CIII. THE ACTION OF TESTOSTERONE PROPIONATE ON NORMAL ADULT FEMALE RATS

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IN our previous papers [Korenchevsky & Dennison, 1936; Korenchevsky *et al.* 1936, 1937] we have shown that the male sexual hormone testosterone and its ester, testosterone propionate, have a powerful effect on the organs of ovariectomized rats. In the present paper the effect of testosterone propionate on normal female rats is described.

Technique

The technique used was the same as that in our previous papers.

Artificial testosterone propionate was prepared and supplied by Messrs Ciba Ltd. Large doses of 0.5 and 1.5 mg. daily were chosen in order to overcome the effect of any antagonistic hormones which might be present in the female organism.

The experiment was performed on 16 rats belonging to 3 litters. The rats were divided in such a way that litter-mates were present in each of the 3 groups. The average final age of the rats was 98 days and the hormone was injected daily for 21 days. The weights of the organs are summarized in Table I. In order to economize space the weights per unit of body weight are not given, but, as in our previous papers, where the conclusions based on these differ from those obtained from the actual weights, this is mentioned in the text.

The detailed histological results will be published elsewhere.

Table I. *Effect of testosterone propionate on normal female rats*

The average actual weights of the organs of rats injected with the hormone compared with those of the organs of uninjected litter-mates

Organs	Weight of organs of		
	Rats injected with testosterone propionate per day		
	Control rats	500 γ	1500 γ
Ovaries (mg.)	75	74	97
Uterus (horns + cervix, mg.)	350	694	955
Cervix of uterus (mg.)	102	268	359
Vagina (mg.)	293	516	550
Female preputial glands (mg.)	103	224	256
Adrenals (mg.)	69	59	64
Thymus (mg.)	261	89	62
Kidneys (g.)	1.48	1.73	1.90
Abdominal fat (g.)	17.6	10.4	9.9
Final body weight (g.)	243	217	227
Gain in body weight (g.)	26	23	28
No. of rats in group	6	4	6

For 1 week before and during the experiment smears were taken from the vagina every day in order to observe any effect of the injections on the sexual cycle.

Effect on the sexual cycle

While normal cyclic changes occurred in the uninjected control rats, from the end of the first week oestrus was completely suppressed in the rats injected with 1500 γ . A number of apparently cornified cells appeared during the 3rd week in the smears of the rats injected with 500 γ , although no cornification was found in histological sections, but only different degrees of mucification. With these exceptions, during the 2nd and 3rd weeks of the injections the smears of the injected rats consisted of clear mucus and varying numbers of leucocytes. Therefore oestrus was completely suppressed with the higher dose of testosterone propionate and at least partially with the lower dose.

Effect on weights of organs

Ovaries. In some of the rats injected with the largest dose (1500 γ) there were quite definite macroscopical changes in the ovaries. The ovaries in these rats were abnormally large and, as judged from their macroscopical appearance, contained more and larger corpora lutea than are present in normal ovaries.

Uterus. Both the horns and the cervix of the uterus were greatly hypertrophied after the injections. Such average weights of the uterus as 955 mg. are only found in normal rats during oestrus and are due chiefly to accumulation of secretion in the lumina of the horns which are thus distended. The largest uterus obtained in the rats injected with 1500 γ weighed 1209 mg. Preliminary histological investigations showed that this increased weight was due to an increase in the thickness of the myometrium and mucosa and not to the accumulation of secretion in the uterus.

The hypertrophy of the uterine horns produced by hormonal action during normal pregnancy can be judged by the size of the "empty" or "sterile" horn, i.e. the horn into which ova did not penetrate and which therefore is without foetuses. This happens in rats only exceptionally. The comparison of such a horn with that produced by testosterone propionate injections showed the latter to be the larger owing to a greater thickness of both the mucosa and the myometrium. Apart from this abnormal hypertrophy and occasional vacuolation of uterine epithelium neither the mucosa nor the myometrium showed any special pathological changes. The histological appearance was reminiscent of but not identical with that of these layers during pregnancy.

Vagina. In the injected rats the vagina was much larger than that found during any period (including oestrus) of the normal sexual cycle. We have not weighed a sufficient number of "pregnancy" vaginas to compare the weights of these with those of the vaginas of the testosterone propionate-injected rats. A preliminary histological investigation has shown that various degrees of mucification of the epithelium were present in the vagina, in some cases reaching that observed during pregnancy. In these rats the development of the muscular and sometimes of the epithelial layer was even greater than that seen in pregnant rats.

The preputial glands were greatly hypertrophied as had already been found in ovariectomized rats (see references above).

Mammary gland and nipples were considerably hypertrophied in the injected rats.

A comparison of the effects of testosterone propionate on the sexual organs of normal and ovariectomized rats. The effect on the weights of the sexual organs of ovariectomized rats was shown in Table II of our previous paper [Korenchevsky *et al.* 1937, p. 479]. If these figures are compared with the weights of the sexual organs of the normal injected rats of the present experiment, it is clear that testosterone propionate, both when injected alone or in combination with oestrone, brought about a much smaller hypertrophy of the uterus and vagina of ovariectomized rats than was obtained with normal rats. For example in ovariectomized rats 700 γ of testosterone propionate in combination with 6 γ of oestrone produced on the average a uterus weighing 236 mg. and a vagina weighing 339 mg., while in the normal rats with 500 γ of testosterone propionate alone the average weight of the uterus was 694 mg. and of the vagina 516 mg. The degree of hypertrophy of the female preputial glands was about the same both in the normal and ovariectomized injected rats.

Adrenals. The size and weight of the adrenals decreased with the daily dose of 500 γ but with the larger dose this effect was not seen. Perhaps therefore it is not accidental that a weaker effect with larger dose was also seen after the injection of testosterone propionate into ovariectomized rats [Korenchevsky *et al.* 1937, p. 479, Table II, cols. IV and V].

Thymus. The rate of involution of the thymus, as has also been previously found in ovariectomized rats, was greatly increased by injection of the hormone.

Other organs. Except in the case of the kidneys, which slightly increased in weight (both in actual weight and per unit of body weight), no definite changes were seen in the other organs.

Fat deposition and gain in body weight. The lower final body weight of the rats in the two injected groups as compared with that of the control group was due to the lower initial weight of the former (injected groups 194 and 199 g. respectively, control group 217 g.). In spite of this initial difference the gain in body weight of the injected rats did not differ greatly from that of the controls, while the fat deposition (as judged by the abdominal fat) was considerably less in all the injected rats. This indicates that in normal rats even such high doses as 1.5 mg. per day bring about increase in the catabolic action on fat and on some of the organs (thymus and perhaps adrenals), but these doses do not interfere with, but even stimulate, the anabolic processes of building of tissue (other than fat) in most organs. The greatly stimulated growth and development of the sexual organs and the effect on the kidneys are examples of the increase in the anabolic effect.

Testosterone propionate was also found to have an effect on the metabolism of ovariectomized rats as was shown in our previous paper [Korenchevsky *et al.* 1937, p. 481].

DISCUSSION

The bisexual properties of testosterone propionate

Our previous and present experiments with testosterone propionate, injected either alone or simultaneously with oestrone, have shown the following noteworthy and rather unexpected facts:

- (1) This male hormone, having the strongest effect on the sexual organs of male rats, has also the strongest stimulating effect on the development of the sexual organs of female rats.
- (2) The effect on the female sexual organs consists in the production of changes similar to those seen during pregnancy, i.e. typical of the most "female sexual" function.

(3) The return towards normal weight of the atrophied sexual organs of ovariectomized rats is greater with testosterone propionate (in the doses used) than with oestrone or oestradiol (up to 30 γ daily).

(4) Both the "method of weights" and histological investigation show that in producing pregnancy changes there is a co-operative activity, and not an antagonistic action, between these "male" and "female" hormones, though the suppression of the cornifying effect of oestrone or of oestradiol by testosterone propionate may be considered as an antagonistic effect.

(5) In female rats, the toxicity of such comparatively small doses of oestrone and oestradiol as 30 γ per day is shown by loss of appetite and fall in body weight, while there is only a check in the gain in body weight when such large doses of testosterone propionate as 1500 γ are administered to ovariectomized rats; and even the latter effect is not seen in normal rats.

The definition of male and female sexual hormones

With few exceptions, bisexual property, though weak in some hormones, must be considered to be one of the common properties of nearly all the sexual hormones. This fact introduces confusion into the classification of the sexual hormones as "male" and "female" hormones.

In the following therefore an attempt at classification from the point of view of male and female activity is made on the results of our experiments on rats with all the most important so-called "male" and "female" hormones.¹ The scheme however should not be considered as a final classification, but as a suggestion for critical consideration, as new data may throw more light upon the subject. Since the sexual functions and conditions in human beings are much closer to those in mammals than in birds, the experimental results obtained on the former are more important than those on the latter from the point of view of human physiology and pathology. Up to the present we have found no reason for changing our attitude, which we have repeatedly expressed in our papers since 1932, namely that mammals and not birds should be used for assays and the characterization of the sexual hormones. This does not of course deny the biological importance and interest of the experiments on birds.

On the basis of the results of our experiments we classified the hormones investigated as follows:

I. Purely "male" and "female" hormones

The definition of a hormone as the "male" hormone or the "female" hormone is only justified if this special hormone (alone or in co-operation with another) has an effect only on the male or only on the female sexual organs, respectively. The results of our experiments with all the sexual hormones that we have investigated up to the present show that such a definition can only be applied to progesterone (these experiments will be published elsewhere), which compound has no definite effect on male rats and which therefore could be classified as a pure female hormone.

II. Partially bisexual hormones

(1) *Hormones with chiefly "male" properties.* In this group may be included those sexual hormones which in castrated males cause a return to or towards normal of all the atrophied sexual organs including the prostate, penis and preputial glands. The hormones of this group also bring about a return towards

¹ See the references to the papers [Korenchevsky *et al.* 1937, pp. 481, 485].

the normal weight of the uterus and vagina of ovariectomized rats. With the same doses, however, the degree of this recovery is smaller than that of the atrophied sexual organs of castrated male rats. To this group belong androsterone, testosterone propionate, androstenediol and possibly Δ^4 -androstenedione.¹

(2) *Hormones with chiefly "female" properties.* These hormones (alone or in combination with another or others) should produce in ovariectomized rats typical female sexual changes such as oestrus, pregnancy changes or a return or considerable approach to the normal condition of the atrophied uterus and vagina, but in castrated male rats should not be able to stimulate to any considerable degree the development of all male sexual organs (in particular prostate, penis and preputial glands). Absence of effect on or only a slight increase in the weight of the prostate, penis and, in both sexes, of the preputial glands is an important differentiating feature.

Oestrone and oestradiol belong to this group. These hormones do not cause any considerable return towards normal of the atrophied prostate, penis and male preputial glands (or even of the female preputial glands, which always respond to the hormones of the previous group), although they produce a definite increase in the weight of the seminal vesicles (with specific histological changes in the latter and also in the coagulating glands and prostate [Korenchevsky & Dennison, 1935]).

III. *True bisexual hormones*

The hormones of this group should cause a return to or towards the normal condition of all the atrophied sexual organs and approximately to the same degree in both male and female gonadectomized rats. To this group seem to belong *transdehydroandrosterone*, *testosterone* and Δ^5 -androstenediol.

The hormone with the most powerful male properties, *testosterone propionate*, while it does not belong to this group, shows some approach to this group, since in suitable doses, in combination with oestrone, it produces progestational changes similar to those observed during pregnancy both in the uterus and in the vagina, and also the return of some of the sexual organs to nearly normal size, while others become supranormal in weight and size.

SUMMARY

1. Experiments were performed on 16 normal adult female rats belonging to 3 litters in order to investigate the effect on the sexual and other organs of prolonged injections of large doses of testosterone propionate.

2. The hormone, in the doses used, suppresses the appearance of normal oestrus.

3. The hormone has a powerful stimulating effect on the uterus, vagina and preputial glands and to a less degree on the mammary gland of normal females, bringing about hypertrophy of these organs to a weight and size much greater than that observed during normal dioestrus.

4. The histological changes produced are reminiscent of those seen during pregnancy, though the hypertrophy of the uterine mucosa and myometrium considerably exceeds the development of these layers in the "empty" uterine horn of a normal pregnant rat.

5. Thus, in agreement with our previous experiments on ovariectomized rats, the present experiments confirm the strong bisexual properties of testosterone propionate.

¹ Further evidence may show that this last belongs to Group III.

6. With regard to the action on the other organs investigated a definite effect is seen on the thymus (a considerable increase in the rate of involution of this gland) and on the kidneys (slight increase in weight).

7. Fat deposition decreases as the result of the injection of the doses used, but the gain in body weight does not show considerable changes, such combination indicating a special effect of this hormone on metabolism.

8. All the sexual hormones investigated by us have been classified into 3 groups on the basis of their male and female sexual activities.

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