The Hormonal Control of Ovulation and Early Development

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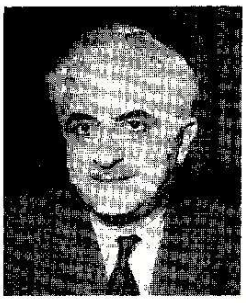

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REPRODUCTION in the female mammal begins with the ripening of the ovarian follicle and the discharge of a fertilizable egg from the follicle. It is now fairly well established for many species of higher mammals that the development of an antrum in young follicles and their growth to preovulatory states are stimulated by an anterior pituitary follicle-stimulating hormone (FSH). The rapid swelling of the follicle, culminating in rupture, requires the synergistic action of FSH and L.H. (anterior pituitary luteinizing hormone). When the ruptured follicle heals and forms the corpus luteum, the secretion of corpus luteum hormone, progesterone, becomes accelerated.

Progesterone serves a dual function: (1) It acts on the oviducts in such a way as to insure normal descent of the ova and their implantation into a properly prepared uterine endometrium, and (2) it inhibits further discharge of L.H., thereby preventing ovulation during pregnancy. In polyestrous species, the marked decline of progesterone secretion occurring at or shortly after parturition withdraws this block to L.H. secretion and a postpartum ovulation occurs. Progesterone is the hormone par excellence of pregnancy; it promotes the proper growth of the uterine ovum, maintains the fetus and placenta, and by its rather specific inhibition of L.H. release insures the absence of supernumerary ova during pregnancy.

Curiously, although the ovulation-inhibiting action of progesterone was established in ex-

TABLE 1

EFFECTS OF ORAL PROGESTERONE ON THREE INDEXES OF OVULATION

MEDICATION	NUMBER	MEAN CYCLE LENGTH	PER CENT POSITIVE FOR OVULATION BY		
			Basal temperature	*Endometrial biopsy*	*Vaginal smear*
None (control)	33	27.9 ± 0.61	100	100	100
Progesterone	69	25.5 ± 0.59	27	18	6

perimental animals over 20 years ago,[1,2] the demonstration was taken for granted but not proved. Progesterone has indeed been used to induce luteal phase changes in the human endometrium and to maintain pregnancy in the face of threatened abortion, but accurate definition of its effects when administered before and during expected ovulation has not been made. Furthermore, there has not been even an attempt at the exploration in women of another effect of progesterone, demonstrated at least for the rabbit, namely, the inhibition of fertilization following administration of *very large doses* of the hormone immediately after ovulation.

Progesterone in the Normal Menstrual Cycle

Several years ago, in collaboration with Dr. John Rock and his colleagues at the Free Hospital for Women in Brookline, Massachusetts, we undertook a study of the effects of progesterone administered to normally menstruating, regularly ovulating women. The hormone was administered by mouth for two reasons: (1) The regular injection of progesterone is difficult due to lack of a depot effect with small doses and the pain accompanying large doses,* and (2) in animal experiments we had found that the oral ovulation-inhibiting dose was much less than the endometrium-stimulating dose.[3] After some preliminary study of dosage effects, we settled on 300 mg. per day as a significantly effective dosage, and this was administered from the fifth day through the twenty-fourth day of the menstrual cycle. In this and subsequent studies we adhered to the "day 5 through 24" regimen because (1) it covers at least 98 per cent of possible ovulation times in the human menstrual cycle,[6] and (2) it insures an approximately normal cycle length since withdrawal bleeding usually takes place within two to six days following the last dose.[3,7] We observed each of 33 volunteer subjects during a control, nontreatment cycle and for one to three successive cycles of medication immediately following the control cycle. As indexes of the occurrence of ovulation, daily basal temperatures and vaginal smears were taken, and at the nineteenth to twenty-second day of the cycle an endometrial biopsy.

Table 1 summarizes our observations of these ovulation indexes. By any single criterion, ovulation occurred in practically every control cycle, but its positive diagnosis could not be made in the great majority of the medication cycles. Since any single index is subject to a degree of observational error, we sought to establish ovulation by combinations of two or all three indexes and emerged with the conclusion that ovulation inhibition occurred in at least 85 per cent of the first cycles of administration and in 95 to 100 per cent of the later medication cycles.[3,7] This deduction appeared to be confirmed by the direct examination during the latter half of a medication cycle of the ovaries of a group of women who underwent laparotomy.

Although we thus demonstrated the ovulation-inhibiting activity of progesterone in normally ovulating women, oral progesterone medication had two disadvantages: (1) the large daily dosage (300 mg.) which presumably would have to be even larger if one sought 100 per cent inhibition, and (2) the occur-

*A depot progesterone is now available—progesterone-17-hydroxy-caproate—and others are being used experimentally,[4,5] but predictable, controllable cyclicity with their administration remains to be established.

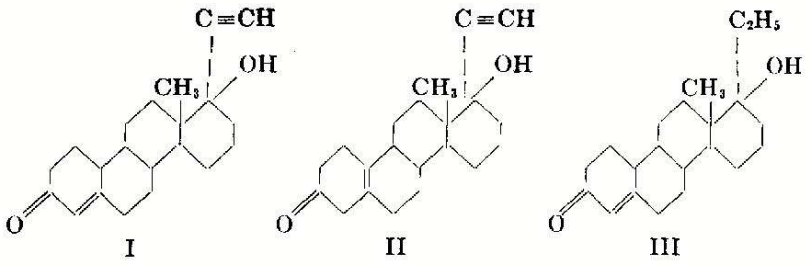

FIGURE 1. I. 17α-ethinyl-19-nortestosterone (Norlutin). II. 17α-ethinylestra-(5,10)-eneolone (Enovid). III. 17α-ethyl-19-nortestosterone (Nilevar).

rence of shortened menstrual cycles, i.e., breakthrough bleeding, in 18 per cent of the medication cycles studied (resulting in the significant decrease in mean cycle length recorded in table 1).

The 19-Nor Steroids

While the foregoing investigation was in progress we were examining a large number of steroidal and nonsteroidal compounds as potential ovulation inhibitors, or inhibitors of early embryo development, in rabbits and rats.[3,8-10] Again, because of ease of administration we sought substances likely to be active by mouth. The most highly active proved to be a group of progestational compounds which we have called 19-nor steroids[11] since they lack carbon No. 19 of the progesterone molecule (see figure 1). From these compounds we selected three for clinical study: 17α-ethinyl-19-nortestosterone (I), 17α-ethinylestra-(5, 10)-encolone (II), and 17α-ethyl-19-nortestosterone (III).*

Using again the day 5 through 24 regimen and a variety of daily dosages, we examined the effects of these compounds on the normal menstrual cycle in 50 volunteer subjects taking the medication ordinarily for three successive cycles. In addition to measuring the ovulation indexes as in the progesterone experiment, we also measured the urinary excretion of 17-ketosteroids and of pregnanediol during days 19 to 22 of the cycle. The latter compound is especially useful since it is an unequivocal measure of the production of progesterone, its metabolic precursor. The data of these experiments have been published in detail.[12,13] Table 2 summarizes our findings on ovulation indexes and urinary steroid excretion. Since essentially similar effects were observed at all dosages of 10 mg. per day and above, the data for these various dosage ranges are averaged. It is apparent from the table that there is clear evidence of ovulation inhibition by all criteria. Since these compounds are thermogenic and have specific progestin-like effects on the vaginal epithelium and endometrium,[14] these indirect indexes may be misleading, but the clear inhibition of pregnanediol output to what are in fact preovulatory levels[15] strongly points to an absence of

*I, norethisterone (NORLUTIN®); II, norethynodrel (ENOVID®); III, norethylsterone (NILEVAR®).

TABLE 2

OVULATION INDEXES AND STEROID EXCRETION IN CONTROL AND MEDICATION CYCLES

MEDICATION	NUMBER	PER CENT POSITIVE FOR OVULATION BY			MILLIGRAMS PER DAY	
		Basal temperature	*Endometrial biopsy*	*Vaginal smear*	*17-Ketosteroids*	*Pregnanediol*
None	56	95	98	89	6.98	2.06
I	21	5	0	0	7.16	0.33
II	36	0	3	7	5.88	0.31
III	17	6	0	11	4.50	0.34

TABLE 3

INCIDENCE OF PREGNANCY IN 265 PUERTO RICAN SUBJECTS

NUMBER OF TABLETS MISSED	NUMBER OF CYCLES	PREGNANCIES PER 100 WOMAN-YEARS
Premedication		62.5 ± 1.35
0	1279	0.0
1-5	282	9.2
6-19	151	25.9
All medication cycles	1712	3.8

ovulation in the medication cycles. Actually, in a series of some 20 laparotomies made in the latter half of medication cycles, complete absence of ovulation from the ovaries was observed.

These compounds appeared to have an advantage over progesterone as ovulation inhibitors since they were completely effective at such low dosages and the incidence of breakthrough bleeding was quite low, particularly with compounds I and II. Further studies demonstrated that such escape bleeding could be virtually eliminated at the 10 mg. dosage by adding a small amount of estrogen.[15,16] Furthermore, a comparative study was undertaken over many months of the effects of 300 mg. of progesterone plus estrogen and II plus estrogen in a group of regularly menstruating and ovulating psychotic women.[17,18] This study demonstrated a remarkable succession of cycles of normal length when II plus estrogen was taken, and a tendency for eventual breakthrough bleeding with oral progesterone despite the added estrogen.

Contraceptive Studies

A little over two years ago, the Family Planning Association of Puerto Rico, through its Medical Director, Dr. Edris Rice-Wray, initiated in collaboration with us a direct study of the contraceptive effects of norethynodrel (II) in volunteer subjects. The organization of the project and a preliminary report of the details of its working have been described by Dr. Rice-Wray.[19] A month's supply of 20 tablets, each containing 10 mg. of II plus 0.15 to 0.23 mg. of ethinyl estradiol 3-methyl ether, was given regularly to each subject when visited by a project worker who obtained details as to the subject's menstrual history in the preceding month. The day 5 through 24 regimen was requested, but the number of days when tablet-taking was forgotten (or omitted) was recorded. Each subject was instructed to use all 20 tablets even if such omissions occurred, unless a menstrual period supervened. Notes were taken on degree of menstrual flow and pain, and on the occurrence of side effects. As the project advanced, various subjects dropped out, but they were immediately replaced so that a population of approximately 125 subjects has been maintained each month.

Various details of the first 18 months of this study have been published.[18,20,21] In approximately 75 per cent of the 1,712 medication cycles the subjects reported regular day 5 through 24 tablet-taking with no omissions; in 16 per cent, one to five days of missed tablet-taking; and in 9 per cent, six or more days of missed tablet-taking. An extremely regular distribution of menstrual cycle lengths was observed in the first group, whereas in the latter two groups a tendency for either short or somewhat lengthened cycles was manifested.

The pregnancy rates in these women before medication and while on medication are presented in table 3. It is clear that in those cycles where tablets were taken according to directions, no pregnancies occurred, and that even where tablet-taking was omitted the pregnancy rate was low and apparently proportional to the degree of tablet-missing. The over-all rate represents roughly a 95 per cent decrease in pregnancy rate over that previously experienced by these women.

Excluding 12 women who were pregnant when they entered the project (and whose course of pregnancy was in no way adversely affected by the medication) and a small number for whom we had inadequate records, we lost an average of seven volunteers per month during the first 18 months. The numbers leaving and the reasons for their leaving are presented in table 4. Those listed as "pregnant with disuse" are women who because of either carelessness or disinterest, or because of al-

TABLE 4

REASONS FOR DISCONTINUING ENOVID MEDICATION, 123 PUERTO RICAN WOMEN

	NUMBER	PER CENT OF TOTAL DISCONTINUING	PER CENT OF TOTAL SUBJECTS
Reactions	37	30.1	14.0
Moved or too distant	19	15.4	7.0
Pregnant with disuse	14*	11.4	5.5
Pregnant during use	5	4.1	2.0
Sterilized	11	8.9	4.0
Separated from husband	10	8.1	4.0
Husband sterilized	7	5.7	2.5
Unrelated illness	7	5.7	2.5
Husband against practice	4	3.3	1.5
No interest	3	2.4	1.0
Religion	1	0.8	0.5
Miscellaneous	5	4.1	2.0
TOTAL	123	100.0	46.5

*Four of these stopped medication because of reactions.

leged adverse side effects, ceased taking the medication and became pregnant within one to three months following discontinuance. Since these pregnancies occurred in women who had taken the medication for from 1 to 18 cycles, they suggest that at least over these medication periods a fairly prompt return of fertility can occur. In our previous studies of medication for three to four month periods, this prompt return to normal ovulatory cyclicity had been observed.[12, 13]

By far the largest single group dropping from the study did so because of alleged disagreeable reactions to the medication (table 4). In reviewing the incidence of such departures, we noted that they were maximal in the first few cycles of medication and that they gradually decreased. Suspecting either a conscious or a subconscious fear of this novel method of contraception as the basis, we undertook an experiment with a new group of volunteers who were using conventional methods of contraception. We asked them *not* to abandon their customary contraceptive practices but to continue them during one or more cycles of tablet-taking. To every other woman in this series, placebos were given in a double-blind study. The data on the reported incidence of "reactions" among those receiving placebos and those receiving true medication are presented in table 5. It is obvious that there is no real difference in the two groups, indicating the origin of the "reactions" to be psychogenic.

To the women in our regular series complaining of such reactions, we gave, also in a double-blind study, antacid tablets or exactly matching lactose placebos. In table 6 are data on the degree of relief experienced from such symptoms as nausea, dizziness, vomiting, headache and abdominal pain. The placebos were 70 per cent as effective as the antacid tablets,

TABLE 5

"REACTIONS" TO ENOVID AND TO PLACEBOS

	PLACEBO	ENOVID
Number of cycles	29	31
Per cent with "reactions"	34	29
Per cent with "nausea"	24	23

TABLE 6

EFFECTS OF PLACEBO AND ANTACID MEDICATION ON "REACTIONS" TO ENOVID

	ANTACID	PLACEBO
Number of cycles	29	23
Improved	26	15
Not improved	3	8
Per cent improved	90	65

which in turn relieved the symptoms in 90 per cent of the cycles during which one or more were taken.

A number of additional studies of these subjects have been made, including steroid excretion, endometrial biopsies from time to time, blood sampling, and, in a limited number of cases, ovarian biopsies of subjects coming to operation.[18,20] Thus far, no pathologic variations have been observed. A remarkably constant cyclic pattern of endometrial response to the medication apparently occurs month after month.[17] This is essentially a sort of premature luteal phase in the endometrial glands and a predecidual development of the stroma during the latter days of the artificial cycle.[12,17,20]

Some Considerations About the Future

The experimental studies I have detailed indicate that a consistent inhibition of ovulation during a succession of apparently normal artificial menstrual cycles may readily be accomplished by orally active progestin fortified by a small estrogen supplement. Thus a method of oral contraception has been devised. When this same material is administered in low doses following ovulation, there appears to be no adverse effect on the developing ovum,[14] and certainly no complete inhibition of endogenous corpus luteum secretion.[15]

A number of questions posed by our finding remain to be answered. First of all, what is the long-run effect of a number of years of such cyclic medication on the uterine endometrium and ovaries? The former appears to respond cycle after cycle in characteristic fashion, and since the normal endometrium exhibits similar cyclic changes throughout reproductive life, we do not anticipate any notable aberrant development. But it remains to be seen. Preliminary studies of ovarian biopsies taken after 2 to 20 cycles of medication reveal a normal complement of ova but an absence of corpora lutea and large follicles.

Secondly, what is the *long-run* effect of cyclic L.H.-release inhibition? We have not been able to examine the pituitary glands of any of the subjects under study, but the indirect indexes of pituitary function employed, e.g., steroid excretion, do not suggest notable deviations in pituitary function. This aspect will certainly be investigated further. However, both constant and cyclic administration of progestin and estrogen have been common clinical practice for many years in both premenopausal and postmenopausal women, and pituitary syndrome has never been reported in these patients.

Finally, what about other means of controlling ovulation and early development? On the basis of data from animal experiments accumulated over many years, the vulnerability of each phase of the reproductive process is well established. For example, large doses of estrogen will inhibit ovulation or ovum implantation or both in a number of species. We long ago demonstrated that certain compounds related to the estrogens may indeed affect the ova without affecting the endometrium, and vice versa.[22] Antiestrogens may be found to disrupt follicular phase phenomena, antiprogestins to counteract endogenous progesterone, and specific antigonadotropins to inhibit not only ovulation but perhaps corpus luteum function. Specific ovicidal substances have been described,[23,24] and intra-uterine processes that are necessary for the ripening of sperm for fertilization may be hormonally controlled.[25]

If we are on the brink of finding numerous methods for the inhibition or disruption of various stages in reproductive processes, it means that we are also armed with means for the repair of naturally occurring defects. Fertility and sterility are two sides of the same coin. As long as scientific inquiry into their control is freely possible, so long will we advance with new means and better insight.

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BOOKS RECEIVED

Books received are acknowledged, and, as space permits, those of principal interest to our readers are reviewed more extensively. Reviews appear in this issue starting on page A-146; additional listings are on pages 614, 637 and A-156.

Progress in Cardiovascular Diseases. A quarterly publication. Progress in Cardiac Surgery. Edited by Charles K. Friedberg, M.D., Cardiologist and Attending Physician for Cardiology, The Mount Sinai Hospital, New York. 108 pages, illustrated. 1958, Grune & Stratton, Inc., New York and London. $3.00.

Illustrated Preoperative and Postoperative Care. By Philip Thorek, M.D., Professor of Surgery, Cook County Graduate School of Medicine, Chicago. 98 pages with 60 illustrations. 1958, J. B. Lippincott Company, Philadelphia and Montreal. $5.00.

Social Psychiatry in Action; a Therapeutic Community. By Harry A. Wilmer, M.D., Naval Medical Research Institute, Bethesda, Maryland. 373 pages, illustrated. 1958, Charles C Thomas, Springfield, Illinois. $8.75.

Pictorial Handbook of Fracture Treatment. By Edward L. Compere, M.D., Professor and Chairman, Sam W. Banks, M.D., Associate Professor, and Clinton L. Compere, M.D., Associate Professor, Department of Orthopaedic Surgery, Northwestern University Medical School, Chicago. Ed. 4. 448 pages with 268 illustrations. 1958, The Year Book Publishers, Inc., Chicago. $7.50.

Sex and the Adolescent. By Maxine Davis. 317 pages. 1958, The Dial Press, New York. $5.00.

Intracardiac Phenomena; in Right and Left Heart Catheterization. By Aldo A. Luisada, M.D., Director, Division of Cardiology, and Chi Kong Liu, M.D., Chief, Laboratory of Catheterization, The Chicago Medical School, Chicago. 179 pages with 82 illustrations. 1958, Grune & Stratton, Inc., New York and London. $9.50.

Clinical Neuroanatomy, Neurophysiology and Neurology; With a Method of Brain Reconstruction. By Louis Hausman, M.D., Professor of Clinical Medicine (Neurology), Cornell University Medical College, New York. 552 pages. 1958, Charles C Thomas, Springfield, Illinois. $9.75.

Cardiovascular Diseases. By David Scherf, M.D., Professor of Clinical Medicine, and Linn J. Boyd, M.D., Professor and Director of Medicine, New York Medical College, New York. Ed. 3. 829 pages with 119 illustrations. 1958, Grune & Stratton, Inc., New York and London. $17.75.

Congenital Anomalies of the Hand and Their Surgical Treatment. American Lecture Series. By Arthur Joseph Barsky, M.D., Professor of Clinical Surgery, Albert Einstein College of Medicine, New York. 165 pages, 77 illustrations. 1958, Charles C Thomas, Springfield, Illinois. $5.75.

Clinical Radiology of Acute Abdominal Disorders. By Bernard S. Epstein, M.D., Chief, Department of Radiology, The Long Island Jewish Hospital, New Hyde Park, New York. 352 pages with 224 illustrations. 1958, Lea & Febiger, Philadelphia. $15.00.

***Hormone* Production in Endocrine Tumours.** Ciba Foundation Colloquia on Endocrinology. Edited by G. E. W. Wolstenholme, O.B.E., and Macve O'Connor, B.A. Vol. 12. 351 pages with 58 illustrations. 1958, Little, Brown & Company, Boston. $9.00.

The Cervical Syndrome. American Lecture Series. By Ruth Jackson, M.D., Clinical Assistant Professor of Orthopedic Surgery, University of Texas Southwestern Medical School, Dallas. Ed. 2. 197 pages with 90 illustrations. 1958, Charles C Thomas, Springfield, Illinois. $6.50.

Applied Medical Library Practice. By Thomas E. Keys, M.A., Librarian, Mayo Clinic, Rochester. 495 pages. 1958, Charles C Thomas, Springfield, Illinois. $10.75.