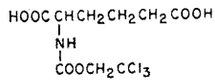
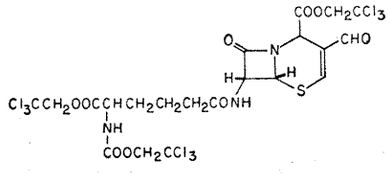
solution with N-β,β,β-trichloroethyloxycarbonyl-D-(−)-α-aminoadipic acid (LXII) in the presence of dicyclohexyl-



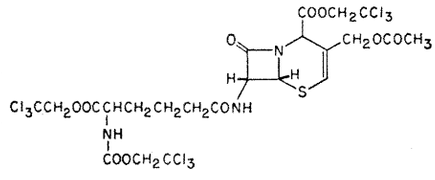
LXII

carbodiimide. The resulting crude reaction mixture was then esterified directly, using β,β,β-trichloroethanol in methylene chloride in the presence of dicyclohexylcarbodiimide and pyridine. This sequence of reactions gave two main products, which were readily separated by chromatography on silica gel using benzene/ethyl acetate (3/1) as eluant. The more polar of the two products was (LXIII), since it was convert-

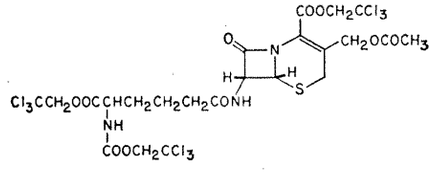


LXIII

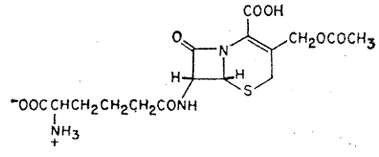
ed by reduction in tetrahydrofurane with diborane, followed by acetylation with acetic anhydride/pyridine to the β,γ unsaturated ester (LXIV). As in the cephalothin series, this unconjugated ester was smoothly equilibrated with the conjugated isomer (LXV) when it was allowed to stand in pyridine at room temperature for three days ($K_{normal/iso}$ = 1/4). Again, the two isomeric esters were readily separated, and the conjugated isomer was reduced by zinc dust and 90% aqueous acetic acid at 0° during two and one-half hours to synthetic cephalosporin C (LXVI ≡ I). The identity of the syn-



LXIV



LXV



LXVI

thetic material was in this case established through examination of its paper chromatographic behavior in several systems as well as through observation of its antibacterial activity against *Neisseria catarrhalis, Alcaligenes faecalis, Staphylococcus aureus,* and *Bacillus subtilis.* Further, the synthetic crystalline barium salt was identical in optical and spectroscopic properties with the salt of natural cephalosporin C.

It remains to express my very warm appreciation of the privilege of having been associated in the work which I have described with an outstanding group of colleagues at the Woodward Research Institute in Basel. Drs. Karl Heusler, Jacques Gosteli, Peter Naegeli, Wolfgang Oppolzer, Robert Ramage, Subramania Ranganathan, and Helmut Vorbrüggen are those whose high experimental skill and unflagging spirit brought this investigation to its successful conclusion, and I am glad to have this opportunity to express my admiration for their achievement.

References

1. G. G. F. Newton and E. P. Abraham, *Nature* **175**, 548 (1955); *Biochem. J.* **62**, 651 (1956).
2. E. P. Abraham and G. G. F. Newton, *ibid.* **79**, 377 (1961).
3. D. C. Hodgkin and E. N. Maslen, *ibid.*, p. 393.
4. *The Chemistry of Penicillin*, H. T. Clarke, J. R. Johnson, R. Robinson, Eds. (Princeton Univ. Press, Princeton, N.J., 1949).
5. J. C. Sheehan and K. R. Henery-Logan, *J. Am. Chem. Soc.* **81**, 5838 (1959); *ibid.* **84**, 2983 (1962).
6. R. R. Chauvette, E. H. Flynn, B. G. Jackson, E. R. Lavagnino, R. B. Morin, R. A. Mueller, R. P. Pioch, R. W. Roeske, C. W. Ryan, J. L. Spencer, E. van Heyningen, *ibid.* **84**, 3402 (1962).
7. G. E. Woodward and E. F. Schroeder, *ibid.* **59**, 1690 (1937).
8. F. Eckstein, *Angew. Chem.* **77**, 912 (1965).

Control of Conception by Hormonal Steroids

There is no substantial evidence that the benefits of oral contraceptives are offset by adverse effects.

Gregory Pincus

It is sometimes difficult to be decisive about the exact date marking the advent of a particular era in experimental science. Although there was a well-established background for its launching, the initiation of the present-day practice of oral contraception may fairly be marked by the publication in 1953 (*1*) of a report by Chang and me on the ovulation-inhibiting potency of progesterone and some of its derivatives as administered by several routes (subcutaneous, intravaginal, oral). Although inhibition of ovulation by administered sex steroids [and particularly by injected progesterone in the rabbit (*2*)] had been previously demonstrated (see *3*), our express objective was to discover a means of oral contraception (*4*). Our findings in the rabbit were soon extended to the rat (*5*) and to humans (*6*). In the course of screening approximately 200 steroids in the rabbit for ovulation-inhibiting activity, we discovered, among the neutral 19-norsteroids with progestational activity, several outstanding ovulation inhibitors that were effective when administered orally (*1*). The establishment of the

The author is research director of the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts. This article was delivered as a talk at the 1965 meeting of the AAAS, Berkeley, California.

Table 1. Number of pregnancies per 100 years of exposure for various methods of contraception.

Method	Pregnancy rate
Douche	31*
Rhythm	24*
Jelly alone	20*
Withdrawal	18*
Condom	14*
Diaphragm	12*
Intrauterine devices	5†
Sequential steroids	5‡
Combined steroids	0.1

* Data of Venning (*47*). † Estimated from data summarized by Pincus (*17*, p. 297). ‡ Data of Mears (*48*).

roles of these inhibitors in the control of human ovulation and menstruation (*8*) led to their use as contraceptive agents (*9*). Projects established in Puerto Rico and Haiti soon demonstrated the extraordinary contraceptive effectiveness of a combination of a 19-norsteroid and an estrogen (*10*), and this finding was confirmed and amplified by projects in Los Angeles (*11*) and England (*12*).

In addition to the 19-norsteroids, a group of highly potent oral progestins was found in various derivatives of 17-acetoxyprogesterone (*13*). Some of these progestins when administered orally inhibit ovulation, particularly when combined with an estrogen (*14*). The progestins now available in contraceptive preparations are derivatives of 19-norsteroids or 17-acetoxyprogesterone. Their structural formulas are presented in Fig. 1. All the available preparations require the action of both progestin and estrogen for maximum contraceptive efficiency, as well as for optimum control of menstrual periodicity. The drugs are administered orally for 20 or 21 days beginning on day 5 of the menstrual cycle. The estrogen employed is either 17α-ethinyl-estradiol or its 3-methyl ether, mestranol (see Fig. 1).

Table 2. Frequency of atresia and densities of follicles in ovarian biopsies from users of Enovid and control patients.

Age range (yr)	Controls			Enovid users		
	No.	Atretic follicles (%)	Follicles per mm²	No.	Atretic follicles (%)	Follicles per mm²
18–25	15	80 ± 3.3	0.79 ± 0.13	9	63 ± 8.0	1.77 ± 0.75
26–29	5	67 ± 8.7	0.39 ± 0.12	8	47 ± 8.2	1.78 ± 0.72
30–33	13	58 ± 6.8	0.38 ± 0.16	9	67 ± 9.0	0.31 ± 0.11
34–37	27	61 ± 4.6	0.22 ± 0.05			
38–42	7	67 ± 4.5	0.09 ± 0.03	5	53 ± 7.3	0.31 ± 0.07*

* Value significantly different from value for controls ($p < .01$).

Table 3. Uterine size in users of Enovid, as determined by manual palpation.

Lunar years of use	No. of patients	Size of uterus			
		Normal (%)	Subnormal (%)	Large (%)	Ill-defined (%)
1–2	901	88.3 ± 1.07	3.3 ± 0.60	5.1 ± 0.73*	3.2 ± 0.59
3–4	837	86.7 ± 1.17	1.7 ± 0.45	8.7 ± 0.98	3.0 ± 0.59
5–9	465	85.4 ± 1.64	0.6 ± 0.36	11.0 ± 1.45	3.0 ± 0.79
Medication discontinued	242	81.0 ± 2.53	2.9 ± 1.08	12.4 ± 2.12	3.7 ± 1.22

* Value significantly different from postmedication value ($p < .02$).

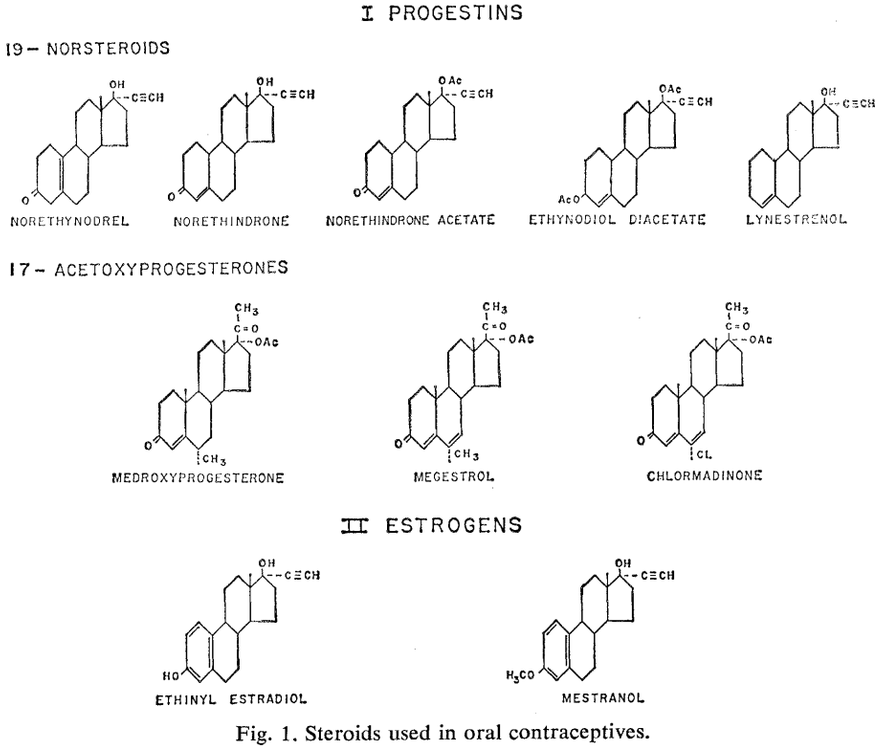


Fig. 1. Steroids used in oral contraceptives.

Methods of Oral Contraception

Progestin-estrogen combinations are used according to one of two regimens, combined therapy or sequential therapy. Combined therapy involves the use of the combination throughout the monthly medication period; sequential therapy, introduced in 1963 (*15*), involves administration of the estrogen alone for 10 or 15 successive days and use of the combination for the remaining period of 10 or 5 successive days. The combined therapy is undoubtedly the most efficient contraceptive method; a somewhat variable failure rate has been reported for the sequential method (see *16*), but on the average it appears to permit ovulation and some pregnancies. The pregnancy rates for a number of contraceptive methods are presented in Table 1, which demonstrates very clearly the superior efficiency of oral contraceptives. Indeed, it has been alleged that the failures reported for the combined administration method are due to patients' failing to follow the directions for regular daily use (*16*). Perhaps the only other experimental contraceptive method which approaches the oral contraceptives in efficiency is that involving the insertion of a plastic or metal intrauterine device. Although long-term data are still being collected, pregnancy rates in users of such devices in various localities vary from approximately 1 to 9 per 100 women-years (*17*).

Special interest attaches to the physiological actions of the oral contraceptives for several reasons. First, they involve the daily use of synthetic steroids

having significant hormonal action. Second, they are being taken by women during their fertile premenopausal years. And third, they are being taken for many months or many years by normal healthy women, not as correctives to or therapy for obvious endogenous hormonal deficiencies or aberrations, but specifically as antifertility agents. Each of these considerations implicates concern for the safety with which these preparations can be used. Apprehension has been expressed because of possible effects of an altered hormonal balance (*18*), with perhaps irreversible effects on pituitary function (*19*). Induced temporary or even permanent sterility has been considered possible (*20*). The occurrence of a variety of "adverse reactions" in women taking or ceasing to take oral contraceptives has led to the allegation of "side effects" varying from thromboembolism to hair loss, from retinopathy to hyperpigmentation. Except for clear and expected hormonal actions of progestins and estrogens I know of no scientifically valid demonstration of significant pathological effects of their use in contraceptive doses and regimens. I have presented a detailed discussion elsewhere (*17*).

Here I propose to present a précis of the available information on the physiological actions of these preparations. Implicit in what follows is that all of the various progestin-estrogen combinations have quite similar biological properties. It should be recognized that the extent to which this is true is limited. For example, we recognized early that in test animals orally administered norethynodrel is a progestin with weak intrinsic estrogenic action whereas norethindrone is a weakly androgenic progestin (*21*). However, in women taking these various preparations at the currently used dosages a number of effects clearly attributable to intrinsic hormonal properties appear to be common properties. These properties are clearly divisible into three categories: (i) actions on reproductive tract and allied organs, (ii) effects on other endocrine systems, and (iii) effects on systems and functions not ordinarily associated with sex hormone action. Most of the data given here are derived from observations made by my colleagues and myself with volunteer patients in several study projects over the past 9 years.

Here I shall consider ovarian, uterine, cervical, vaginal, and mammary-gland functions.

We have observed oocyte numbers and cytology in ovaries from experimental animals receiving various dosages of oral contraceptives over periods ranging from months to years. In rats, for example, we have seen no significant differences between ovaries from controls and those from females to which drugs have been administered (*22*, *23*, *24*). Reporting on a 3-year period of cyclical administration of norethynodrel-mestranol (Enovid, 10 milligrams per 60 kilograms of body weight) to prepubertal female rhesus monkeys, Kar *et al.* (*25*) state that Enovid "has not been found to cause

Table 4. Analysis of endometrial biopsies in users of Enovid; "normal" endometrial states.

Lunar years of use	Patients				
	No.	Endometrial state (%)			Hormonal effect
		Proliferative	Secretory	Menstrual	
0	2013	54.6 ± 1.11	19.4 ± 0.88	5.4 ± 0.51	5.9 ± 0.53
1–2	492	9.2 ± 1.30*	10.2 ± 1.37*	6.1 ± 1.08	72.4 ± 2.02*
3–4	434	13.8 ± 1.66*	14.7 ± 1.70*	8.1 ± 1.31	58.3 ± 2.37*
5–9	258	11.2 ± 1.97*	12.3 ± 2.05*	4.3 ± 1.27	65.5 ± 2.97*
Medication discontinued	134	33.5 ± 4.09*	24.6 ± 3.73	6.0 ± 2.06	25.4 ± 3.78*

* Values differ significantly from premedication values ($p < .05$).

Table 5. Analysis of endometrial biopsies in users of Enovid; "abnormal" endometrial states.

Lunar years of use	Patients					
	No.	Percentage with abnormalities				
		Cystic and adenomatous hyperplasia	Atrophy	Endometritis	Anaplasia	Carcinoma in situ
0	2013	5.0 ± 0.49	0.9 ± 0.21	8.6 ± 0.63	0.14 ± 0.08	0.09 ± 0.07
1–2	492	0.8 ± 0.40*	0.6 ± 0.35	0.6 ± 0.35*	0.0	0.0
3–4	434	2.8 ± 0.79*	0.9 ± 0.45	1.2 ± 0.52*	0.20 ± 0.22	0.0
5-9	258	3.5 ± 1.15	1.9 ± 0.85	0.8 ± 0.56*	0.40 ± 0.39	0.0
Medication discontinued	134	6.0 ± 2.06	1.5 ± 1.05	3.0 ± 1.48*	0.0	0.0

* Values differ significantly from premedication values ($p < .05$).

Table 6. Cycle lengths in users of Enovid. Controls used vaginal spermicidal jellies and foams as contraceptives. All values differ significantly from those for controls ($p < .05$).

Lunar years of use	No. of patients	No. of cycles	Percentage of patients			
			Cycle < 25 days	Cycle 25–30 days	Cycle 31–36 days	Cycle > 37 days
1–2	2,682	25,158	6.9 ± 0.49	84.5 ± 0.70	7.6 ± 0.51	1.1 ± 0.20
3–4	2,310	15,636	4.0 ± 0.41	91.2 ± 0.51	4.4 ± 0.43	0.5 ± 0.15
5–10	952	8,652	4.2 ± 0.65	90.6 ± 0.95	4.9 ± 0.70	0.4 ± 0.20
Control	455	6,242	10.5 ± 1.44	56.0 ± 2.33	27.3 ± 2.09	7.8 ± 1.26

Table 7. Menstrual phenomena reported by volunteer long-term users of Enovid. Controls, who used vaginal contraceptives, reported data for up to 4 years.

Lunar year of use	No. of patients	Cycles		
		No.	Breakthrough bleeding* (%)	Amenorrhea (%)
1st	1,591	14,957	7.4 ± 0.20	0.8 ± 0.07
2nd	1,091	12,480	5.0 ± 0.20	0.7 ± 0.07
3rd	827	8,875	3.8 ± 0.18	0.9 ± 0.10
4th	655	7,492	3.3 ± 0.21	0.9 ± 0.11
5th	474	4,692	2.7 ± 0.24	1.0 ± 0.15
6th	283	2,763	2.4 ± 0.29	1.1 ± 0.20
7th	124	1,054	3.0 ± 0.53	1.6 ± 0.39
8th	48	387	3.6 ± 0.95	1.8 ± 0.68
9th	19	125		
10th	6	22		
Total	5,118	52,847	4.8 ± 0.09	0.9 ± 0.04
Controls	455	6,809	10.0 ± 0.36	4.7 ± 0.26

* Cycles less than 25 days in length.

any change in the ovary except a consistent increase in weight. The follicular development proceeds uninterrupted, culminating in the appearance of typical Graafian follicles towards the terminal stage of the drug regime. No noteworthy effect on the number of primordial oocytes has been observed." In ovarian biopsies taken at laparotomy from women using Enovid and from control non-users we have found no differences in the proportions of atretic follicles and have found a tendency in Enovid users toward increased density of oocytes, attributable to the absence of corpora lutea and of large follicles (Table 2). Effects of oral contraceptives on the secretory activity of the ovaries have been reported by Loraine *et al.* (*26*) and are illustrated in Fig. 2. A decrease in the urinary output of estrogens and pregnanediol is not accompanied by a decrease in the total amount of gonadotropic activity detectable in the urine, and a direct action on the secretory tissues of the ovaries is deducible. However, specific inhibition of pituitary luteinizing-hormone secretion may underlie a diminished ovarian steroid production.

Because it is a major target organ for the ovarian hormones, the uterus and its functions have been subjects of especial study in users of oral contraceptives. In Table 3 are presented data on uterine size as determined by manual palpation. Except for a significant low incidence of large uteri in 1- to 2-year users of Enovid, no significant differences have been found between long-term and short-term users and a group of former users examined some time after discontinuance of use. Our data on the nature of the endometria of users of Enovid as determined from biopsies taken before use, during use, and after cessation of use are presented in Tables 4 and 5. The relative reduction in proliferative and secretory specimens and the increase in the proportion of specimens exhibiting "hormonal effect" are obvious. This "effect" is characterized by a relative predominance of stromal edema and by a degree of glandular involution. Most significant is that the proportions of these endometria are identical in 1- to 2-year users and in longer-term users. The hormonal effect tends to persist in uteri after discontinuance of medication, but a lessening in incidence is obvious. The data of Table 5 are noteworthy in indicating that the incidence of cystic and adenomatous hyperplasia and of endometritis tends to be diminished in users of Enovid, the decrease in endometritis persisting into the period after medication has been discontinued. These may be considered therapeutic actions.

Uterine function is reflected in the nature of the menstrual cycles. That the use of oral contraceptives from day 5 through day 24 imposes a fairly specific pattern of menstrual periodicity was evident early. This is clearly illustrated in the data on relative frequencies of various cycle lengths in users of Enovid and in a group of control patients in the same locality who used vaginal spermicidal jellies and foams as contraceptives (Table 6). The tendency in Enovid users for the great majority of cycle lengths to fall between 25 and 30 days is obvious, as is the relatively narrow distribution of frequencies about this mode. Again, it should be noted that no significant differences in cycle-length distributions are seen between long-term and short-term users.

Table 7 presents data on two phenomena that have been associated with the use of oral contraceptives, breakthrough bleeding, and amenorrhea. "Breakthrough bleeding" refers to menstrual spotting or bleeding occurring during medication days and presumably representing incomplete control of endometrial vasculature by the drug. Amenorrhea refers to the absence of the menstrual flow that usually occurs in days 1 to 7 following the taking of the last dose in any given month (if no flow occurs by the seventh day, medication is reinstituted). The data of Table 7 exhibit the reduction, noted by many investigators, in frequency of breakthrough bleeding after the first year of use and the relative stabilization in the frequency from the end of the second year on. The frequency of amenorrhea, on the other hand, is identical from year to year over the 8 years of study. We have attributed this first-year frequency of breakthrough bleeding in part to forgetfulness in pill-taking (a "withdrawal" flow may occur if pills are missed for even 1 day and almost certainly after two or more days) which characterizes the period of

Table 8. Incidence of suspicious Papanicolaou smears in subjects with negative smears before the use of various contraceptives.

Contraceptive	Patients	
	No.	Percentage with suspicious smears
Vaginal	208	7.2 ± 1.8
Enovid	580	2.6 ± 0.6*
Ovulin	188	2.1 ± 1.0*
Orthonovum	105	2.8 ± 1.6
Intrauterine	500	5.4 ± 1.0

* Incidence differs significantly ($p < .05$) from that for users of vaginal contraceptives.

Table 9. Urinary excretion of gonadotropin in six patients.

Age of patient	Cycle		Activity (mg/day)*	Remarks
	No.	Days		
		No medication		
37	†	6–8	0.53	Mid-cycle rise
		16–18	3.14	
		22–24	1.41	
41	†	7–9	0.58	Mid-cycle rise
		18–20	.75	
		25–27	.47	
		Enovid, 10 milligrams		
24	98	7–9	0.25	No mid-cycle rise
		13–15	.12	
		23–25	.29	
42	96	11–13	0.26	No mid-cycle rise
		14–16	.22	
		23–25	.21	
34	61	7–9	0.77	No mid-cycle rise
		14–16	.35	
		22–24	.29	
36	107	5–7	0.11	No mid-cycle rise
		16–18	.10	
		21–23	.36	

* Activity is given in estrone equivalents.
† Ovulatory cycle.

Table 10. Protein-bound iodine (PBI) in the blood of users of Enovid.

Lunar years of use	No. of patients	Percentage of patients		
		PBI < 4.0 μg/100 ml	PBI 4.0–8.8 μg/100 ml	PBI ≥ 9 μg/100 ml
1–2	69	0	82.7 ± 4.59	17.3 ± 4.59*
3–4	113	0	84.1 ± 3.46	15.9 ± 3.46*
5–9	58	1.7 ± 1.71	75.8 ± 5.67*	22.4 ± 5.52*
Medication discontinued	49	4.1 ± 2.86	93.9 ± 3.45	2.0 ± 2.02

* Values differ significantly from post-medication values ($p < .05$).

Table 11. Comparison of average thyroid uptake in patients who used Enovid for 3 years or longer and in control groups (users of vaginal contraceptives).

No. of patients	Medication	Average uptake (% of administered dose)
50	Enovid, 5 mg	19.97 ± 0.59*
56	Enovid, 2.5 mg	20.88 ± 1.07*
52	Control	20.84 ± 0.81*

* Mean ± standard error.

learning to use the pills properly, and in part to a physiological adjustment of the endometrial vasculature to the imposed levels of circulating progestin and estrogen. It should be noted, however, that in the control patients breakthrough bleeding (cycles shorter than 25 days) occurs more frequently than in users of oral contraceptives. This illustrates the relatively tight control of menstrual bleeding exercised by the exogenous steroid. Indeed, the use of oral contraceptives in the control of hypermenorrhea is now commonplace. We have several times presented data demonstrating reduced duration of menstruation in users of Enovid, and also a clear reduction in the quantity of menstrual fluid (*17*, *27*). This has been observed in users of practically all the presently available preparations, and there is a tendency for the amount of the effluvium to decrease with increasing dose. The rather constant frequency of "amenorrhea" observed from year to year probably also reflects a constant level of progestin and estrogen month in and month out. The higher frequency of amenorrhea in users of vaginal contraceptives may reflect in part the occurrence of pregnancy but primarily reflects the degree of irregularity encountered in menstrual cyclicity in our Puerto Rican subjects.

Our studies of vaginal and cervical responses to the oral contraceptives have involved primarily the study of Papanicolaou smears and of cervical biopsies from patients with confirmed suspicious smears. We have elsewhere discussed the type of data obtained (*28*). Table 8 illustrates the incidence of suspicious smears for users of vaginal contraceptives, of three oral contraceptives, and of plastic intrauterine devices. The lower rates for the oral contraceptives may be due to lack of irritative or inflammatory reactions that occur in some users of local materials, or there may be a genuine suppression by the steroids of dysplasia or anaplasia such as is found in the uterine endometrium (Table 5). Our data on the occurrence of carcinoma in cervical biopsies are too scant to afford statistically significant figures; thus far, users of oral contraceptives have had the lowest rates (see *17*, p. 257).

If we take the breasts as reproductive-system target organs, the available data for users of oral contraceptives indicate the following: (i) there is no significant average increase in breast size, although some individuals claim a mild hypertrophy or an increase in breast sensitivity (*17*, pp. 260–261); (ii) there is no increase, over a 9-year period, in the occurrence of mastalgia (observed in about 0.5 percent of the subjects); (iii) lactation appears to be reduced by large doses but is unaffected at the lower contraceptive doses, although certain preparations appear to be much less potent inhibitors than others (see *16*, pp. 64–65); (iv) the frequency of breast nodularities is significantly reduced compared to the frequency before medication and to that in users of vaginal contraceptives; this suggests inhibition of a potential precancerous state.

Table 12. Concentration of cortisol in plasma in pregnant and in nonpregnant women taking oral estrogens and progestins.

Group	No. of patients	Cortisol	
		Endogenous (μg/ml)	Bound (% of tracer dose)
Control	8	13.7 ± 4.3*	78 ± 3.7
Pregnancy, 3rd trimester	3	40.0 ± 1.8†	86 ± 2.5‡
Enovid, 10 mg/day	8	30.8 ± 8.4‡	91 ± 1.5‡
Ethinylestradiol-3-methyl ether, 0.1 mg/day	5	24.0 ± 3.7‡	85 ± 5.1‡
Ethinylestradiol-3-methyl ether, 0.3 mg/day	5	32.0 ± 4.7†	89 ± 2.1†
Progesterone, 300 mg/day	5	7.5 ± 4.2	75 ± 3.0
Norethynodrel, 10 mg/day	5	25.5 ± 6.1‡	88 ± 3.6†

* Standard error. † Significantly different from control group ($p = .01$). ‡ Significantly different from control group ($p = .02$).

We have data on aspects of the activity of three endocrine organs, the anterior pituitary, the thyroid, and the adrenal cortex. I early reported effective suppression of urinary gonadotropin activity in users of a norethynodrel-estrogen combination (*29*), especially in postmenopausal women, in whom gonadotropin output is relatively high. Other preparations have appeared to be

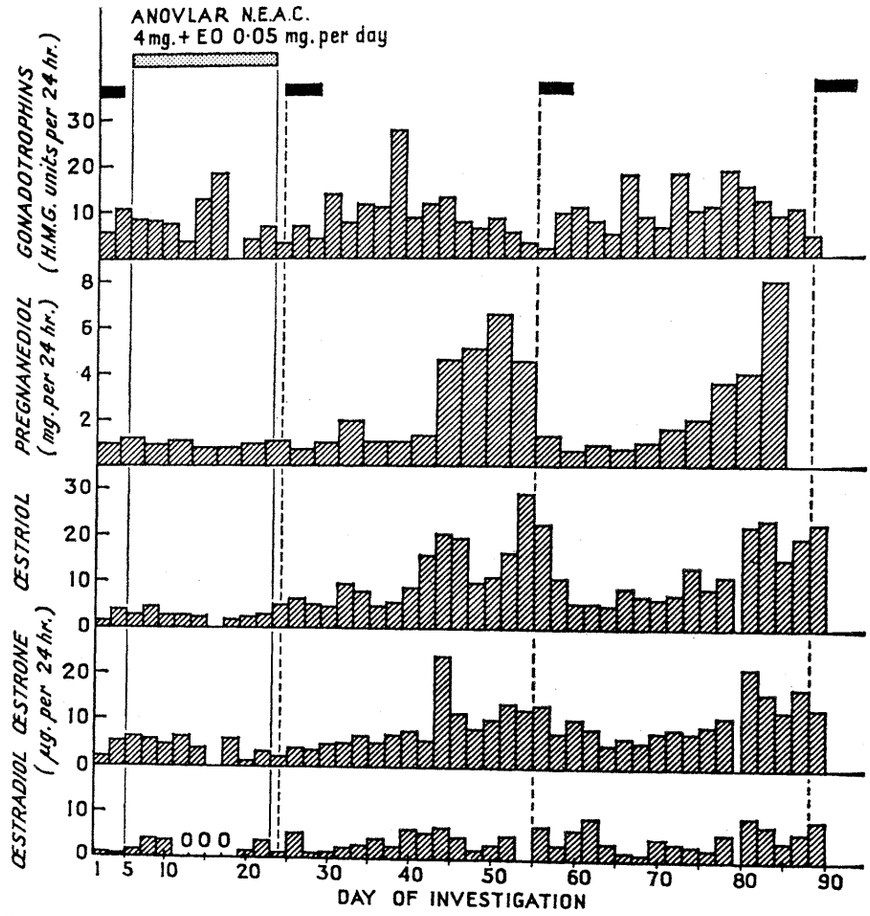


Fig. 2. Excretion of hormones in urine of a patient treated with Anovlar. Black areas indicate the duration of menstrual bleeding [From Loraine *et al.* (*26*)].

Table 13. Rates of cortisol production in long-term users of Envoid and in control patients.

Months of therapy	Cortisol (μg/hr)
Control	412
Control	536
Control	493
Control	731
Mean ± S.D.	543 ± 135
93	540
59	294
104	460
94	861
Mean ± S.D.	538 ± 238

ineffective suppressors of urinary gonadotropic activity. Since, however, this activity is the result of the combined action of follicle-stimulating and luteinizing gonadotropins, the method of assay may affect the findings. Most observers agree that excretion of luteinizing gonadotropins tends to peak most markedly at mid-cycle, and separate assay for partially purified luteinizing gonadotropins shows abolition of this peak in users of synthetic progestins or progestin-estrogen mixtures (*30*). Data on long-term users of Enovid certainly indicate absence of this mid-cycle peak (Table 9), which is evident in the control patients. Whatever the degree of suppression, a rapid recovery following cessation of use of oral contraceptives is clearly indicated by the practically immediate return of normal ovarian secretory activity (*26*) and the almost hypernormal fertility (*31*).

I know of no studies of the production or secretion of other anterior pituitary hormones. It should be noted that young animals fed fairly high doses of progestin-estrogen combinations continuously for many months tend to have lower body weights than control animals. This is in large measure due to an inhibition of appetite, as our feeding records have shown (*24*). However, a limited inhibition of production or release of pituitary growth hormone cannot be excluded. Studies of the histology and cytology of pituitaries taken from rats fed varying doses of a combination of ethynodiol diacetate and mestranol over a 2-year period disclosed no significant differences from controls (*23*).

Two measures of thyroid function commonly used in human subjects have been applied to users and nonusers of Enovid. The first of these, the amount of protein-bound iodine in blood, exhibits an average increase during medication (*17*, p. 266). This skewing of frequencies toward higher values is shown in Table 10. There is no systematic alteration with increasing years of use. Presumably this is a characteristic estrogen effect seen in pregnancy (*32*) and after administration of exogenous estrogen (*33*) and reflects the activity of the estrogen component in increasing the amount of thyroxin-binding globulin in the blood (*34*). The prompt return to normal values which we have noted on discontinuance of use has been confirmed in users of several oral contraceptives (*34*) and indicates that there is no permanent alteration in the iodine-binding systems.

The second measure of thyroid function that we have studied is the uptake of administered radioactive iodine. Here we have seen no difference in percentages of uptake between users of oral contraceptives and users of vaginal contraceptives. Moreover, as is evident in the data of Table 11, there is no significant difference between long-term users and controls. Employing much larger than normal dosages of medroxyprogesterone alone or in combination with estradiol, Maneschi *et al.* (*35*) have found a decrease in uptake of radio-iodine and other evidence of depressed thyroid function.

Our data on adrenocortical function in users of oral contraceptives tend to parallel those obtained on thyroid function. First of all, at high doses of Enovid or either of its components, there is a clear increase in concentrations of cortisol in plasma. As may be seen in Table 12, this increase appears to be attributable to an increase in plasma transcortin, the cortisol-binding protein. In contrast, in several long-term users, there has been no apparent change in the rate of cortisol production (Table 13) as determined by studies with radioisotopes. Furthermore, responsivity to administration of adrenocorticotropic hormone is undiminished in Enovid users (*36*).

Other Potential Targets

Since all of the 19-norsteroids and estrogens combined in contraceptive preparations are alkylated at carbon 17 of the steroid skeleton, an effect on one aspect of liver function as determined by retention of bromsulfophthalein may be expected (*37*). This is indeed shown in the data of Table 14 by the average increase in the percentage of bromsulfophthalein retained. In contrast, two other tests of liver function show no alteration during medication, and the data for bromsulfophthalein clearly indicate no very marked alteration. Return to lower percentages of bromsulfophthalein retained is prompt on cessation of medication (*16*, p. 49). Other tests of liver function (for example, thymol turbidity, cephalin flocculation) give no indication of significant change during use of oral contraceptives. An odd exception appears to occur in the first cycle of use by postmenopausal women in Finland, but this "may indicate no more than that the livers of postmenopausal Finnish women are less well adapted to

Table 14. Results of three tests of liver function in users of Ovulen.

Test	No. of patients	Before medication	No. of patients	During medication
Bromculfophthalein retention (milligrams per 100 ml at 45 minutes)	44	3.1 ± 0.40‡	32	4.9 ± 0.56‡
Transaminase (TransAc units)*	46	12.3 ± 0.62‡	63	14.6 ± 0.98‡
Alkaline phosphatase (K B R units)†	45	3.3 ± 0.10‡	62	3.7 ± 0.20‡

* Twenty-five TransAc units are defined as the amount of enzyme that will form 25 micromoles of oxalacetic acid per minute per liter of serum under the specific conditions (*p*H 7.40, 37°C). † Klein-Babson-Read unit is the number of micrograms of phenolphthalein released when phenolphthalein phosphate is incubated with serum or plasma at 37°C for 30 minutes. The free phenolphthalein is liberated by the alkaline phosphatase present. ‡ Mean ± standard error. All values are within the range for normal individuals.

Table 15. Blood pressures in users of Enovid.

Lunar years of use	Patients			
	No.	Hypotensive (%)	Normotensive (%)	Hypertensive (%)
1–2	342	1.5 ± 0.66	97.4 ± 0.86	1.2 ± 0.59
3–4	467	1.1 ± 0.48	97.2 ± 0.76	1.7 ± 0.60
5–9	394	0.8 ± 0.45	95.7 ± 1.02	3.5 ± 0.93
Medication discontinued	134	1.5 ± 1.05	93.2 ± 2.18	5.2 ± 1.93

estrogen inactivation than those of women during the reproductive years" (*16*, p. 48).

We have seen no very consistent changes in hematological functions studied in users of oral contraceptives. Thus the hematocrit values and the white blood counts are fairly constant from year to year in any given woman, and no meaningful trends occur in long-term users. In view of the claims of thromboembolic effects of progestin-estrogen combinations, a number of studies have been made of clotting factors in the blood of women taking oral contraceptives. Most observers agree that the concentration of certain clotting factors (for example, the factor VII-X complex) in the blood increases (see *38*). The general conclusion from the available data has been well expressed by Miller *et al.* (*39*): "Although the occurrence of thromboembolic phenomena suggests a disturbance of the blood coagulation system, no reliable evidence has established a connection between increase in coagulation factors and an increased incidence of such phenomena. Thus, although the present study demonstrates changes in the coagulation mechanism similar to those seen in pregnancy, no implications can be drawn regarding the relationship of these findings to the clinical tendency toward thromboembolic disease." Indeed, apprehensions expressed by some of thromboembolic effects of oral contraceptives (*40*) are unsupported not only by these coagulation-factor studies but also by careful analysis of the epidemiology of thromboembolism. Winter (*41*) has reviewed the data on thromboembolic mortality rates in Enovid users and in the female population generally for the years 1961, 1962, and 1963. He concludes that "no significant increase in the risk of thromboembolic death from the users of Enovid has been demonstrated," which is the conclusion also of the Food and Drug Administration committee which reviewed the 1962 data for the United States (*42*).

Studies of the cardiovascular system have revealed no significant trends in blood pressures. This is illustrated in Table 15, which lists the proportions of patients classifiable as hypotensive, normotensive, and hypertensive in our long-term study of Enovid. There are no statistically significant trends in these data. Table 16 demonstrates that there tend to be fewer varices in Enovid users than in women who have ceased taking Enovid. We have elsewhere noted this phenomenon and attributed it to the absence of pregnancy (*43*).

A number of miscellaneous physical and physiological conditions have been studied in users of oral contraceptives, particularly when an occasional report of phenomena such as hair loss or increased hirsutism is made. Thus degrees of hirsutism and chloasma have been attributed to the use of these drugs, but adequately controlled studies give no support to such actions. A tendency to lowered glucose tolerance has been reported (*44*) in some users of Enovid, with a high frequency in women with a family history of diabetes. This is the type of change in glucose tolerance that occurs in pregnancy and may be attributed to an estrogen-progestin balance similar to that in pregnancy. In any event, the diagnosis of true diabetes is not justified. A number of uncontrolled observations of users of oral contraceptives have led to claims of the induction of leg cramps, various types of headache, edema, and so on. Exceptional weight gain has been attributed to some but not all of the oral contraceptives. This is illustrated in Table 17, taken from Satterthwaite (see *27*), which summarizes significant weight gains and losses (2.7 kilograms or more) at annual examination of three groups of women taking the three drugs indicated. Orthonovum is more obviously stimulative of weight gain than of weight loss.

The psychological effects of the use of oral contraceptives have been much discussed. A good proportion of the "reactions" reported by users appear to be of this nature. The types of such reactions and the proportions of women reporting them are presented in Table 18 (*27*). Listed as subjective have been such complaints as headache, nervousness, and so on. In the first year about one-fourth to one-third of the women complain of undesirable effects.

I have been struck by the finding of practically all investigators that these complaints are most evident early during the use of the drugs and tend to be reported at a rather low, fairly constant level thereafter (*17*, p. 301). This is illustrated by the data of Table 19 for women in Rio Piedras, Puerto Rico. Every one of the alleged reactions to Enovid declines in frequency after the first year. We therefore suspected that apprehension occasioned by the use of a new (and often unknown) drug might underlie these symptoms. Accordingly, we tested the putative psychogenic action (i) in 15 women in a city many kilometers away from Rio Piedras who were offered an effective contraceptive to be taken by mouth, with no admonition concerning possible side effects, and (ii) in 28 women in Rio Piedras who were asked not to abandon their use of vaginal (diaphragm and jelly) contraceptives for several months and to

Table 16. Varices in users of Enovid.

Lunar years of use	Patients	
	No.	With varices (%)
1–2	108	24.1 ± 4.13
3–4	320	23.1 ± 2.36
5–9	380	22.9 ± 2.16
Medication discontinued	96	33.3 ± 4.84

Table 17. Weight changes in users of oral contraceptives after 1 year of use. Weight changes of less than 2.7 kilograms are not included (from *26*).

Drug	Patients		
	No.	Gaining (%)	Losing (%)
Enovid, 2.5 mg	95	19	20
Orthonovum, 2 mg	106	24	6.6
Ovulen, 1 mg	87	18	22

Table 18. Results of a comparative study of symptoms produced by three oral contraceptives during the first year of use. Patients were the same as those for whom data are reported in Table 17.

Symptom	Patients complaining (%)		
	Enovid	Ortho-novum	Ovulen
Gastrointestinal	13	10	10
Subjective	20	19	14
Vaginal and lower abdominal distress	6	7	6
Breakthrough bleeding	6	1	2
All symptoms*	28	32	28

* Some patients complained of more than one symptom; therefore this is not additive of the data in the preceding columns.

Table 19. Complaints by patients taking Enovid in Rio Piedras, Puerto Rico.

Lunar year of use	No. of patients	Cycles		
		With nausea (%)	With headache (%)	With dysmenorrhea (%)
0				52.0
1st	744	1.20	2.83	27.5
2nd	450	0.57	2.24	25.0
3rd	321	0.65	1.85	22.3
4th	237	0.26	0.84	23.9
5th	176	0.33	0.98	16.9
6th	118	0.25	0.91	12.8
7th	68	0.00	1.08	20.5
8–10th	65	0.40	0.40	9.2

Table 20. Results of administration of Enovid with and without admonitions and of placebos with admonitions.

Group	No. of patients	Cycles				
		No.	Mean length (days)	Reactions* (%)	Breakthrough bleeding (%)	Amenorrhea (%)
No admonition, effective drug	15	48	29.2	6.3 ± 3.5†	2.1 ± 2.1*	0.0
Admonition, placebo	15	41	31.6	17.1 ± 5.9	4.9 ± 3.2	9.8
Effective drug	13	30	25.5	23.3 ± 7.7	16.7 ± 6.8	3.3

* Includes complaints of physical ill-being such as nausea, vomiting, headache, vertigo, gastralgia, and malaise. † Standard error.

take pills by the usual regime to see if the pills were "fit" to continue such use. They were asked to report any untoward reactions. By random assignment, 15 of the women in the second group received placebos identical in appearance with the true medication taken by the other 13 in the group. The data from this study, presented in Table 20, demonstrate no statistically significant difference in the percentage of reactions reported by placebo users as compared to the 13 matched, randomized Enovid users, a much reduced "reaction rate" in women who had not been admonished, and definite occurrence of breakthrough bleeding in those who had been given placebos and had been admonished.

A number of additional effects with psychological bases have been studied. Thus premenstrual tension has been found to decrease in users of oral contraceptives (*45*), and both increases and decreases in libido have been reported, although generally the frequency of coitus tends to increase and to remain somewhat higher than prior to use of oral contraceptives. For comments on subjective symptoms, see Puddy (*46*).

Conclusions

Experience with the use of progestin-estrogen preparations as oral contraceptives indicates that they inhibit fertility by preventing ovulation and that their action is clearly a physiological process. Their efficiency as antifertility agents is extraordinary. An examination of their physiological effects over many years of use indicates that actions to be expected from the hormonal properties of the steroidal components occur but that none of these constitute pathological phenomena. Rather, we observe a state conditioned by the rather constant hormonal milieu resulting from the particular dosages and regimes of use. Thus far, despite use by millions of women, none of the alleged pathological adverse reactions (such as thromboembolism) to the drugs have been established as more than coincidental. Clearly beneficial effects are inherent not only in the avoidance of risks attendant upon pregnancy but also in improvements in menstrual function (regularity, reduction of hypermenorrhea and dysmenorrhea) and perhaps in a prophylactic effect upon certain abnormalities of the reproductive tract (cervical anaplasia, endometrial cystic and adenomatous hyperplasia). Psychogenic phenomena due to use of oral contraceptives are difficult to measure, but beneficial effects would seem to outweigh adverse ones.

References and Notes

1. G. Pincus and M. C. Chang, *Acta Physiol. Latinoam.* **3**, 177 (1953).
2. S. W. Makepeace, G. L. Weinstein, M. W. Friedman, *Am. J. Physiol.* **119**, 512 (1937).
3. J. W. Everett, in *Sex and Internal Secretions* W. C. Young, Ed. (Williams and Wilkins, Baltimore, 1961), vol. 1, p. 497.
4. Experimental work was supported by a grant from the Planned Parenthood Federation made possible through the kind offices of Margaret Sanger.
5. R. F. Slechta, M. C. Chang, G. Pincus, *Fertil. Steril.* **5**, 282 (1954).
6. G. Pincus, in *Proc. Intern. Conf. Planned Parenthood, 5th, Tokyo, 1955*, p. 175.
7. ———, M. C. Chang, E. S. E. Hafez, M. X. Zarrow, A. Merrill, *Science* **124**, 890 (1956).
8. J. Rock, G. Pincus, C. R. Garcia, *ibid.*, p. 891; J. Rock, C. R. Garcia, G. Pincus, *Recent Progr. Hormone Res.* **13**, 323 (1957); C. R. Garcia, G. Pincus, J. Rock, *Am. J. Ostet. Gynecol.* **75**, 82 (1958).
9. G. Pincus, J. Rock, C. R. Garcia, E. Rice-Wray, M. Paniagua, I. Rodriguez, *Am. J. Obstet. Gynecol.* **75**, 82 (1958).
10. G. Pincus, C. R. Garcia, J. Rock, M. Paniagua, A. Pendleton, F. Laraque, R. Nicolas, R. Borno, V. Pean, *Science* **130**, 81 (1959).
11. E. T. Tyler and H. S. Olson, *J. Am. Med. Assoc.* **169**, 1843 (1959).
12. E. Mears, *Brit. Med. J.* **1961-I**, 1318 (1961).
13. T. Miyake and G. Pincus, *Endocrinology* **63**, 816 (1958).
14. G. I. M. Swyer and V. Little, *Proc. Roy. Soc. Med.* **55**, 861 (1962).
15. J. W. Goldzieher, J. Martinez-Manatou, N. B. Livingston, Jr., L. E. Moses, E. Rice-Wray, *Western J. Surg.* **71**, 187 (1963).
16. E. Mears, Ed., *Handbook on Oral Contraception* (Little, Brown, Boston, 1965).
17. G. Pincus, *The Control of Fertility* (Academic Press, New York, 1965).
18. E. C. Dodds, *J. Endocrinol.* **23**, 1 (1961).
19. R. L. Holmes and M. Mandl, *Lancet* **1962-I**, 1174 (1962).
20. M. J. Whitelaw, V. F. Nola, C. F. Kalman, *J. Am. Med. Assoc.* **195**, 781 (1966).
21. G. Pincus, M. C. Chang, M. X. Zarrow, E. S. E. Hafez, A. Merrill, *Endocrinology* **59**, 695 (1956)
22. G. Pincus and A. Merrill, in *Control of Ovulation*, C. Villee, Ed. (Pergamon, New York, 1961), p. 37.
23. L. W. Self, *Metabolism* **14**, 311 (1965).
24. S. M. Husain and G. Pincus, *Am. Zoologist* **5**, 660 (1965).
25. A. B. Kar, R. P. Chandra, V. P. Kamboj, F. R. Choudhury, *Indian J. Exp. Biol.* **3**, 69 (1965).
26. J. A. Loraine, E. T. Bell, R. A. Harkness, E. Mears, M. C. N. Jackson, *Lancet* **1963-II**, 902 (1963).
27. C. R. Garcia, A. P. Satterthwaite, G. Pincus, *Addendum Proc. Intern. Planned Parenthood, Fed. Intern. Congr. Ser. 72* (Excerpta Medica Foundation, Amsterdam, 1964), p. 3.
28. G. Pincus and C. R. Garcia, *Symp. Cancer Utero, 16th. Rev. Inst. Nac. Cancerol.* (Mexico City, 1964), p. 3. *Metabolism* **14**, 344 (1965).
29. G. Pincus, *Proc. Symp. 19-Nor Progestational Steroids* (Searle Research Laboratories, Chicago, 1957), p. 105.
30. M. L. Taymor, *J. Clin. Endocrinol. Metab.* **24**, 803 (1965); V. C. Stevens, N. Vorys, P. K. Besch, R. D. Barry, *Metabolism* **14**, 327 (1965).
31. C. R. Garcia, in *Human Fertility and Population Problems*, R. O. Greep, Ed. (Schenkman, Cambridge, Mass., 1963), p. 43; S. W. Goldzieher, E. Rice-Wray, M. Schulz-Conteras, A. Aranda-Rosell, *Am. J. Obstet. Gynecol.* **84**, 1474 (1962).
32. J. P. Peters, E. B. Man, M. Heinemann, in *The Normal and Pathological Physiology of Pregnancy* (Williams and Wilkins, Baltimore, 1948).
33. J. T. Dowling, N. Freinkel, S. H. Ingbar, *J. Clin. Endocrinol. Metab.* **16**, 1491 (1965).
34. W. H. Florsheim and M. A. Faircloth, *Proc. Soc. Exp. Biol. Med.* **117**, 56 (1964).
35. M. Maneschi, E. Cittadini, P. Quartararo, *Sicilia Sanit.* **4**, 135 (1962).
36. E. E. Wallach, C. R. Garcia, R. W. Kistner, G. Pincus, *Am. J. Obstet. Gynecol.* **87**, 991 (1963).
37. I. M. Arias, in *Ciba Symposium on Protein Metabolism* (Springer, Berlin, 1962), p. 434.
38. O. Egeberg and P. A. Owren, *Brit. Med. J.* **1963-I**, 220 (1963); C. Hongie, R. N. Rutherford, A. L. Banks, W. A. Coburn, *Metabolism* **14**, 411 (1965); J. Donayre and G. Pincus, *ibid.*, p. 418.
39. S. P. Miller, S. L. Lee, N. Ritz, *ibid.* **14**, 398 (1965).
40. W. Jordan, *Lancet* **1961-II**, 1146 (1961); W. F. Minogue, I. C. Halperin, J. Soler-Bechara, P. Varricle, F. B. Flood, *New Eng. J. Med.* **266**, 1037 (1963).
41. I. C. Winter, *Metabolism* **14**, 418 (1965).
42. Special Report: FDA on Enovid: Ad hoc Advisory Committee for the Evaluation of a Possible Etiologic Relation with Thromboembolic Conditions, *J. Am. Med. Assoc.* **185**, 776 (1963).
43. G. Pincus, *Advan. Chem. Ser.* **49**, 177 (1965).
44. H. Gershberg, C. Z. Javier, S. M. Halse, *Diabetes* **13**, 378 (1964).
45. A. Wiseman, in *Recent Advances in Ovarian and Synthetic Steroids*, R. P. Shearman, Ed. (G. D. Searle, High Wycombe, 1965), p. 206.
46. E. M. Puddy, *ibid.* p. 233.
47. G. R. Venning, *Metabolism* **14**, 457 (1965).
48. E. Mears, in *Biological Council Symposium on Agents Affecting Fertility*, C. R. Austin and J. S. Perry, Eds. (Churchill, London, 1965), p. 211.