the dosage regimens could not be carried out. Endometrial biopsies were obtained between cycle days 15-21 of the second 21-day course of estrogen administration. 93% of the 141 biopsies were taken on cycle days 16, 17 or 18. A 7-day interval elapsed between the two cycles of administration. Subjects were interrogated at the end of each cycle to ascertain the reliability of drug intake. Tissue was fixed in formalin, processed in the usual manner, stained with hematoxylin-eosin and submitted as coded slides (together with the no-drug IUD biopsies) to be read "blind" by one of us (M.M.). The histological material was categorized as proliferative or secretory. Proliferative endometria were classified as early, intermediate, or late (given numerical values of 1, 2 or 3 respectively), and as to the presence or absence of cystic glandular hyperplasia (0 or 1) and mitotic activity (0 to 3). A numerical score for each biopsy was obtained. Biopsies showing secretory changes or insufficient tissue for accurate evaluation were excluded from the study. The numerical scores of the biopsies were tabulated by drug and by dose and analyzed by the nonparametric G-test (9). (The G-test and the more commonly used χ^2 test yield approximately the same results, but the G-test has certain general theoretical advantages over χ^2; moreover the property of additivity of the G-test is an advantage in the present context).

RESULTS

The endometrial "score", an index of the estrogenic response during cycle days 16-18 of the second cycle of exposure, ranged from a minimum value of 2 to a maximum of 6. The frequency distribution of these scores, by drug and by dose, is given in Table 1 for a total of 121 biopsies. The G-test was applied to these data and again after the data were compressed into 3 categories (those with scores of 3 or less, 4, or 5 or more). The G-value of 2.750 (8 degrees of freedom) gave a probability of $0.5>P>0.2$, indicating that the null hypothesis (no significant difference between the various drug-dose groups) could not be rejected. Finally, between-drug and between-dose-level comparisons were performed. The statistical evaluation is shown in Table 2. No histological differences could be demonstrated between different dose levels of the same drug (i.e. 50 versus 80 versus 100 µg of mestranol or 50 versus 80 µg of ethynylestradiol). No differences could be demonstrated between equal doses of the two estrogens (50 µg mestranol versus 50 µg ethynylestradiol or 80 µg mestranol versus 80 µg ethynylestradiol).

DISCUSSION

In assessing the effects of estrogen on the endometrium, it is most important to keep the conditions of the experiment in mind and not generalize to other situations. The response of the reproductive-age endometrium may not be the same as that of the castrate or post-menopausal individual. Previous hormonal exposure, endogenous or

exogenous, is known to affect tissue responsiveness[10], and may introduce an important variable. The response of vaginal epithelium to a given estrogen level is known to plateau after 7-10 days, but comparable information for the endometrium is not well documented, nor is the influence of repeated treatment cycles at the same dose level.

The present study indicates that, under the given experimental conditions (which were intended to simulate contraceptive steroid regimens), a level of 50 µg/day of either mestranol or ethynylestradiol has reached the endometrial response plateau, and that the tissue is relatively insensitive to further dosage increments in 2-cycle exposures. In other words, a dose-response relationship, and differences in the relative endometrial potency of mestranol and ethynylestradiol can only be established at doses below 50 µg/day. Other treatment regimens might, of course, display different response characteristics. (Vaginal epithelium, in a single 10-day exposure, has already been shown to have a different dose-response). Continuous (as contrasted to cyclic) treatment is known to promote the development of cystic glandular hyperplasia; the relative potency of the two ethynyl estrogens in inducing such changes cannot be predicted from the present findings. In this regard, the relative importance of duration of exposure versus "estrogenic potency" has not been adequately explored.

The use of an IUD to protect the subjects taking 50 µg estrogen per day introduces a new variable in this group. Extensive studies of the effect of IUDs on endometrial morphology[11,12] indicate that the maturation of the <u>secretory</u> endometrium is delayed, and that there

is an increased tendency toward predecidualization. There are in addition, endometritis and stromal fibrosis in the immediate vicinity of the device. None of these changes would have affected the assessment of estrogenic effects, and in any event would have tended to reduce the hormonal response, a finding which was not observed.

The "antiestrogenic" activity (on the endometrium) of progestational steroids used in combination-type oral contraceptives influences the histological expression of the ethynyl estrogen. All the dose levels of progestational steroids in marketed preparations are sufficient to suppress completely estrogen-induced endometrial growth and to produce a characteristic involuted endometrium. A detailed study of the interaction of these two hormonal responses would require different doses and proportions than have been examined hitherto. The findings of the present study are at variance with the planimetric measurements of Delforge and Ferin. Only one dose level of each estrogen, made up of material of unspecified origin and unknown bioavailability, was examined. Moreover, the tedium of the histoplanimetric technique severely limited the sample size. Large standard deviations were observed, as might be expected, and this suggests caution in interpretation of the results. Taken all together, the results of primate studies indicate an equipotence of mestranol and ethynylestradiol on the endometrium, or possibly some modest increase in potency of the latter over the former. The differences do not appear to be as great as indicated in certain experiments with laboratory rodents. Once again, this emphasizes the danger of uncritical extrapolation of laboratory-animal results into the clinical area.

The factor of bioavailability of the test drugs has been emphasized in this presentation. Its critical importance in the pharmacology of orally-ingested preparations is common knowledge, but it has been almost totally ignored both in pharmacological studies and in clinical trials of contraceptive steroids. It becomes particularly relevant when the active drug is a relatively insoluble material, used in microgram amounts. Significant variations in contraceptive effectiveness, almost surely due to differences in manufacture and quality control, have been observed in clinical trials of oral contraceptives over the years (13). For methodological reasons, it has been difficult to assess this factor in the past, but radioimmunoassay methods now make such investigations feasible. Future studies of the effects of ethynyl estrogens (and other contraceptive steroids) may be far more meaningful if this factor receives appropriate attention.

SUMMARY OR ABSTRACT

Reproductive-age women were given identically-prepared mestranol or ethynylestradiol orally for two consecutive 21-day cycles in doses ranging from 50 to 100 micrograms per day. Endometrial biopsies were obtained at the end of the second cycle and assessed for estrogenic effect. At these dose levels and with this treatment regimen, no differences could be detected between doses or between drugs, indicating that a plateau in endometrial response was reached.

TABLE I

COMPOUND & DOSE	NUMBER OF PATIENTS IN CLASSES 2-6, BY TREATMENT: 2	3	4	5	6	Total # Pts.
Mestranol, 50 µg/day	-	5	9	8	1	23
Ethynylestradiol, 50 µg/day	-	2	8	4	2	16
Mestranol, 80 µg/day	2	4	10	7	-	23
Ethynylestradiol, 80 µg/day	1	8	11	8	1	29
Mestranol, 100 µg/day	3	4	14	7	2	30
	6	23	52	34	6	

TABLE II

STATISTICAL ANALYSIS OF SUBSETS OF THE DATA

COMPARISON	G-VALUE	SIGNIFICANCE
Mestranol 50 µg vs Ethynylestradiol 50 µg	0.72796	0.7>P>0.5
Mestranol 80 µg vs Ethynylestradiol 80 µg	0.20850	0.95>P>0.9
Mestranol 50 µg vs 80 µg	0.39434	0.9>P>0.8
Mestranol 80 µg vs 100 µg	0.07020	0.98>P>0.95
Mestranol 50 µg vs 100 µg	0.50334	0.8>P>0.7
Ethynylestradiol 50 µg vs 80 µg	2.08828	0.5>P>0.3

REFERENCES

1). Edgren, R.A.: In Lednicer, D., editor: Contraception; the chemical control of fertility, New York, 1969, M. Dekker, p.46.

2). Stupnicki, R. and Teter, J.: J. Reprod. Fertil. 22:563, 1970.

3). Teter, J. and Krzywdzinski, K.: Rev. Fr. Endocrinol. Clin. 12:9, 1971.

4). Teter, J. and Stupnicki, R.: Acta Cytol. (Baltimore) 15:167, 1971.

5). Teter, J.: Acta Cytol. (Baltimore) 16:366, 1972.

6). Henzl, M.R., Moyer, D.L., Townsend, D., Valand, R.S., and Segre, E.J.: Am. J. Obstet. Cynecol. 115401, 1973.

7). Schane, H.P., Anzalone, A.J., and Potts, G.O.: Fertil. Steril. 23:745, 1972.

8). Delforge, J.P. and Ferin, J.: Contraception 1:57, 1970.

9). Sokal, R.R., and Rohlf, F.J.: Biometry; the principles and practice of statistics in biological research, San Francisco, 1969, W.H. Freeman, p. 599.

10). Phelps, D.: J. Clin. Endocrinol. Metab. 7:611, 1947.

11). Wynn, R.M.: Fine structural effects of intrauterine contraceptives on the human endometrium. Fertil. Steril. 19:867, 1968.

12). Nicolaisen, H.H., Pedersen, H., Guttorm, E., and Rebbe, H.: Postovulatory endometrial development in women with IUD. Acta Obstet. Bynec. Scand. 52:253, 1973.

13). Goldzieher, J.W.: Unpublished studies.

COMPARATIVE STUDIES OF THE ETHYNYL ESTROGENS USED IN ORAL CONTRACEPTIVES
II. ANTIOVULATORY POTENCY

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The anti-ovulatory effect of oral contraceptives is considered to be their major mechanism of action. This property of both progestational and estrogenic substances has been known for about 50 years; since 1959 the investigations of Rudel, Goldzieher and their associates ( ) have established the disproportionately high pituitary-inhibiting potency of the ethynyl estrogens as compared to natural compounds such as estradiol, estriol, conjugated estrogens, and also to nonsteroidal synthetics such as stilbestrol (Table 1). Previous studies, using urinary pregnanediol excretion as an index of ovulation, demonstrated that a dose-level of about 80 mcg/day of ethynyl estrogen, by itself, was required for acceptable antiovulatory efficacy. The sample size at the .05 mg/day level was too small for a meaningful evaluation of its efficacy (Table 2). Although there are published opinions regarding the required dose, no actual study has been performed, nor has there been any valid clinical comparison of the relative effectiveness of the two ethynyl estrogens, ethynylestradiol and mestranol. Animal experiments have suggested that the former of these compounds is more potent, but studies on rodents ( ) are often not comparable to effects in man. It appears hazardous to extrapolate the animal data to clinical usage, as is commonly done. In order to examine this problem further, we undertook a comparative study of the antiovulatory activity of the two ethynyl estrogens, with and without concomitant synthetic progestational steroids.

MATERIALS AND METHODS

For the comparative dose-response studies of ethynylestradiol and mestranol, bulk steroids were obtained and tablets containing .05, .08 or

0.1 mg of mestranol and .05 or .08 mg of ethynylestradiol were prepared by Wyeth Laboratories, using identical formulas for each tablet strength except for adjustment of the amount of diluent due to differences in the amount of active ingredient. The tablet formula and method of manufacture were the same as those used for a currently-marketed contraceptive containing ethynylestradiol. Complete quality control checks were performed, including tablet-to-tablet content uniformity and dissolution, thereby assuring appropriate control of dose content and bioavailability. In a similar manner, tablets containing the same doses of these estrogens, combined with various amounts of norethindrone, dl-norgestrel, or megestrol were prepared. Commercial clinical-trial material of formulations of ethynylestradiol with norgestrel, ethynylestradiol with norethindrone acetate, mestranol with chlormadinone acetate, and a sequential preparation of ethynylestradiol and dimethisterone were made available by Mead, Johnson and Co., Parke, Davis and Company, Syntex Laboratories and Wyeth Laboratories. The clinical samples derive primarily from studies carried out at our facility. In addition, some blood samples from collaborative clinical trials with the ethynylestradiol-norethindrone acetate combinations were provided by other investigators. Since these investigations extended over a considerable period of time, no randomized drug assignment was possible. Moreover, in subjects studied at the .05 mg/day level of ethynyl estrogen alone, supplemental contraceptive protection was provided by the use of an IUD. These individuals were a select group willing to use two contraceptive modalities, and are therefore not homogeneous with the other groups receiving higher doses of mestranol or ethynylestradiol alone. The estrogen-alone regimens (groups A-F, Table 3) consisted of 21-day cycles of tablets with a 7-day rest

period, for a maxiumum of 6 cycles. The relatively poor cycle control produced a significant dropout rate. Those who completed the 6 cycles continued on into combination-type regimens (series G), maintaining the same type and dose of estrogen for an additional maximum of 6 cycles. The commercial preparations (E,H-O were also in the form of 21-day drug cycles.

The women who participated in the ethynyl estrogen dose-response studies were menstrually regular, fertile women of reproductive age who had not used steroidal contraceptives previously. In the other study groups previous contraceptive use (with a rest period prior to the initiation of this study) was permitted. Fully informed, written consent was secured in all instances.

Plasma samples were obtained during the last 7 days of contraceptive agent intake. The progestin level was measured by a competitive protein binding method ( ) without chromatographic purification. The procedure yields somewhat higher values than a specific assay for progesterone. In a very large experience we have found that anovulatory cycles or the pre-ovulatory phase of normal cycles yield plasma progestin values of less than 1ng/ml. We consider a value of 1 to 2 ng/ml "possibly ovulatory" and values of 2 ng/ml or more a positive indication of progesterone production by a corpus luteum and therefore operational evidence for ovulation.

Every effort was made to insure reliable medication intake by intensive, monthly monitoring at our clinical facility. However, undisclosed errors can and probably do exist both in our studies and in the material obtained at collaborating institutions.

For the purposes of the study, "possibly ovulatory" and definitely

ovulatory cycles were lumped together, and the overall percent of presumably ovulatory cycles was calculated. Next, for any two groups that were to be compared, a calculation was performed to see what sample size would be required to demonstrate a difference with a 90% certainty of detecting differences (if they exist) at the P=.05 level. If the actual sample size exceeded this figure, a t-test showing statistical significance could be accepted with some confidence. If the sample size was less than the minimum calculat requirement, then the significance of t-test calculations becomes questionable. On the assumption that the data for any particular regimen represents a random sample of a larger universe, a 95% confidence limit was calculated for the percent of ovulations in that particular regimen and sample. Tests for significance at the P=.05 and P=.01 levels were carried out by means of the formula $ts = \frac{\text{arc sin } (P1)^{\frac{1}{2}} - \text{arc sin } (P2)^{\frac{1}{2}}}{(820.6(1/N1+1/N2)^{\frac{1}{2}}}$ where P= the percentage of ovulatio decimal form) and N= sample size.

RESULTS

Two control series of fertile, reproductive age women using different contraceptive modalities are shown for comparison purposes (Table 3). The frequency of ovulatory cycles is within the accepted range for this population; the difference reflects differences in age distribution of the two groups, and probably inherent population differences as well; these are known to exist between pill choosers and IUD choosers.

The three dose levels of mestranol (.05, .08 and 0.1 mg/day) reveal a progressive increase in effectiveness of ovulation inhibition (15.4, 5.7 and 1.1% ovulatory cycles). The differences between the groups are significant at the P=.01 level, but a larger sample size (200 in each group)

would be desirable for the comparison of the .05 and .08 mg/day levels.

The difference between the two dose levels of ethynylestradiol (.05 and .08 mg/day) is also significant at the P=.01 level; the sample size is adequate. The difference between the two .05 mg/day ethynylestradiol formulations (series D and E) is also significant at P = .01. It may represent a difference in bioavailability in the two formulations, an effect of the dimethisterone, or some unknown factor.

The comparison of mestranol and ethynylestradiol on a dose-for-dose basis is the first of its kind. At the .05 mg/day level, the ovulation frequency for ethynylestradiol is 25.2%, that for mestranol only 15.4%. However, the difference is not statistically significant at the P=.05 level. A sample size of 350 in each group would be required for an evaluation that meets our specifications. There is much less difference between the two steroids at the .08 mg/day level - 5.7% for mestranol, 4.3% for ethynylestradiol. It is not significant at the P=.05 level. A sample size of 5400 in each group would be required to test this small difference adequately.

Thus, these results do not confirm animal studies which attribute a significantly higher potency (2 to 5-fold) to ethynylestradiol. In fact, they suggest that further experiments at .05 mg/day and lower dose levels might demonstrate a higher antiovulatory potency for mestranol.

The various combined preparations yielded ovulation frequencies which cluster around 4% or around the region of less than 1%. In the group of ethynylestradiol-norethindrone acetate formulations there is no statistically significant difference between the ovulation frequencies of 10.2% (the .02/0.4 formulation), 4.2% (the .02/1.0 formulation), 2.4% (.03/0.6) and 2.2% (.04/2.0). The small sample size in the .02/0.4 series produces a very large uncertainty (±7.7) in the observed value of 10.2%. Series J (.02/1.0) included groups

of samples received from different sources over a period of time. One subset of 138 samples included 14 of the 15 values in the ovulatory range. The odds are more than 100:1 against this having occurred simply by chance, suggesting that unadmitted irregularities in drug intake were a factor. Excluding this subset yielded a value of 0.8% for the ovulatory frequency associated with this formulation, a result almost identical with that found for the .03/1.5 formulation (series M) or the norgestrel group (series H). The difference between the .03/1.5 (series M) and the .04/2.0 (series N) dose levels was significant at P=.05, but groups of 1125 samples each would be required for a satisfactory test of the difference. The small sample size in the mestranol-chlormadinone acetate formulation also prevents an adequate comparison. The practical difficulties in trying to obtain meaningful evaluations at these high antiovulatory efficiencies are enormous: It would require 2 groups of at least 904 samples each for a reliable comparison of series L with M, 1509 each for J versus N, and 2009 for J versus L. For this reason also, it would be impossible to detect any trend in antiovulatory efficiency in these various norethindrone acetate formulations, should one exist. In practical terms, all the values are in the range associated with clinically satisfactory contraceptive effectiveness.

DISCUSSION

These studies provide the first clinical analysis of the antiovulatory dose-response and relative potencies of ethynylestradiol and mestranol under conditions of standardized bioavailability. A distinct improvement in ovulation inhibition was seen as the dose of mestranol was increased from .05 to .08 to 0.1 mg/day, and the same was observed for ethynylestradiol

from .05 to .08 mg/day. In a dose-for-dose comparison of the two estrogens, mestranol appeared to be more effective than ethynylestradiol at the .05 mg/day level, but the sample sizes did not provide a 90% certainty of seeing a difference, and the observed, large difference was not statistically significant at P=.05. At .08 mg/day, the effectiveness of the two steroids appeared to be identical. These results are strikingly different from potency estimates derived from animal studies; furthermore, they contradict the indirect estimates made by Dickey ( ) and others with respect to their relative effectiveness in man. The numerical difficulties in measuring a difference in potency at .08 mg/day appear to be prohibitive, but studies at .05 mg/day or lower (with adequate supplemental contraceptive protection) might be desirable to explore further the question of the relative potency of these estrogens in the lower dose range. The data suggesting that mestranol is more potent than ethynylestradiol are compatible with the observation that the action of mestranol is prolonged due to the necessity of hydrolysing it to the biologically active form, ethynylestradiol. Studies of the metabolic clearance rates ( ) of the two compounds are not helpful, as they do not measure the biologically active form of mestranol. However, comparative studies of plasma levels of ethynylestradiol after oral administration of both parent compounds are now feasible, and should yield the necessary pharmacodynamic insight.

No significant difference is demonstrable between the antiovulatory effectiveness of the estrogens alone at .08 or 0.1 mg/day and that of combination agents which yielded antiovulatory rates of 2-4%. Very large samples are required for such analyses of small differences, even under optimal conditions, but when the possibility of patient error as a cause

of elevated progestin levels enters the picture, the numbers required to demonstrate differences in drug effectiveness become astronomical. Calculations we have made for comparisons of pregnancy rates ( ) apply also in the present context. In any event, it is clear from the data that very small amounts of ethynyl estrogens, in combination with relatively small amounts of progestins, have as much antiovulatory activity as far larger quantities of ethynyl estrogens by themselves. It is well known that "microdoses" of progestins, even if given continuously, have relatively little antiovulatory activity. Therefore a synergism between the two types of steroid must exist at the hypothalamo-pituitary level. Extensive clinical trials with these low-dose combinations have demonstrated their contraceptive effectiveness; our data provide an insight into the major mechanism of action. Qualitative differences in the effect of estrogens and progestins on the gonadotropic mechanism have been described previously ( ), and studies carried out in conjunction with the present experiments will be reported separately ( ).

These studies serve to point up some of the problems encountered in apparently simple clinical pharmacological investigations. The problem of bioavailability of the drugs under test has been alluded to previously ( ), and may well be crucial in studies of orally ingested steroids. Neglect of this factor may cause substantial errors in potency estimates. Another factor which has a major influence on the validity of the results is the degree of patient reliability in taking medication as prescribed. Human unreliability in this regard is well documented, and although clever medication intake monitors have been described ( ), it has not been possible so far to convince the sponsors of such research that the perfection and use of

a monitoring device would represent a major step forward in clinical pharmacology. Finally, it should be pointed out that the simplistic use of t-tests and similar procedures may be quite misleading. It must be demonstrated, before tests of significance are applied, that the sample size is sufficient to insure that a difference will be detected, with a specified degree of assurance, if it exists. Such calculations may also demonstrate that the testability of certain differences may be impractical, because of the time or resources required to acumulate the necessary number of measurements.

ANTIOVULATORY ACTIVITY OF VARIOUS ORAL ESTROGENS

BY URINARY PREGNANDIOL ASSAY

Compound	Dose mg/day	Number of Cases	Number of Cycles	Percent Ovulatory
Ethynyl estradiol	.02	10	20	10
" "	.05	20	44	2.3
Mestranol	.02	10	20	10
"	.08	18	60	1.7
Estradiol	1.0	4	11	45
"	2.0	10	18	39
"	5.0	10	24	13
Estriol	5.0	5	7	86
Premarin	1.25	10	18	67
"	3.75	15	17	5.9
Stilbestrol	5.0	6	12	8.3

ANTIOVULATORY ACTIVITY OF VARIOUS ORAL ESTROGENS

BY URINARY PREGNANDIOL ASSAY

Drug and Dose (mg/day)	No. Cycles	No. with Elevated Pregnandiol Values	% Cycles Considered Ovulatory ± 95% C. L.
Ethynyl estradiol .02 x 20 days	12	3 (4 pregnancies in 68 cycles)	25
Ethynyl estradiol .05 x 20 days	59	2	3.4 ± 4.6
Mestranol - Chlormadinone acetate (.08 + 2) sequential	398	14	3.5 ± 1.8
Mestranol - Lynestrenol (.075 + 2.5)	170	10	5.9 ± 3.5
Mestranol - Norethindrone (.06 + 10)	88	6	6.8 ± 5.3

TABLE 3

	DRUG AND DOSE (MG/DAY) FOR 21-DAY CYCLES	NO. CYCLES WITH PLASMA PROGESTIN VALUE (NG/ML) IN A GIVEN RANGE: < 1	1 - < 2	≥ 2	TOTAL NO. OF CYCLES	PERCENT CYCLES CONSIDERED OVULATORY (PLASMA P ≥ 1 NG/ML), ± 95% C.L
	CONTROL CYCLES					
	1. PILL CHOOSERS	28	13	106	147	81.0 ± 6.3
	2. IUD USERS	18	14	251	283	93.6 ± 2.9
	MESTRANOL					
A.	.05 MG/DAY	121	3	19	143	15.4 ± 5.9
B.	.08	166	4	6	176	5.7 ± 3.4
C.	.10	174	1	1	176	1.1 ± 1.5
	ETHYNYLESTRADIOL (EE)					
D.	.05 MG/DAY	83	1	27	111	25.2 ± 6.7
E.	WITH DIMETHISTERONE (SEQ.) .05 + .25	345	5	36	386	10.6 ± 3.1
F.	.08	155	4	3	162	4.3 ± 3.1
	EXPERIMENTAL COMBINED E + P PREPARATIONS					
G.	VARIOUS	486	1	19	506	4.0 ± 1.7
	EE + NORGESTREL					
H.	.03/0.3	266	0	0	266	0.0 + 1.4
	EE + NORETHINDRONE ACETATE					
I.	.02/0.4	53	1	5	59	10.2 ± 7.7
J.	.02/1.0	362	1	15	378	4.2 ± 2.0
K.	.02/1.0 *	238	1	1	240	0.8 ± 1.1
L.	.03/0.6	525	4	6	535	1.9 ± 1.1
M.	.03/1.5	625	1	3	629	0.6 ± 0.6
N.	.04/2.0	314	1	3	318	1.3 ± 0.2
	MESTRANOL + CHLORMADINONE ACETATE					
O.	.05/0.5	99	1	6	105	7.3 ± 5.0
P.	.10/1.0	22	0	0	22	0.0 + 15.3

* Recalculation of series J, with one subset including 14 ovulations in 138 cycles omitted.