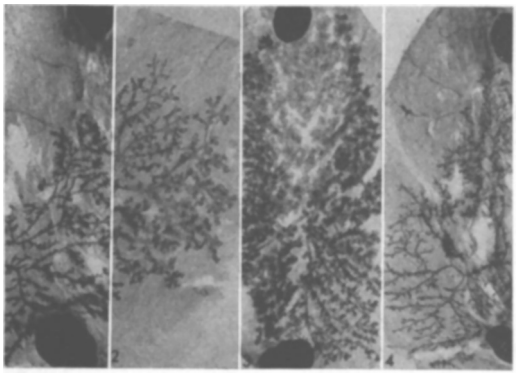

FIG. 1. Outgrowth from a piece of mammary duct from a 4-mo-old nulliparous pregnant A mouse transplanted into a female A × C3H hybrid. Host hypophysectomized-ovariectomized at 5 wk of age and treated with estradiol + progesterone + somatotropin for 21 days. Note presence of a few alveoli. Hematoxylin. × 3.5.

FIG. 2. Host gland from A × C3H female, hypophysectomized-ovariectomized at 5 wk of age and treated with estradiol + progesterone + somatotropin for 21 days followed by cortisol + somatotropin for 5 days. Note presence of some milk-filled alveoli. Hematoxylin. × 5.

FIG. 3. Outgrowth from a piece of mammary duct from a 4-mo-old nulliparous pregnant C3H female, transplanted into the same hybrid host described in Fig. 2. Note presence of large number of milk-filled lobules. Hematoxylin. × 3.5.

FIG. 4. Outgrowth from a piece of mammary duct from a 4-mo-old nulliparous pregnant A mouse transplanted into the same hybrid host described in Fig. 2. Note absence of milk-filled alveoli. Hematoxylin. × 3.5.

similar conclusion in their studies of the strain differences in estrogen sensitivity of the vaginal mucosa of mice.

Summary and conclusions. Mammary tissues from A/Crgl and C3H/Crgl strains of mice were transplanted into mammary gland-free fat pads of female hybrids (C3H × A, A × C3H). The hosts were hypophysectomized-ovariectomized, and treated with estradiol + progesterone + somatotropin for 21 days. Half of them received an additional 5 days treatment with cortisol + somatotropin. On the basis of the lobuloalveolar mammogenic and lactogenic responsiveness of the A, C3H, and hybrid mammary tissues to the hormone combinations used, the following general conclusions were drawn: 1. There is a quantitative difference in lobuloalveolar mammogenic response of A/Crgl and C3H/Crgl mammary tissues to the somatotropin-containing hormone combination, degree of response being greater in the C3H/Crgl mammary gland. 2. There appears to be a qualitative difference in lactogenic response of A and C3H mammary tissue to the somatotropin-containing combination. 3. The occurrence of milk secretion in mammary glands of hybrids (A × C3H, C3H × A) and in C3H mammary transplants, and its absence from A transplants, indicate that the genetic determinant for mammary gland sensitivity to the somatotropin-containing hormone combination must lie in the mammary tissue itself, and that this can be transmitted by either male or female C3H parent to the hybrid offspring.

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Effect of Enol Etherification on Antiestrual and Contraceptive Activity of Some Progestins in Rats. (26827)

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A series of several enol ethers of Δ^4-3-ketosteroid hormones recently synthesized in our laboratories(1) has shown more potent hormonal activity when administered orally than their respective parent compounds(2-4). Enhancement of the activity was dependent upon the nature of the alcohol chain. The present investigation was designed to study

TABLE I. Influence of Progesterone on Estrous Cycle of Rats.

Compound	Dose/animal/day		No. of rats with estrous cycle*		Length of cycle (days)
	mg	μmoles	Maintained	Inhibited	
Sesame oil	—	—	10	0	4.0 ± .2†
Progesterone	.628	2	10	0	5.9 ± .2
	1.256	4	10	0	12.8 ± 2.3
	2.512	8	0	10	—

* 10 animals/group.

† Mean ± stand. error.

the change in activity produced by enol etherification of some progestational steroid compounds on the estrous cycles of rats. The best results in earlier experiments were obtained using cyclopentyl derivatives of 17α-methyltestosterone(2,3) and 17α-acetoxyprogesterone(4); therefore, cyclopentyl enol ether derivatives were also used for these experiments.

Materials and methods. The experimental animals were laboratory-bred, albino rats, weighing 160-180 g, fed a normal laboratory diet with unrestricted access to water. The females were selected at random, marked, and distributed 5 to a cage. Their vaginal smears were studied for 2 to 3 weeks in order to ascertain the regularity of their estrous cycles; female rats showing irregular cycles were discarded. On the 16th day of steroid treatment, 2 males were placed in each cage of 5 females and were withdrawn after 10 days of cohabitation. Steroid treatment was terminated on the 31st day, and the females were observed until the 50th day, in order to allow the pregnant ones to reach delivery time. Daily vaginal smears were obtained with a suitable pipette after lubricating the vagina with a drop of saline. This procedure was used to minimize trauma. The smears were stained with methylene blue; cornified epithelial cells without leucocytes were regarded as evidence of estrus, and the presence of spermatozoa was considered an indication of mating. The steroids used were the following: progesterone, 17α-acetoxyprogesterone (AP), 6α-methyl-17-α-acetoxyprogesterone (MAP), 17α-ethynyl-19-nortestosterone acetate (ENTA), and the cyclopentyl enol ethers of the 3 latter compounds (APc-5, MAPc-5 and ENTAc-5 respectively).*

The progestins were dissolved in 0.2 cc of sesame oil and administered daily by gastric tube, with the exception of progesterone which was given subcutaneously.

Results and discussion. Table I shows the effect of graded doses of progesterone on the estrous cycle of the rat. At a dose of 2 μmoles/animal/day there was a slight prolongation of the interval between 2 estrus-positive smears; increasing delay was obtained using 4 μmoles/day, and complete inhibition with 8 μmoles/day. These results, showing that diestrus can be lengthened in proportion to amount of progesterone administered, are essentially in agreement with the findings reported by others(5-8).

Table II compares the effects of AP, MAP, and ENTA with those of their respective cyclopentyl enol ethers. Neither AP at 1-2 μmoles/day, nor its enol ether derivative even at 20 μmoles/day, showed any inhibitory effect on the estrous cycle, mating, or fertilization confirming earlier findings(4) that AP cyclopentyl enol ether was endowed with strong oral progestational activity but devoid of contraceptive effects. On the other hand, MAP proved to be a potent inhibitor of estrus in the rat. At 0.5 μmole/day more than half of the animals showed inhibition of the cycle; in the remaining ones, there was a marked prolongation of the cycle. At 1 μmole/day all the females failed to have their estrus. Furthermore, both dosage levels of MAP prevented mating. Thus the results, reported by Sala *et al.*(9) on subcutaneous administration of MAP, can be obtained also by oral administration. Interestingly, enol etherification of MAP with cyclopentylic al-

* The physical constants of these 3 compounds are: APc-5, m.p. 158-159.5°, $[a]_D = -142°$; MAPc-5, m.p. 169-171°, $[a]_D = -172°$; ENTAc-5, m.p. 181-183°, $[a]_D = -215°$.

TABLE II. Influence of 17α-acetoxyprogesterone (AP), 6α-methyl-17α-acetoxyprogesterone (MAP), 17α-ethynyl-19-nortestosterone acetate (ENTA) and of Their Cyclopentyl Enol Ethers on Estrous Cycle, Mating and Fertilization of Female Rats.

Compound	Dose/animal per day		No. of rats with estrous cycle*		Length of cycle (days) †	No. rats mated	No. of matings per rat in 10 days		No. of deliveries
	mg	μmoles	Maintained	Inhibited			Mean	Range	
Sesame oil	—	—	10	0	4.0 ± .1‡	10	1.1	(1-2)	10
AP	.372	1	10	0	4.6 ± .4	9	.9	(0-1)	7
	.745	2	10	0	4.7 ± .2	9	.9	(0-1)	8
APc-5	.881	2	10	0	4.6 ± .4	10	1.0	(1-1)	8
	4.406	10	10	0	4.6 ± .4	10	1.2	(1-2)	9
	8.812	20	10	0	5.1 ± .3	10	1.3	(1-2)	10
MAP	.193	.5	4	6	7.2 ±2.3	2	.2	(0-1)	0
	.387	1.0	0	10	—	0	.0	(0-0)	0
MAPc-5	.227	.5	10	0	4.3 ± .1	9	1.0	(0-2)	8
	.455	1	10	0	4.3 ± .1	10	1.0	(1-1)	10
	.909	2	10	0	4.5 ± .3	10	1.0	(1-1)	10
	1.819	4	5	5	4.6 ± .5	6	.6	(0-1)	6
	3.637	8	0	10	—	9	1.0	(0-2)	7
ENTA	.170	.5	10	0	4.0 ± .1	10	1.3	(1-2)	7
	.340	1	10	0	4.3 ± .3	10	1.1	(1-2)	9
	.681	2	10	0	4.8 ± .3	10	1.1	(1-2)	4
	1.362	4	10	0	6.9 ±1.4	9	1.1	(0-3)	0
	2.724	8	0	10	—	9	2.2	(0-5)	0
ENTAc-5	.204	.5	10	0	4.4 ± .3	9	1.0	(0-2)	3
	.409	1	10	0	4.0 ± .4	9	1.3	(0-2)	0
	.817	2	1	9	9.0	10	2.4	(1-5)	0
	1.634	4	0	10	—	9	2.4	(0-5)	0
	3.268	8	0	10	—	8	2.1	(0-5)	0

* 10 animals/group. † In the animals showing estrus. ‡ Mean ± stand. error.

cohol brought about a marked reduction of its previously described anti-estrual activity —a dose of 8 μmoles of MAPc-5 was necessary to inhibit estrus in all the animals. It is noteworthy, however, that at lower dosage levels the cycle was apparently not prolonged; the doses which inhibited the cycle did not interfere with copulation and pregnancy in most cases. Thus it can be assumed that the only action of MAPc-5 was to inhibit epithelial cornification of the vagina, without affecting the occurrence of 'heat' (considered as willingness to mate), ovulation and fertilization. However, we know that sexual receptivity has been reported occasionally in old rats(10) or in rats likely to suffer from abnormalities of the reproductive tract(11) or in x-irradiated animals(12) at times other than typical 'estrus'.

ENTA and its derivative ENTAc-5 had different effects than the other progestins reported here. Table II shows that 2 μmoles of ENTA/day adversely affected ovulation and fertilization, although neither estrus nor mating was affected. Inhibition of estrus increased with dosage level—4 μmoles prolonged the cycle, and 8 μmoles inhibited it completely. These high dosages of ENTA did not hinder mating; in fact, in some of the animals mating was more frequent than expected. Treatment with ENTA, therefore, did not suppress 'heat' in these non-fertilizable females but probably enhanced it.

The cyclopentyl enol ether of ENTA, ENTAc-5, proved to be 4 times more potent than ENTA, for inhibition of estrus was produced by 2 μmoles of ENTAc-5 as compared with 8 μmoles of the parent compound; in addition, low dosage levels, *i.e.* 0.5-1.0 μmole, controlled fertility (*i.e.*, as measured by number of deliveries). However, sterility was only temporary because after termination of treatment the majority of females caged with males were fertilized within 10 days (data not tabulated).

Thus, ENTAc-5 appears to be a potent

inhibitor of estrus and conception in the female rat; in addition, it does not interfere with mating, nor have permanent effects on fertility.

Enol etherification by cyclopentylic alcohol, while reducing the anti-estrual and contraceptive activity of MAP, enhances those of ENTA. It is possible that these changes of activity reflect parallel changes in other biological properties; however, the progestational potency of MAPc-5 and of ENTAc-5 as judged by the Clauberg test, and of MAPc-5 as shown by the pregnancy maintenance test in ovariectomized rats†, appears to remain practically unmodified in comparison with their respective parent compounds. Enol etherification of both MAP and ENTA produces a striking dissociation in the contraceptive and progestional activities of the parent compounds; MAPc-5 is a less active contraceptive and anti-estrual agent than MAP, while ENTAc-5 is 4 times as active as ENTA in this respect. On the other hand, enol etherification of AP neither enhances nor decreases any inhibitory effects on estrus, mating or fertilization within the limits of the experiment.

Summary. Cyclopentyl enol ethers of 17α-acetoxyprogesterone, 6α-methyl-17α-acetoxyprogesterone and 17α-ethynyl-19-nortestosterone acetate were tested for ability to inhibit estrus, mating and fertilization in female rats with the following results: The first compound showed no inhibitory effect; the second blocked the estrous cycle, without interfering with mating and fertilization; the third proved to be a potent antiestrual and contraceptive agent, although it did not disturb sexual receptivity or cause permanent sterility. Thus, enol etherification reduces the antifertility effects of 6α-methyl-17α-acetoxyprogesterone, but strongly enhances those of 17α-ethynyl-19-nortestosterone acetate.

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† Unpublished observations (Falconi, G.)

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Comparison of Lipid Metabolism of Chicken Embryo Organs and Cells in Culture.* (26828)

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Differences exist in amino acid and carbohydrate metabolism(1,2) between primary cultures and established cell lines. Little is known of the relationship between the metabolism of primary cultures and parent organs. Therefore, a comparison of lipid me-

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