A TECHNIC SUPPRESSING DEVELOPMENT OF REPRODUCTIVE FUNCTION AND SENSITIVITY TO ESTROGEN IN THE FEMALE RAT*

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In many recent investigations of the rôle of sex hormones in the differentiation and development of sexual organs the heterologous sex hormone has been administered. In general, two technics have been used, indirect treatment of the fetus by injections into the pregnant female or into the amniotic cavity[3, 4, 5, 6, 9, 10, 11, 12, 16, 17, 18, 19, 22, 23] or, alternatively, direct treatment of the newborn.[1, 2, 8, 14, 21, 23, 25, 26]

Conspicuous alterations of structure have been reported, but relatively little attention has been given to the capacity of treated animals for normal reproductive function. Hamilton and Gardner[12] observed in mature female rats treated prenatally with 500 gamma of testosterone propionate that some degree of ovarian function was retained, as evidenced by the state of uteri, vaginae, and mammary glands, by the presence of follicles and corpora lutea in the ovaries, and by fluid in the uteri and vaginae. Turner[22] states that as little as 2 mg. of testosterone propionate administered to the mouse during intra-uterine development impairs ovarian function, as is indicated by the absence of cyclic estrous periods and by the failure of ovulation and luteinization. Selye[21] found that injections of 1 mg. of testosterone daily into one litter of female rats during the first 30 days of life resulted in deficient development of the ovaries, uterus, and vagina; prevention of the onset of puberty is ascribed to persistence of this condition. The present investigation was undertaken in an attempt to extend the knowledge relating to the functional capacities of the female reproductive system when subjected during developmental stages to the action of male hormone.

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Methods

The animals were divided into three groups depending on the time at which testosterone propionate* was administered. Rats in one group were subjected prenatally and postnatally to relatively small quantities of male hormone. Eight pregnant females were injected with testosterone propionate daily for 3 days beginning the 16th day of gestation. The total quantity of injected androgen varied from 1.5 to 6 mg. (Table 1). In all litters parturition occurred at term on the 22nd day. On the day following birth the young were given 1 mg. of male hormone, and this procedure was repeated 3 times weekly for 4 weeks; thus, each newborn animal received 12 mg. during the first 28 days of extra-uterine life.

Animals of the second group were treated only prenatally. Fourteen pregnant females were given injections of male hormone on 3 successive days, starting on the 14th, 15th, or 16th day of pregnancy. The total dose was from 6 to 50 mg. (Table 1). Parturition at normal term occurred only in the 2 animals that received 6 and 15 mg., respectively, beginning on the 16th day. The 12 animals receiving 30 mg. or more showed a tendency toward delayed parturition, the delay sometimes being as much as 5 days. Abnormal gestation in 7 instances was further indicated by the fact that the fetuses were macerated and partly resorbed, or the young, although born alive, survived no more than a few hours. Nevertheless, litters of viable young were obtained from 5 females past term which, along with the 2 litters born at normal term, became available for the observations and experiments described below.

Animals in the third group were injected postnatally. Beginning the day after birth, females from 10 untreated litters were injected 3 times weekly for 4 weeks with quantities of testosterone propionate totalling from 3 to 36 mg. (Table 1). The rate of increase in body weight of the injected rats was of the same magnitude as that in control rats of the same age. For prenatal injections testosterone propionate was dissolved in 0.25 cc. of peanut-oil, for postnatal treatment in 0.05 cc. of peanut-oil. All injections were made subcutaneously.

When the 91 experimental females in the 3 groups listed above were from 61 to 135 days of age, they, together with 18 normal females of approximately the same age, were examined at hourly intervals, day and night, for at least 24 days for signs of heat.[13] Three to four weeks later 37 representative animals including individuals from each of the 3 groups were spayed. The ovaries were weighed, fixed in Bouin's fluid, and embedded in paraffin. One ovary from each animal was then serially sectioned at 10μ and stained with Delafield's hematoxylin and eosin. Ten days after ovariectomy a biopsy from the middle of the right uterine horn was taken from 17

* Testosterone propionate, under the trade-name Perandren, was furnished through the courtesy of Ciba Pharmaceutic Products, Inc.

TABLE 1

THE EFFECTS OF PRENATALLY AND POSTNATALLY ADMINISTERED ANDROGEN ON SPONTANEOUS MATING BEHAVIOR

Litter No.	Hormone treatment in mg.		Age at beginning of observations (in days)	Number of females per litter	Mating behavior			
	pre-natal	post-natal			Normal	Moderate intensity; non-cyclic	Low intensity; non-cyclic	Absent
A1	1.5	12	83	5	0	0	0	5
A5	2.5	12	83	4	0	0	0	4
A9	4.5	12	81	6	0	0	0	6
A12	5.0	12	80	2	0	0	0	2
A13	3.0	12	80	4	0	0	0	4
A15	5.0	12	80	8	0	0	0	8
A18	6.0	12	73	3	0	0	0	3
A22	6.0	12	61	6	0	0	0	6
C1	6.0	..	73	4	3	0	0	1
C2	15.0	..	bred	5	5	0	0	0
C3	30.0	..	135	1	0	0	0	1
C4	30.0	..	135	5	0	2	0	3
C12	30.0	..	85	1	0	0	1	0
C30	50.0	..	72	6	0	4	0	2
D4	...	3	103	4	0	0	1	3
D3	...	6	104	6	0	0	3	3
D1	...	12	106	2	0	0	0	2
D2	...	12	105	2	0	0	1	1
D7	...	18	98	3	0	0	2	1
D12	...	18	76	4	0	0	0	4
D10	...	24	87	1	0	0	0	1
D5	...	36	102	2	0	0	0	2
D6	...	36	99	1	0	0	0	1
D11	...	36	80	6	0	0	0	6

representative animals. Four days later 100 R.U. of estradiol benzoate* were injected. After 72 hours a second biopsy from the middle of the left uterine horn was taken for microscopic study for comparison of the responsiveness to estrogen of this tissue with that of uterine tissue from spayed control animals.

The remaining 20 spayed animals were used in testing the sensitivity of the mechanism responsible for estrous behavior to the conditioning action of estrogen. The first series of injections consisted of 10 R.U. of estradiol benzoate followed 48 hours later by 0.4 I.U. of progesterone.* A second series of injections, given after an interval of 7 days, consisted of 100 R.U. of estrogen followed 48 hours later by 0.4 I.U. of progesterone. Another week was allowed to elapse before beginning a third series composed of 4 daily injections of 100 R.U. of estradiol benzoate followed on the fifth day by 0.4 I.U. of progesterone. The interval between injection of progesterone

* Estradiol benzoate (Progynon-B) and progesterone (Proluton) were made available through the courtesy of the Schering Corporation.

and the first elicited response is designated as the latent period; that between the first and the last response as the period of heat. Following the first two procedures, all rats were examined for the copulatory response at hourly intervals for 15 hours after the time of injection with progesterone. During the third procedure examinations for mating behavior were made at 8-hour intervals from the time of the first injection of estrogen until progesterone was given in order to detect behavioral responses induced by estrogen alone. Subsequent to administration of progesterone, hourly examinations were made for 20 hours. Control data were obtained from 15 animals spayed as adults and from 2 spayed at birth.

Experimental

Estrous behavior. The extent to which estrous behavior occurred in the 91 experimental animals is shown in Table 1. During the entire 24-day period of observation no responses which could be interpreted as sexual behavior were displayed by rats which received both prenatal and postnatal treatment with testosterone propionate.

The effects of prenatal treatment with the doses employed were less severe. Three of four animals from litter C_1, in which the dose of androgen was smallest, came into heat at normal cyclic intervals. The length of estrus and the intensity of the responses were similar to those shown by the 18 control animals. The members of this litter were subsequently placed with males. The three which had come into estrus bore litters, but for an undetermined reason the fourth was sterile. The five females from litter C_2, which were subjected to 15 mg. of androgen, were allowed to remain with brother siblings. All bore litters before they were 90 days old and therefore have been listed as normal. Animals in the four litters which received from 30 to 50 mg. of testosterone propionate either failed to show mating behavior or they displayed estrous responses which were abnormal from the standpoint of intensity, duration, and regularity of occurrence. Six of the 13 were not found in heat, one gave occasional single responses of a low grade, three displayed several non-cyclic estrous periods of short duration and moderate intensity, and three tended to display mating behavior of a low intensity with short intermittent lapses throughout the 24-day period of observation.

Postnatal injections alone had an effect which was not much less pronounced than that seen in the first group of animals which received androgen both prenatally and postnatally. Twenty-four of the 31 females in this group exhibited no sexual behavior. The

remaining seven displayed neither cyclic estrus nor separate periods of normal duration and intensity, but did give occasional single responses of a very low grade. Within the group there is no precise correlation between the total dose and response, but it is noted that the animals from litters which received more than 18 mg. of hormone failed to give any type of copulatory reflex.

The failure of most of these animals to display normal estrous behavior has been interpreted to mean that the androgen administered during early life impaired the assumption of reproductive function, either as a whole or in some essential part. In an attempt to determine the nature and extent of any alterations that might be involved, the ovaries and uteri were examined for morphological deviations, and the sensitivity of the behavioral mechanism and the uterus to injected estrogen was tested.

Condition of ovaries. An examination of ovaries from experimental animals and from control animals of a comparable age (100 to 140 days) indicates that the ovary is concerned, at least indirectly, in the failure to show normal cyclic changes. Animals in the group receiving prenatal and postnatal treatment possessed ovaries the weight of which was less than half that of ovaries from animals in the diestrum which served as controls (Table 2). Microscopic study

TABLE 2

EFFECT OF PRENATAL AND POSTNATAL ANDROGEN ADMINISTRATION ON OVARIAN WEIGHT AND CORPUS LUTEUM FORMATION

Time and quantity of androgen treatment	*Number of animals*	*Average weight of both ovaries in mg.*	*Animals showing corpus luteum formation*
1.5 to 6 mg. prenatally + 12 mg. postnatally	13	26.2	0
30 to 50 mg. prenatally	5	56.2	2
3 to 36 mg. postnatally	7	33.6	0
diestrous controls	6	56.0	6
estrous controls	6	63.1	6

revealed that growing and atretic follicles were present. Many normal-appearing follicles attained the size at which the preovulatory swelling ordinarily begins, but no evidence of luteinization was seen. In this respect they resemble those described by

Fevold, Hisaw, and Leonard,[7] Wallen-Lawrence,[24] and Rinderknecht and Williams,[20] all of whom considered such a condition a consequence of follicle-stimulating hormone action in the absence of stimulation by the luteinizing hormone.

Females subjected prenatally to male hormone had ovaries which were within the range of weight for normal ovaries. In 2 of 5 ovaries examined, follicular maturation and luteinization appeared to have been normal (Fig. 1), but in the others no corpora lutea were present (Fig. 2). The latter condition resembles that in ovaries removed from animals to which androgens had been administered both prenatally and postnatally.

Of the 20 pairs of ovaries removed from the animals injected postnatally, only 7 could be satisfactorily dissected from the capsule and other masses which surrounded them. In 13 cases the ovaries were completely enclosed in an abnormal mass of tissue which apparently resulted from excessive distention of the Fallopian tubes. An accumulation of caseous or highly viscous material had distended the tubes to a diameter of as much as 5 mm. This condition resembles pyometra. The 7 ovaries which were only slightly or not at all involved weighed considerably less than those of control animals. As in those removed from animals given prenatal and postnatal treatment with androgen and those from some animals treated prenatally, growing and atretic follicles were seen, but no corpora lutea were present (Fig. 3).

Condition of uterus. Uterine horns from rats to which androgen was administered both prenatally and postnatally were removed from 10 animals prior to ovariectomy and from 5 animals 10 days after ovariectomy. In contrast to the effects of ovariectomy in untreated females (Figs. 4 and 5), little difference was seen that can be attributed to the absence of the ovaries in the androgen-influenced animals (Figs. 6 and 7). The average cross-sectional area, which was 3.6 sq. mm. prior to ovariectomy, was 2.7 sq. mm. after ovariectomy, but in uteri removed from an animal during the diestrum and from an animal 10 days after spaying the cross-sectional areas were 4.3 sq. mm. and 2.3 sq. mm., respectively. No visible structural differences were seen after ovariectomy of the androgen-affected animals. Indeed, either set of tissues may be used as a basis for describing the structural changes which resulted from the androgenic treatment.

The cross-sectional area of such uterine horns (Figs. 6 and 7)

was less than that of untreated diestrous animals (Fig. 4), but greater than that of spayed untreated females (Fig. 5). Only a few glands were found and these were always atrophic. In 4 of the 5 uteri examined large metaplastic areas were seen in which the usual columnar cells of the mucosal epithelium were replaced by stratified cuboidal epithelium (Fig. 8). All the uteri appear to have been subjected to some form of stimulation, because the degree of development exceeded that in uteri of animals spayed at birth (Fig. 9). It is apparent, however, that the stimulus was not of a sort to produce a condition comparable to that in the intact diestrous animal.

Uterine tissue was not removed from the animals given androgen prenatally only or postnatally until 10 days after ovariectomy. It is compared, therefore, with that removed from the prenatally and postnatally treated females 10 days after spaying. As would be expected from the behavior and from the ovarian condition of the females given testosterone propionate prenatally only, the uterine horns of such animals (Fig. 10) were less modified than were those of animals which received postnatal injections as well. The cross-sectional area which averaged 4.6 sq. mm. was larger and approximated that of the diestrous uterus. A columnar epithelium was retained and many glands were present in the endometrium, although most were atrophic.

The uteri of animals treated with androgens postnatally only were even more abnormal than were those of animals given small doses both prenatally and postnatally. The cross-sectional area was smaller, averaging only 1.8 sq. mm. Metaplasia of the epithelium was more extensive and the stratified cuboidal epithelium seen in 2 of 6 uteri contained many cystic structures (Fig. 11). In 1 of the 6 uteri many desquamated epithelial cells and leukocytes were in the lumen.

Sensitivity to estrogen: (*A*) *Behavioral mechanism.* The possibility that the sensitivity to the action of estrogen might have been modified was investigated by ascertaining the behavioral and uterine responses of spayed animals in these groups to normal and increased quantities of estrogen. The data bearing on the behavioral responses are summarized in Table 3.

Animals that had received prenatal and postnatal androgen as well as those that received only postnatal treatment failed to respond to 10 R.U. of estrogen followed by 0.4 I.U. of progesterone, while the 15 control animals spayed as adults exhibited estrous periods

TABLE 3

THE EFFECT OF PRENATALLY AND POSTNATALLY ADMINISTERED ANDROGEN ON THE SENSITIVITY OF THE ESTROUS BEHAVIORAL MECHANISM TO ESTROGEN

	Androgen treatment in mg.		*Mating behavior following varied injection procedures*					
			10 RU estradiol b. 0.4 IU progesterone		*100 RU estradiol b. 0.4 IU progesterone*		*400 RU estradiol b. 0.4 IU progesterone*	
Animal number	*pre-natal*	*post-natal*	*latent period in hrs.*	*heat period in hrs.*	*latent period in hrs.*	*heat period in hrs.*	*latent period in hrs.*	*heat period in hrs.*
5A1	1.5	12	∞	0	∞	0		
14A9	4.5	12	∞	0	∞	0		
15A9	4.5	12	∞	0	∞	0		
16A12	5.0	12	∞	0	∞	0		
17A12	5.0	12	∞	0	∞	0		
8C4	30	..	7	6	5	7	4	7
11C4	30	..	3	11	3	14	*	20
12C12	30	..	∞	0	∞	0	*	0
15C30	30	..	∞	0	∞	0	∞	0
18C30	50	..	6	8	5	9	*	14
11D4	..	3	∞	0	∞	0	6	7
14D4	..	3	∞	0	∞	0	3	8
7D3	..	6	∞	0	∞	0	∞	0
1D1	..	12	∞	0	∞	0	6	9
3D2	..	12	∞	0	∞	0	1	18
20D7	..	18	∞	0	∞	0	1	15
28D12	..	18	∞	0	∞	0	∞	0
21D10	..	24	∞	0	∞	0	∞	0
15D5	..	36	∞	0	∞	0	1	15
22D11	..	36	∞	0	∞	0	7	4
15 controls spayed as adults	..	..	4.5	8.2	3.4	12.2	*	17.8
2 controls spayed at birth	..	..	7.0	6.5	3.5	12.5	*	19

* Came into heat on estrogen alone, before progesterone was injected.

averaging 8.2 hours in length and the two controls spayed at birth remained in heat for an average of 6.5 hours. Of the 5 prenatally treated females tested, 3 responded to 10 R.U. of estrogen followed by 0.4 I.U. of progesterone with heat periods comparable in length and in intensity to those of the controls. When the estrogen dose was increased to 100 R.U. the results were similar to those obtained with the smaller quantity, except that the length of the period of heat was generally longer. Injection of 400 R.U. of estradiol ben-

zoate was followed by the display of estrogen-induced periods of heat in 3 animals which had received androgens prenatally only and in all the control animals. Injection of 400 R.U. of estradiol benzoate followed by 0.4 I.U. of progesterone induced estrous behavior in a fourth animal which had received androgen prenatally and in 7 of the 10 animals which had been treated with androgen postnatally and which had not shown such behavior in consequence of previous injections of smaller quantities of estrogen.

It is clear from these results that male sex hormone in a total quantity as small as 3 mg. administered during the first 28 days of extra-uterine life was sufficient to render the adult rats, of this strain at least, insensitive to injected estrogen in an amount ten times that sufficient to condition spayed control animals for estrus. When the standard dose of 10 R.U. of estradiol benzoate was increased 40 times and was followed by 0.4 I.U. of progesterone, the majority of postnatally treated females responded, although there was no correlation between the quantity of androgen given postnatally and the responsiveness of an individual to injected estrogen. Animals receiving only prenatal androgen treatment of 30 to 50 mg. retained normal sensitivity to estrus-producing hormones in 3 of 5 instances.

Sensitivity to estrogen: (*B*) *The uterus*. The uteri from androgen-treated animals likewise failed to respond normally to quantities of estrogen which were adequate to stimulate changes in the control animals (Table 4). The lumina which had been narrow and slit-shaped (Fig. 12) became large and ovate with serrated margins (Fig. 13). A moderate amount of distention resulted from accumulation of fluid secreted by activated glands. The low columnar epithelium became the tall columnar type. An extensive proliferation and hypertrophy of glands occurred. Mitotic figures were numerous, especially in the surface epithelium and glands.

Except for the group of experimental animals to which androgen was administered prenatally only, injections of estradiol benzoate produced little change in the uterus comparable with that seen in the controls (Table 4), and even in these animals the increase was still far below that shown by the control animals. The result is significant in view of the intermediate position occupied by this group of animals with respect to spontaneous and induced mating behavior, the ovarian condition, and the structure of the uterus prior to estrogen administration.

A similar relationship was found when these uteri were studied

TABLE 4

EFFECT OF PRENATALLY AND POSTNATALLY ADMINISTERED ANDROGEN ON THE RESPONSIVENESS OF THE UTERUS TO INJECTED ESTROGEN

Time and quantity of androgen treatment	*Number of animals*	*Average cross-section area in sq. mm.*		*Average percentage increase*
		before injection	*after injection*	
5 to 6 mg. prenatally + 12 mg. postnatally	5	2.7	3.3	22
30 to 50 mg. prenatally	2	4.6	8.8	91
3 to 36 mg. postnatally	6	1.8	2.5	38
controls spayed as adults	6	1.9	5.1	168
controls spayed at birth	5	0.4	2.8	600

microscopically. In the mice to which androgen was given prenatally and postnatally, and in those treated postnatally only the uteri showed none of the changes seen in the controls. The lumina remained small and irregular (Figs. 7 and 14), except in cases of distention by masses of desquamated epithelial cells and leukocytes (Figs. 15 and 16). No instance of distention due to glandular secretion was observed. The endometrium retained its stratified epithelium and in some instances, particularly in the animals which had been injected postnatally only, the extent of stratification was greater (Figs. 15 and 16). Glands remained few and atrophic and mitotic figures were scarce throughout the endometrium. The uteri in animals which had been given androgen prenatally only showed a more nearly normal response (Figs. 10 and 17). The epithelium was converted from a low to a high columnar type. The glands, however, were not hypertrophic and there was no accumulation of fluid either in the glands or in the lumen of the uterus. Mitotic figures were abundant throughout the endometrium.

It is clear from the above data that the normal responsiveness of the uterus to injected estrogen was abolished by the quantities of testosterone propionate which were administered. The possibility that this refractoriness should be attributed to the absence of ovarian estrogen during postnatal development, rather than to the presence of androgen, is excluded by the growth stimulated by estrogens given to adult animals which had been spayed on the day of birth (Table 4). Like uteri in animals spayed as adults and injected with

estrogen, the microscopic structure of the uteri in these animals was normal (Fig. 18).

Condition of the external genitalia. A modification of the reproductive tract and external genitalia has been seen by all who have administered androgens during gestation or immediately after birth (*loc. cit.*). In addition to the condition of the ovaries and uterus described above, our observations of structural changes are concerned chiefly with differences between the effect of prenatally and postnatally administered hormone. At the age of 60 days females treated prenatally (excepting litters C_1 and C_2) were found to possess no externally patent vagina, a small clitoris, no hypospadias of the preputial fold, no *os penis*, and a configuration of hair on the perineum characteristic of the male. Postnatally treated females examined at a corresponding age likewise showed no externally patent vagina, but possessed a large hyperemic clitoris that was extensile, a well-developed *os penis*, a hypospadiac preputial fold, and the characteristic female distribution of hair on the perineum. The distance between the anus and clitoris was a few mm. longer in androgen-affected animals than in normal females.

Discussion

The results of these experiments demonstrate that the development of reproductive function is markedly impaired by postnatally administered testosterone propionate and to some extent by prenatal treatment. Existing data are not sufficient to permit a complete analysis, but it appears that one or both of two causes may be involved. The endocrine system responsible for reproductive function, in consequence of some defect, may not be capable of stimulating normal sexual activity, or the organism may have been rendered insensitive to stimulation even in the presence of adequate quantities of hormone.

As regards the failure of endocrine factors, little can be learned from the present investigation. The ovaries possessed growing follicles which reached pre-ovulatory size, but estimation of the production of follicular hormone was not possible because the uterus and vagina were not responsive to normal estrogenic stimulation. In most animals, luteinization did not occur and it may be assumed that normal quantities of corpus luteum hormone were absent. The failure of ovulation and luteinization indicates either that development of a normal pituitary-gonadal relationship had been prevented

or that the ovaries were insensitive to gonadotropic substances. Had Pfeiffer been able to produce masculinized pituitaries which secrete only follicle-stimulating hormone by means of injected androgens as well as by implanted testes,[15] a plausible explanation for the results we have obtained could be given. Injection of androgen into newborn rats, however, has not had this effect (Pfeiffer, personal communication) and, unless the androgen he injected is different from that secreted by the testis, another explanation must be sought.

The suggestion that at least localized parts of the organism may have been rendered insensitive to estrogen stimulation is supported by data obtained from the present study. The tissues of the uterus were incapable of normal response to estrogen. In addition, the behavioral mechanism was wholly insensitive to 10 times the quantity of estrogen that produced a response in 100 per cent of control rats and only partially sensitive to 40 times the amount causing a complete response in controls. These observations indicate that androgenic treatment shortly before or shortly after birth alters the normal threshold of sensitivity to estrogen, but it is not known whether, with respect to behavior, it does so by raising the threshold to estrogen or by preventing the acquisition of normal sensitivity during the first 30 days of extra-uterine life (Wilson and Young, unpublished data). Furthermore, it is not known whether this result is produced by a direct action of androgen on the tissues or through an intermediary organ such as the pituitary. The possibility that absence of stimulation by ovarian estrogen might account for the low sensitivity is excluded by the behavioral and uterine responses shown by animals spayed on the day of birth and injected with estrogen as adults.[27]

The effect of experimentally administered androgen on the sensitivity to estrogen is regarded as clearly shown. Less certain is the likelihood that the changes in sensitivity which take place as an animal develops[27] are controlled by androgen present in the organism during this period. The demonstration has still to be made that androgen has this rôle in the normal female animal.

Summary

Female albino rats were placed under the influence of testosterone propionate during the late prenatal and early postnatal periods and then allowed to mature. The capacity for reproductive function was tested by observation of spontaneous mating behavior,

by microscopic examination of the ovaries and uterus, and by the behavioral and uterine responses to injected estrogen.

Postnatal treatment, in particular, was followed by (1) failure to display spontaneous cyclic changes in behavior, (2) a failure of ovulation and of corpus luteum formation, although the follicles attained the size at which the pre-ovulatory swelling ordinarily begins, (3) abnormal differentiation of the uterine endometrium, and (4) following the injection of estrogen, absence of (a) normal responses of growth in uterine tissues and (b) normal mating behavior. The manner of action of testosterone propionate in producing these modifications is discussed.

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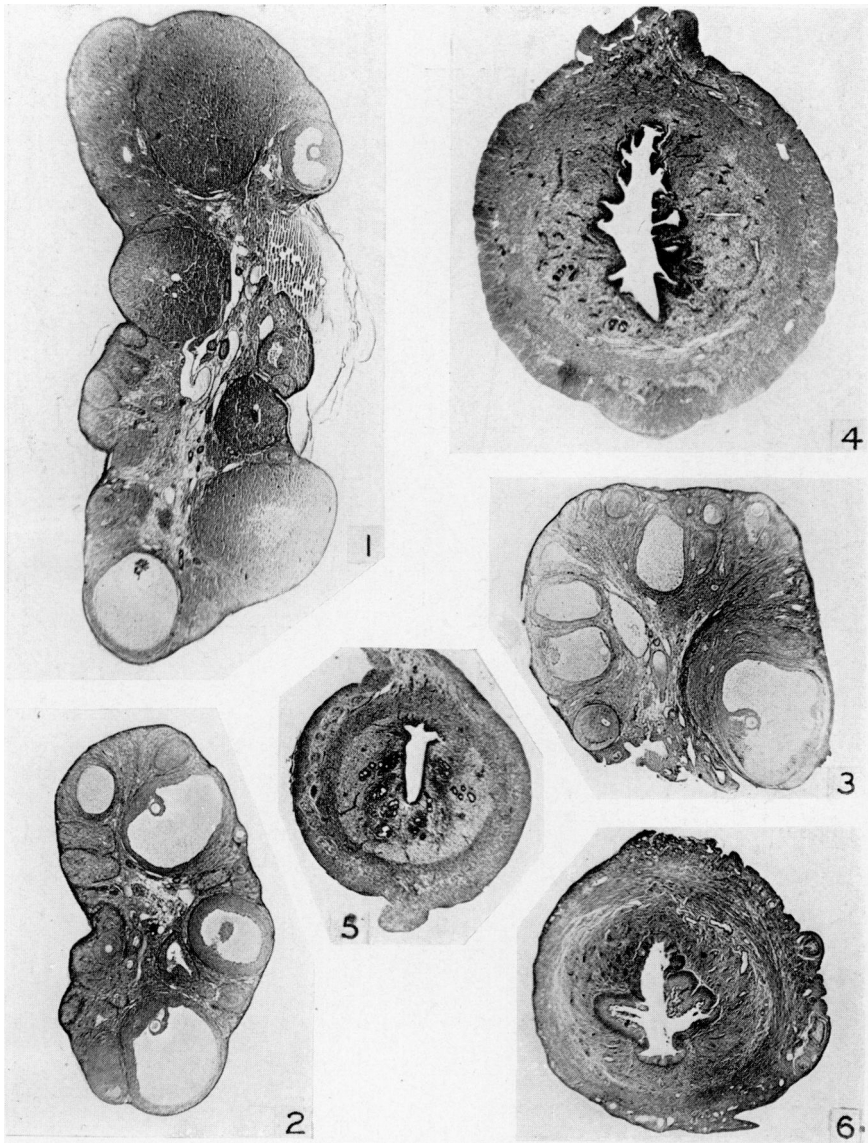

FIG. 1. Ovary from a rat treated prenatally with testosterone propionate. Follicular growth and corpus luteum formation appear to be normal. × 17.

FIG. 2. Ovary from a rat receiving same treatment as that described in Fig. 1. Although follicular growth is extensive, no corpora lutea are present. × 17.

FIG. 3. Ovary from an animal injected postnatally with androgen, showing growing and atretic follicles but no corpora lutea. × 17.

FIG. 4. Uterine biopsy from untreated, intact animal in diestrum. × 19.

FIG. 5. Uterine biopsy 10 days after ovariectomy to illustrate retrograde changes in untreated animals. (For comparison with Fig. 4.) × 19.

FIG. 6. Uterine biopsy from intact animal that received prenatal and postnatal treatment with androgen. × 19.

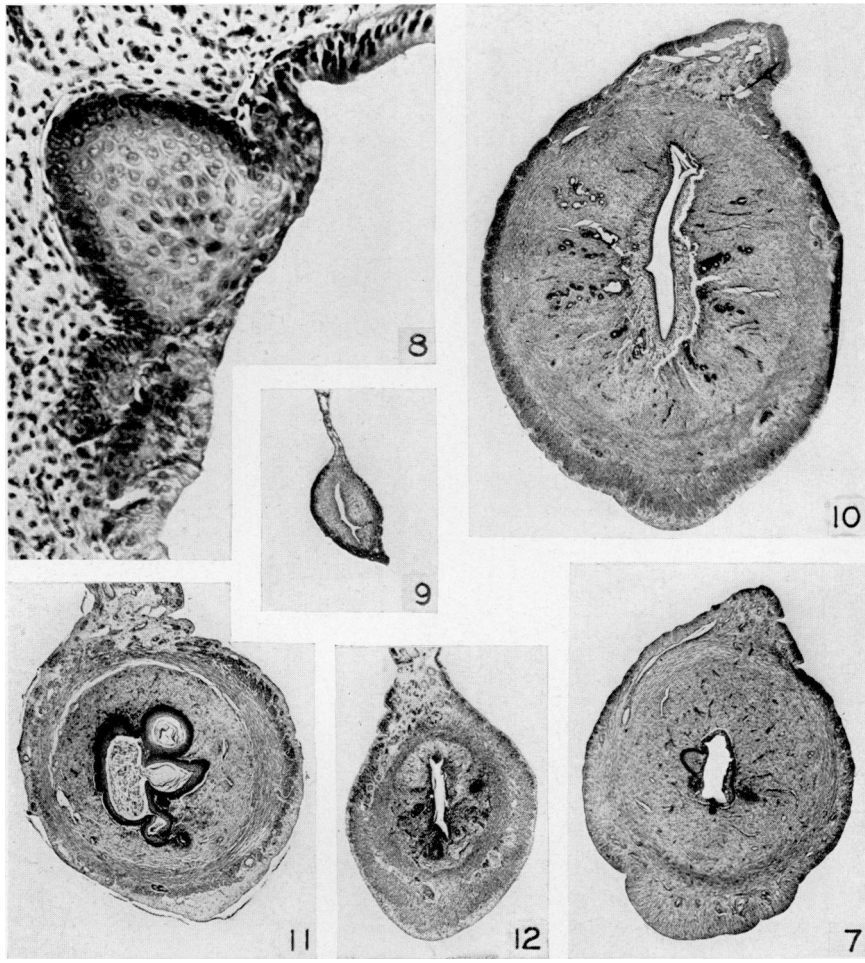

FIG. 7. Uterine biopsy 10 days after ovariectomy from a rat given androgen prenatally and postnatally. (For comparison with Fig. 6.) × 19.

FIG. 8. Area of metaplastic epithelium from mucosa of uterus shown in Fig. 7. × 200.

FIG. 9. Uterine biopsy from untreated adult female, spayed the day of birth. × 19.

FIG. 10. Uterine biopsy 10 days after ovariectomy from an animal given androgen prenatally. For comparison of results following other types of androgen treatment see Figs. 7 and 11. × 19.

FIG. 11. Uterine biopsy 10 days after ovariectomy from a rat injected postnatally with male hormone. For comparison of effects produced by other types of androgen treatment see Figs. 7 and 10. × 19.

FIG. 12. Uterine biopsy from an untreated rat ovariectomized as an adult 3 months previously. × 19.

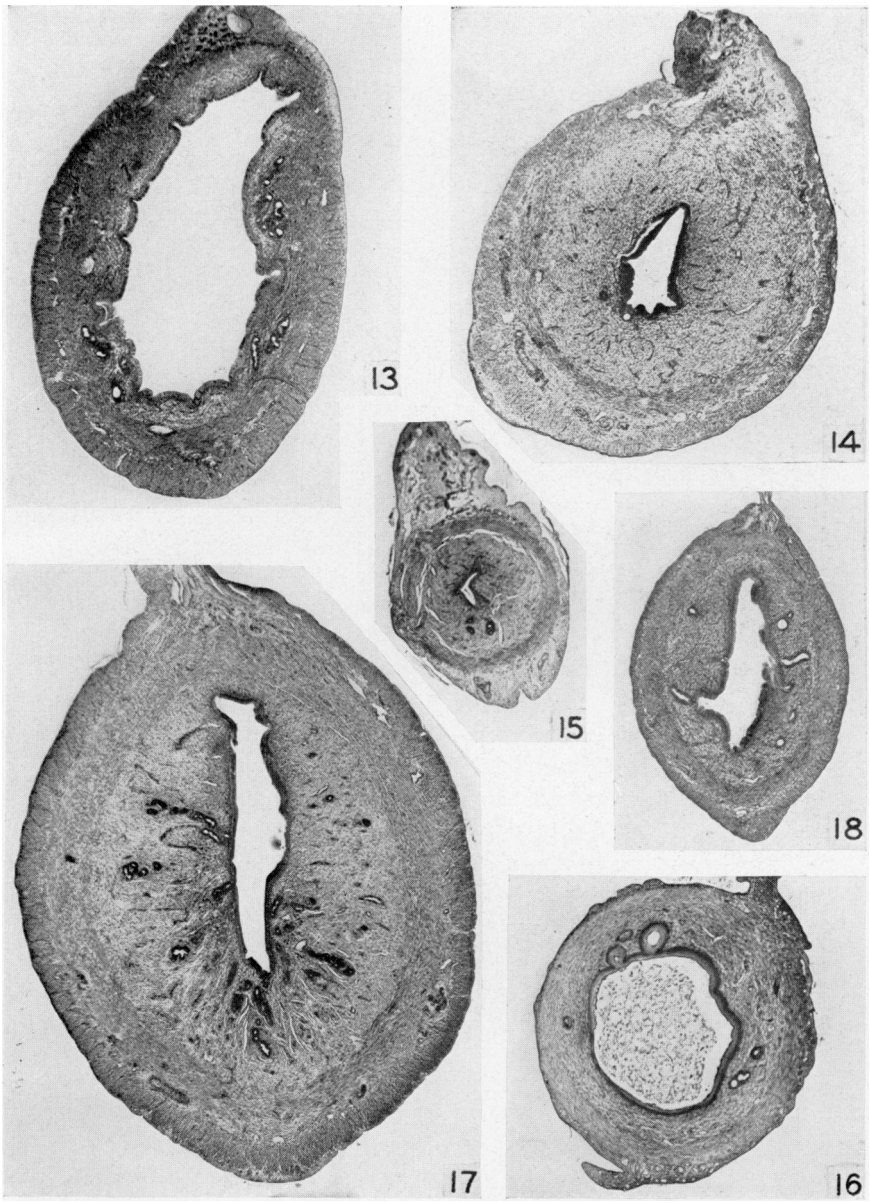

FIG. 13. Uterine biopsy from the spayed animal described in Fig. 12, 3 days after injection with 100 R.U. of estradiol benzoate. × 19.

FIG. 14. Uterine biopsy 3 days after estrogen injection and 15 days after ovariectomy. From the prenatally and postnatally treated animal described in Fig. 7. × 19.

FIG. 15. Uterine biopsy 10 days after ovariectomy. From a rat treated postnatally with androgen. × 19.

FIG. 16. Uterine biopsy from the animal described in Fig. 15, 3 days after injection with estrogen. × 19.

FIG. 17. Uterine biopsy from the prenatally-treated, spayed rat described in Fig. 10, 3 days after estrogen injection. × 19.

FIG. 18. Uterine biopsy taken 3 days after estrogen injection. From the animal spayed the day of birth and described in Fig. 9. × 19.