for estrogenic activity, II has this same order of activity, whereas III is inactive.

We have been unable to demonstrate deciduomagenic activity (C) of I in doses up to 10 mg and of II in doses up to 15 mg. Compound III is about 4 times as active as progesterone, and IV has the same activity as progesterone.

Our best means of comparing the conception-inhibiting action of these compounds in rats (D) is on the basis of oral administration. Compound II is the most active of the substances studied. It should be pointed out, however, that progesterone given by mouth does not exhibit a true dosage/response curve over the range 2 to 50 mg (*2*, *4*), whereas by subcutaneous injection it does and exhibits an M.E.D. of less than 5 mg. The means whereby conception is inhibited by II in the rat appears to be dual in nature. In certain animals a clear inhibition of ovulation has been determined by inspection of the ovaries and noting the failure to form corpora lutea. In other animals ovulation was observed, but fertilization failed to occur. This is reminiscent of the inhibition of fertilization by progesterone administration in artificially ovulated rabbits reported by Dutt and Casida (*8*).

All of these compounds are effective inhibitors of ovulation in the rabbit. Although accurate quantification of this effect is difficult, the data of Table 1 (E) suggest that I and II are the most active in this test and that III and IV have the order of magnitude of activity of progesterone. We have previously reported that certain compounds active in this test in the rabbit may be inactive as conception-inhibitors in the rat (*4*). In this instance activity is exhibited in both species.

By their activity in the Clauberg test, these 19-nor compounds would be classed as progestins. One would expect, therefore, that they, like progesterone, should be effective in maintaining pregnancy. Actually, as is evident from the data of Table 1 (F), this is not completely demonstrable. Thus in doses up to 2 mg per day per rabbit II fails to induce implantation; III acts very much like progesterone, but a somewhat higher dosage is required. Compound I has behaved very peculiarly in this test. Tested at the 0.25-mg-per-day level it has induced implantation, albeit accompanied by much fetal degeneration. At dosages up to 10 mg per day very limited implantation has occurred in one-fourth of the animals and no implantation in the remaining three-fourths. We have no adequate explanation for this phenomenon but have considered the possibility that among the various effects exerted there may be combined in one molecule both progestational and antiprogestational potentialities. We have not been able to test this with I, but certain experiments conducted with II are suggestive. Rabbits pretreated with II for 3 days were mated to fertile bucks; at the same time ovulation was insured by the intravenous injection of a pituitary gonadotrophic extract. Examination of the tubal ova of several females indicated that most of the ova were fertilized. Nonetheless, in no instance were young born of such rabbits, and palpation failed to disclose normal implantation.

When administered to fertile female rats in 2-mg dose every other day for 70 days, II completely inhibited fertility during the period of administration, although matings occurred with males kept with the females. Following withdrawal of administration, a sterile period ensued which averaged 26 days in length. Examination of the daily vaginal smears disclosed fairly regular vaginal cycles as well as mating, suggesting that II did not alter completely the fundamental endogenous secretory rhythm but affected either ovulation or ovum development.

On the basis of these animal studies, we find here a group of substances which, by reason of certain similarities to progesterone, may be classified as progestins. Only one of them, 17α-ethyl-19-nortestosterone (III), appears to have typical progesteronelike activity in all of the tests employed, but it is quantitatively more active than progesterone in certain tests (for example, A and C) and less active than progesterone in others (for example, F). With these compounds one may inhibit normal reproductive processes in one aspect (for example, ovulation) or stimulate them in others (for example, endometrial proliferation). The fact that certain of them are active at quite low concentrations suggests potential therapeutic usefulness.

Table 1. A comparison of estimated minimal active doses of progesterone and the 19-nor steroids in six tests of activity.

Compound	(A) M.E.D. in Clauberg assays (mg)	(B) Minimum uterotrophic dose in mice (μg)	(C) Minimum deciduomagenic dose in rats (mg)	(D) Minimum oral antifertility dose in fertile rats (mg)	(E) Minimum ovulation-inhibiting dose (mg)	(F) Minimum implantation sustaining dose in rabbits (mg/day)
Progesterone	1–2	87	2	5–10+	1–2	0.5–1.5
I	0.1–0.2	29	> 10	5	0.25	0.25*
II	2–4	0.24	> 15	2	> 0.2 < 1.0	> 2.0
III	0.1–0.2	± 100†	0.5	5	> 1.0 < 5.0	2.0
IV	0.1+	19	2	Not studied	0.5–2.0	Not studied

* Less active at higher dose. † Data of Drill, Saunders, and Edgren (*7*).

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References and Notes

1. G. Pincus and M. C. Chang, *Acta Physiol. Latinoamer.* 3, 177 (1953).
2. R. F. Slechta, M. C. Chang, G. Pincus, *Fertility and Sterility* 5, 282 (1954).
3. G. Pincus, *Aspectes du Metabolisme des Steroides Hormonaux* (Masson, Paris, 1955).
4. ———, *Proc. 5th Inter. Planned Parenthood Conf.*, in press.
5. The work described in this paper was aided by grants from the Planned Parenthood Federation of America and G. D. Searle & Co. The compounds used were contributed by G. D. Searle & Co. and the Chemical Specialties Co., Inc. We are indebted for valuable technical assistance to Pauline Longo, Mrs. A. Dorfman, and P. Bodurtha.
6. G. Pincus *et al.*, *Endocrinology*, in press.
7. V. Drill, F. Saunders, R. A. Edgren, personal communication (1956).
8. R. H. Dutt and L. E. Casida, *Endocrinology* 43, 208 (1948).

6 September 1956

Effects of Certain 19-Nor Steroids on the Normal Human Menstrual Cycle

In an accompanying paper (*1*) are described the effects evoked by certain 19-nor steroids on the reproductive processes of rodents. The present report deals with three of these compounds, 17α-ethinyl-19-nortestosterone (I), 17α-ethinyl-5(10)-estraeneolone (II), and 17α-ethyl-19-nortestosterone (III) (*2*). These were administered by mouth in dosages of 5 to 50 mg per day to 50 women from days 5 through 25 of the menstrual cycle. The subjects' ages ranged from 22 to 39 years (average, mean, and mode, all about 29 years).

During treatment, only rare instances of moderate side-effects occurred. Medication was given for inexplicable childlessness, since previous use of progesterone in high dosages had been helpful (*3*). On the basis of past history, these patients were known to ovulate regularly and, hence, to menstruate regularly and normally. During most con-

Table 1. Effects of 17α-ethinyl-19-nortestosterone (I), 17α-ethinyl-5(10)-estraeneolone (II), and 17α-ethyl-19-nortestosterone (III) on cycle lengths and indices of ovulation in normally ovulating women.

Compound and dosage (mg/day)	No. of cycles	Mean cycle length (days)	Incidence of ovulation										
			Basal temp.			Endometrial biopsy			Vaginal smear			Pregnanediol (mg/day)	
			(No.)	(% ±)	(% −)	(No.)	(% ±)	(% −)	(No.)	(% ±)	(% −)	(No.)	(Mean)
Control	40	27.2 ± 0.51	39	6	6	37	0	0	31	3	0	35	3.4 ± 0.27
(I) 10	21	28.9 ± 5.56	20	5	95	21	14	86	20	25	75	20	0.24 ± 0.037
(I) 20	36	28.6 ± 0.57	36	3	89	25	20	76	35	17	83	32	0.35 ± 0.073
(I) 40	5	26.8 ± 1.16	5	0	100	4	50	25	4	0	100	3	0.97 ± 0.97
(I) 10–40	62	28.5 ± 0.68	61	3	92	50	20	76	59	19	81	55	0.34 ± 0.066
(II) 10	28	26.5 ± 0.58	28	14	79	22	0	96	26	19	65	26	0.25 ± 0.052
(II) 20	6	27.7 ± 0.26	6	0	100	6	0	83	6	0	100	6	0.53 ± 0.335
(II) 10–20	34	26.7 ± 0.48	34	12	82	28	0	93	32	16	72	32	0.30 ± 0.074
Control	10	25.7 ± 0.91	9	10	0	11	0	0	8	0	0	10	3.1 ± 0.23
(III) 10–50	16	25.3 ± 2.24	13	0	100	11	0	91	10	0	100	10	0.31 ± 0.038

trol cycles, and ordinarily throughout one or more succeeding cycles of treatment, a careful study was made of (i) length of menstrual cycle; (ii) daily basal body temperature; (iii) endometrial biopsy (within cycle-days 19–24); (iv) daily vaginal smear; (v) 48-hour pregnanediol excretion (within cycle-days 17–23). Creatinine values indicated that these collections were complete. Analytic methods are described elsewhere (*4*).

From ii, iii, and iv, indirect diagnoses of the incidence of ovulation were made, the data in each category being classified as positive (+), doubtful (±), or negative (−), for ovulation. From the values of v, the degree of corpus-luteum activity was deduced (*5*).

In Table 1 are summarized the data in 50 control cycles of 50 subjects and in 112 medication cycles of 48 of these same women. Two are omitted from this presentation but are included in other reports (*4, 6*). They were given only 5 mg per day of compound I, which is now considered below the minimal effective dose. The subjects receiving I and II are regarded as a single group, hence their control values are averaged, for these compounds had almost identical effects. The test values i to v for each dosage in treated cycles are also averaged, since these women were sufficiently alike in pertinent respects.

By any single indirect criterion, doubtful or negative diagnoses for ovulation occurred in only minor percentages of the control cycles. If any two positive indirect criteria of ovulation are considered sufficient, 100 percent of the control cycles were ovulatory. In contrast, doubtful or negative indices of ovulation characterized the great majority of medication cycles, regardless of which of the three compounds was used. With compound III, there was interim spotting or break-through bleeding during treatment in eight of 16 cycles, without relation to dosage. Endometrial biopsies in these eight cycles disclosed hypoplasia, as though a needed estrogen were lacking.

In two cycles, subjects receiving II gave a positive sign of ovulation by each of two indirect criteria, so they might have been adjudged ovulatory, but the excretion of pregnanediol in each instance was 0.2 mg per day, indicating that even had ovulation occurred, corpus-luteum secretion was, at least largely, inhibited.

The nature of the endometrial histology is described elsewhere (*4, 6*). It suffices here to say that both I and II appear strongly to stimulate stromal development, even occasionally to a decidual stage, but endometrial glands do not function proportionately. Furthermore the mucosa is thin.

The mean data on pregnanediol excretion among all patients in the first, second, and third treated cycles, at each dosage-level of each compound, indicate a consistent and significant depression of output. Only two cycles with I and II, respectively, exhibited excretion values lying within two standard deviations of the mean control value, but in these cycles, the indirect criteria were negative for ovulation. Moreover, it is noteworthy that in seven cases of required laparotomy in patients who had previously taken compound I from 1 to 3 months, no recent corpora lutea were seen. In five of these instances, the observations in gross were confirmed by microscopic examination of ovarian tissue.

A follow-up has been possible for from 1 to 3 months after medication in 28 of the subjects who had taken compounds I and II. Although detailed analyses of these untreated cycles have not been feasible, each subject furnished us with postmedication basal temperature charts, which have been studied for ovulation time and cycle length. The relevant data are presented in another publication (*4*). They demonstrate (i) a statistically significant increase (of not more than 8 days) in the mean length of the first posttreatment cycles in both medication groups, and in the second cycle after therapy with I; (ii) a return to normal mean cycle length in the second and third postmedication cycles in subjects receiving II; (iii) a significantly delayed mean ovulation time in the first postmedication cycle in the case of both compounds, with a return to a normal mean time thereafter. In first and second postmedication cycles, there is a slight (statistically insignificant) increase in the percentage of doubtful ovulations over that in the control cycles. Observations made in posttreatment cycles showed that medication did not prevent a return of ovulation to any appreciable extent. When cycle prolongation occurred, it was the result of delayed ovulation. This suggests the need of a latent period of several days for complete ovarian recuperation.

The results, as shown in Table 1, indicate that all three compounds are effective ovulation-inhibitors in women. In dosages of 10 mg per day and higher, I and II, by their intrinsic action on the endometrium and/or by their only partial suppression of ovarian secretion, can maintain the endometrium in such a state that a catamenialike withdrawal bleeding occurs after cessation of therapy. In contrast, III, at dosages up to 50 mg per day, fails to exhibit such support in half of the cases studied.

Except for four habitual aborters, as well as for three patients with secondary infertility and one with primary infertility, who had been exposed to conception for not more than 1.5 years, exposure had prevailed for 2 to 10 years. Ten women with infertile exposure of 2 to 6 years had been previously pregnant. None of the 32 others had ever conceived, although their average exposure was 5 to 6 years.

Despite adequate coitus, none of the 50 women became pregnant during the months of medication. Their long-standing infertility may make this zero figure of no import. Nevertheless, it seems of at least passing interest that within only 5 months of the last treated cycle, seven patients conceived. Two of these, however, had secondary infertility, and each had been exposed for only 1.5 years. The remaining five had been inexplicably

barren for 3, 4.5, 4.5, 5, and 6 years, respectively. Hence in these seven instances, at least, it appears that the steroids not only did not damage the ovaries but, on the contrary, may have been helpful in the relief of sterility (*7, 8*).

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References and Notes

1. G. Pincus *et al.*, *Science* 124, 890 (1956).
2. For convenience, the numbers I, II, and III are used to denote these respective steroids.
3. J. Rock *et al.*, in preparation.
4. G. Pincus *et al.*, in preparation.
5. 17-Ketosteroid determinations carried out for the purpose of ascertaining the possible effect of these compounds on adrenocortical secretion showed but a slight (statistically insignificant) reduction in output.
6. J. Rock *et al.*, in G. Pincus, Ed., *Recent Progress in Hormone Research, Proc. 1956 Laurentian Hormone Conf.* (Academic, New York, in press), vol. XIII.
7. This investigation was aided by grants from the Planned Parenthood Federation of America, Inc., and G. D. Searle & Co. The compounds used were contributed by G. D. Searle & Co. and the Chemical Specialties Co., Inc.
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Aftereffect in the Degradation of Cellulose and Pectin by Gamma Rays

Desoxyribonucleic acid is degraded by x-rays, and after the termination of irradiation a further degradation occurs (*1*). The latter phenomenon has been called an "aftereffect." Similarly, an aftereffect has been observed in the irradiation inactivation of trypsin (*2*), pepsin (*3*), and bacteriophage (*4*). The present communication (*5*) describes an aftereffect in the degradation of cellulose and pectin by gamma rays.

Solka Floc (Brown Company BW-200), which is a purified wood cellulose containing approximately 99.5 percent alpha-cellulose, and NF Citrus Pectin, a highly purified product used for pharmaceutical purposes, were chosen for these studies. The air-dry samples were reduced to lower moisture contents by being placed over phosphorus pentoxide in a desiccator which was evacuated by a Hyvac oil pump for periods up to 120 hours. Moisture contents were determined by the loss in weight at 70°C for 6 hours at 100 mm pressure. Samples that were heated were not used in further studies.

Solka Floc and pectin were placed in screw-cap glass vials and exposed to gamma radiation from cobalt-60. The dosages were controlled by placing the samples at fixed distances from the source for known periods of time, as has been previously described (*6*).

An estimate of the degradation of these samples was obtained from solution viscosity measurements in Ostwald-Cannon-Fenske viscometers at 25°C for cellulose and 30°C for pectin. Cellulose solutions were made up in cuprietheylenediamine which was 0.5*M* with respect to copper and in which the ratio of ethylenediamine to copper was 2/1 (*7*). Intrinsic viscosities were obtained by plotting n_{sp}/C as a function of the concentration, C (0.250, 0.125, and 0.0625 g/100 ml), and extrapolating the lines to zero concentration. Relative viscosities were determined for pectin at a concentration of 0.100 g/100 ml in a solution containing 0.2 percent Calgon and 0.8 percent sodium chloride and adjusted to *p*H 6.0 (*8*). In order to determine the primary effect of radiation, the viscosities of the irradiated and control (unirradiated) samples were measured as soon as possible after the end of irradiation. The solid samples were stored in vials at room temperature until they were needed for subsequent viscosity determinations.

Cellulose (*9*) and pectin (*8, 10*) are known to be degraded by ionizing radiation, and the extent of degradation (percentage change in viscosity) as measured immediately after the irradiation is proportional to the log of gamma-ray dosage (*8, 11*). To our knowledge, no aftereffect has been described for any polysaccharides.

The results in Fig. 1 were obtained on cellulose and pectin at moisture contents of 0.32 percent and 0.75 percent, respectively, irradiated in air. The degradation continued in the solid (dry) samples beyond that indicated by solution viscosities determined as soon as practicable after irradiation. With both polysaccharides, the aftereffect continued for at least 2 weeks, after which it became difficult to distinguish between small changes and experimental variations. In the specific instances illustrated, the aftereffect amounted to 106 percent and 25 percent of the primary effect for cellulose and pectin, respectively. The apparent dissimilarity is in part due to the fact that the particular dosage for cellulose (103.5×10^3 r) is much smaller than that for pectin (2030.0×10^3 r) so that in the case of cellulose the primary effect was relatively small. Thus, for example, at 552.3×10^3 r the aftereffect for cellulose was 59 percent of the primary effect.

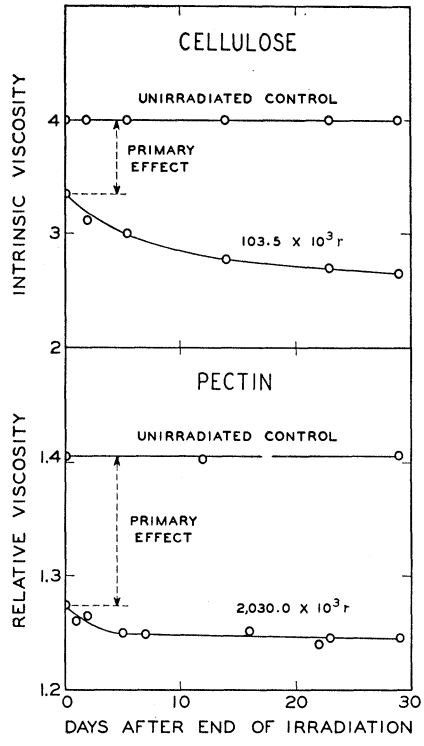

Fig. 1. Changes in solution viscosities of cellulose and pectin irradiated and stored in the solid (dry) state at room temperature. The primary effect is the change induced in the viscosity of the original sample by gamma rays and ascertained as soon after the end of irradiation as practicable (within 5 hr). The aftereffect is the change that occurs after the first viscosity measurement on irradiated samples.

The phenomenon reported here is unique in that the aftereffect occurred in solid (dry) samples, whereas those previously reported (*1–4*) involved substances irradiated in the presence of water. In fact, cellulose and pectin samples of various moisture contents were investigated and the aftereffect was observed only in the afore-mentioned samples which were of the lowest moisture levels studied.

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References and Notes

1. B. Taylor, J. P. Greenstein, A. Hollaender, *Science* 105, 263 (1947) and *Arch. Biochem.* 16, 19 (1948); J. A. V. Butler and B. E. Conway, *J. Chem. Soc.* 1950, 3418 (1950); M. Errera, *Bull. soc. chim. biol.* 33, 555 (1951); M. Daniels, G. Scholes, J. Weiss, *Nature* 171, 1153 (1953).
2. M. R. McDonald, *Brit. J. Radiol.* 27, 62 (1954).
3. R. S. Anderson, *ibid.* 27, 56 (1954).
4. T. Alper, *Discussions Faraday Soc.* No. 12, 234 (1952).
5. Approved by the director of the New York State Agricultural Experiment Station, Geneva, as journal paper No. 1041, 12 June 1956. This paper reports research sponsored by the Quartermaster Food and Container Institute for the