LXV. THE PROLONGED TREATMENT OF CASTRATED AND OVARIECTOMIZED RATS WITH TESTOSTERONE PROPIONATE

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MIESCHER *et al.* [1936, 1, 2] found that the activity of testosterone on male sexual organs was greatly increased by esterification. Of the various esters prepared the propionate, as judged by short-duration experiments, proved to be the most effective. It brought about a greater increase in the size of the male sexual organs in castrated rats and also a longer duration of the effect than was obtained with other esters; e.g. after a single injection of 2 mg. of testosterone propionate, dissolved in 2 ml. of sesame oil, or after two injections of 1 mg. in 1 ml. of sesame oil with a 5-day interval, the maximum effect was obtained on about the 11th day after the first injection, the activity lasting at least 2-4 weeks. With testosterone the activity had disappeared by the 11th day, the maximum effect being observed a few days after the injection. Parkes [1936] also confirmed the results quoted above in short-duration experiments on the prostate and seminal vesicles.

Such properties of the propionate would be important from a therapeutic view-point and it is also possible that testosterone is normally present in the body as an ester.

On the basis of the experiments with the esters of testosterone Miescher *et al.* [1936, 2] confirmed the conclusion which we have repeatedly emphasized in our papers since 1932 [Korenchevsky, 1932; Korenchevsky *et al.* 1933; Korenchevsky & Dennison, 1934, 2; 1935, 2; Korenchevsky *et al.* 1935, 1] and also at the "Second Conference on the Standardization of Sex Hormones" (1935, London), that the capon unit is insufficient for the standardization of the male sexual hormones and that a rat unit should also be introduced. In addition to the results of our experiments Miescher *et al.* gave a good example in favour of our conclusion, namely that in the capon test testosterone benzoate was practically inactive, whilst in the rat test it was active.

In our previous papers we have shown (1) that for the characterization of the hormones it is essential to obtain the results of prolonged treatment, (2) that the male hormones, in particular testosterone, have many of the important properties of the female hormones and (3) that the sexual hormones have an important influence on various organs other than the sex organs. Therefore in the following experiments prolonged treatment with testosterone propionate was carried out not only on males but also on females, various organs, in addition to the sex organs, being investigated.

TECHNIQUE

The general technique was the same as that used in our previous experiments. Artificial testosterone and testosterone propionate were prepared and kindly supplied by Messrs Ciba Ltd., and oestrone by Prof. Girard. The hormones were

dissolved in sesame oil, the daily dose being injected in half-doses (0.1 ml. of oily solution) twice a day. In the present experiments all injections were made for the prolonged periods of 23 days in the case of male rats and 21 days in that of the female rats, in order that the results obtained might be compared with those previously published [Korenchevsky *et al.* 1936; Korenchevsky & Dennison, 1936, 2]. For the same reason the particular doses used were selected for the present experiment.

Further details of the technique used are given at the beginning of the description of each experiment. In order to economize space only the actual weights of the organs are given in the tables, reference being made in the text where the weights per unit of body weight (200 g.) suggest another explanation of the results obtained than that given by the actual weights.

1.2 mg. of testosterone propionate are equivalent to 1.005 mg. of testosterone.

THE COMPARISON OF THE ACTIVITY OF TESTOSTERONE PROPIONATE WITH THAT OF TESTOSTERONE ON MALE RATS

In each group of rats the results obtained both from the weight of the organs and from the preliminary histological investigation were not only sharply defined, but consistent.

Therefore, 8 litters containing 31 rats were sufficient for the experiment. The average age of the rats at death was 89 days and the rats were divided into 5 groups (Table I, cols. II–VI) in such a way that litter-mates or rats of similar age were present in each of the groups. In order to compare the activity of testosterone propionate with that of testosterone the results are given (Table I, cols. VII–IX) of a previous experiment on 12 castrated rats injected for 23 days with quantitatively the same doses of testosterone as those of testosterone propionate used in the present experiments. We also wished to confirm the claim, made by Miescher *et al.*, that after injection the activity of the propionate not only persists, but the maximum effect is seen on the average on the 11th day after injection. For this purpose in half of the rats in each group (cols. II, III and IV) the injections were started 9 days earlier than in the rats of the other half of the group. As all the rats were injected for the same period, the injection of the rats of the first group ceased 9 days before they were killed, and thus the rats in the two half-groups were killed at the same age. The number of days between the last injection and death is given in Table I, col. I.

Since the “testosterone rats” (aged 98 days) were older than the “testosterone propionate rats”, the data given for the normal and castrated litter-mates (cols. V and VI) of the “testosterone propionate rats” refer only to the latter. Similar data for the normal and castrated controls to the “testosterone rats” can be found in Table III, cols. I and II of our previous paper [Korenchevsky *et al.* 1936, p. 563]. However, as the ages of the two groups of rats happen to be not greatly different, the control weights of the organs do not show great differences and in most cases the data given for the control rats in the present paper may be taken for comparison with both the “propionate” and the “testosterone” rats.

Sexual organs. Rats killed 1 day after the last injection. As can be seen from Table I, cols. II–VI, the injection of 500 γ of testosterone propionate for 23 days brought about a return of the sexual organs to the normal weight, only the penis being slightly less than normal. The variation in the normal weight of the preputial glands is considerable and such a weight as 154 mg. may be considered normal for rats of the age of those in the present experiments. The largest daily

Table I. *Effect on male rats of testosterone propionate compared with that of testosterone*

The average actual weights of organs of castrated rats injected with testosterone propionate compared with those of the organs of normal and castrated litter-mates and with those of testosterone-injected rats

Organ	I	II	III	IV	V	VI	VII	VIII	IX
	No. of days between last injection and death	Castrated rats injected with testosterone propionate per day			Control rats		Castrated rats injected with testosterone per day		
		167 γ	500 γ	1400 γ	Normal	Cas- trated	167 γ	500 γ	1400 γ
Seminal vesicles (mg.)	1	766	1228	1441	1005	14	81	180	276
	9	204	284	507	—	—	—	—	—
Prostate (mg.)	1	887	1242	1165	1167	63	230	367	554
	9	463	614	774	—	—	—	—	—
Penis (mg.)	1	283	301	322	336	78	208	243	277
	9	267	276	295	—	—	—	—	—
Preputial glands (mg.)	1	153	154	226	188	67	120	129	185
	9	118	118	165	—	—	—	—	—
Adrenals (mg.)	1	55	59	55	62	76	65	65	60
	9	55	59	48	—	—	—	—	—
Hypophysis (mg.)	1	14.3	12.5	15.1	12.7	15.8	14.1	13.3	15.5
	9	13.0	13.3	13.1	—	—	—	—	—
Thymus (mg.)	1	356	219	110	425	617	500	409	382
	9	376	291	120	—	—	—	—	—
Liver (g.)	1	11.4	12.1	12.5	12.9	10.7	12.9	12.6	13.8
	9	12.2	11.5	11.6	—	—	—	—	—
Kidneys (g.)	1	1.92	2.03	2.30	2.21	1.72	2.01	1.98	2.22
	9	1.99	1.84	2.22	—	—	—	—	—
Heart (mg.)	1	963	932	1062	1001	974	976	919	1023
	9	929	927	873	—	—	—	—	—
Retroperitoneal fat (g.)	1	9.1	12.2	10.1	15.9	14.3	15.6	17.1	17.2
	9	10.8	13.1	10.9	—	—	—	—	—
Final body weight (g.)	1	301	316	327	355	317	356	348	377
	9	294	318	322	—	—	—	—	—
Gain in body weight (g.)	1	99	77	64	91	74	85	85	92
	9	74	61	28	—	—	—	—	—
No. of rats in group		9	8	4	4	6	4	5	3

dose of propionate used, 1.4 mg., caused an increase in the weight of the seminal vesicles and preputial glands to a weight greater than normal, the weight of the penis increased to normal, while the weight of the prostate remained approximately the same as with the smaller dose of 0.5 mg.

Rats killed 9 days after the last injection. As is shown by the second line for each organ (Table I), after an interval of 9 days there was considerable atrophy of the seminal vesicles and prostate, which were respectively about 3 to 4 times and twice the weight of these organs in rats killed 1 day after the last injection. These lower weights, however, still represent considerable hypertrophy of the sexual organs as compared with those in castrated rats (col. VI). The corresponding decrease in weight was less in the case of the penis and preputial glands.

Comparison with testosterone. A comparison of the data in cols. II, III and IV with those in cols. VII, VIII and IX shows clearly that the activity of the propionate is much greater than that of testosterone. This difference in the effects is

greatest with the smaller doses (167 γ) and is less pronounced with the larger doses of 500 and 1400 γ .

The degree of restorative effect on the different sexual organs differed with the two hormones, the propionate restoring both the seminal vesicles and the prostate in a normal ratio to or towards normal, while testosterone had much less effect on the seminal vesicles than on the prostate. This difference is much less pronounced in the case of the penis and still less so in that of the preputial glands.

Adrenals. These glands which hypertrophy after castration return to the normal weight after testosterone injections but become slightly subnormal in weight after propionate injections, this effect persisting even 9 days after the last injection.

Thymus. The rate of involution of the thymus, decreased by castration, returned to normal after the injection of testosterone and of the small doses of propionate, but 500–1400 γ of this ester brought about a pathologically rapid involution, which was still maintained 9 days after the last injection.

Liver and kidneys. The slight atrophy of the liver and kidneys observed after castration, partially or completely disappeared after the injection both of the propionate and of testosterone, but this effect did not seem in all cases to last 9 days after the last injection.

Heart. In the present experiment it was not found that the heart of the castrated control rats (Table I, col. VI) was significantly less than that of normal rats (col. V), though in rats castrated for a longer time than those in the present experiment the weight of the heart is almost always subnormal. Consequently the changes produced by the propionate were not so clear as in the testosterone experiment [Korenchevsky *et al.* 1936, p. 563, Table III, cols. I–VI]. If however any significance can be attached to such small changes as those in the present experiment, these are of the same nature as those which we have previously observed after the injection of male hormones into rats castrated for a longer period. In agreement with this the largest heart was seen in the rat injected with the largest dose of the propionate (as in the case of testosterone) but 9 days after the cessation of the injections the slight decrease in the weight of this organ had reappeared.

Thyroid, hypophysis and spleen did not show definite or constant changes in weight and, therefore, before the histological investigation is completed, no conclusions can be drawn with regard to these organs.

Fat deposition and gain in body weight. As in our other experiments, it was found that the gain in body weight of castrated male rats (col. VI) was less than that of normal rats (col. V), but contrary to our usual observation no significant change in fat deposition was observed. In our previous experiments it was found that testosterone injections caused an improvement in the gain in body weight without any increase in the deposition of fat [Korenchevsky *et al.* 1936, pp. 564–5]. It was therefore concluded that this gain in body weight was due to the anabolic processes of building new tissues and not to an increase by fat deposition. Small doses (167 γ , col. II) of the propionate had a similar effect, but the largest dose caused a small decrease in the gain in body weight. It is remarkable that this decrease was more pronounced 9 days after than immediately after the last injection of the propionate and that with the smaller doses the gain in body weight also decreased. The decreased gain in body weight is possibly due to an increase in the catabolic processes in the organism caused by excessive doses of the propionate, while small doses may increase only the anabolic processes.

THE COMPARISON OF THE EFFECTS OF TESTOSTERONE PROPIONATE WITH
THOSE OF TESTOSTERONE OR OESTRONE ON FEMALE RATS

Experiments were performed on 35 female rats, belonging to 6 litters, divided into 7 groups as shown in Table II, cols. I-VII. These data were compared with those previously obtained with 20 rats injected with testosterone. As only part of these data has been published elsewhere [Korenchevsky *et al.* 1936; Korenchevsky & Dennison, 1936, 2], in this paper the complete data are given in Table II, cols. VIII-XI. An investigation was made of the effect both of the male

Table II. *Effect on female rats of testosterone propionate compared with that of testosterone*

The average actual weights of organs of spayed rats injected with testosterone propionate alone or in combination with oestrone compared with those of the organs of normal and spayed uninjected litter-mates and with those of testosterone-injected rats.

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
	Spayed rats injected with										
Organ	Control rats		Oest- rone 6γ	Testosterone propionate		Oestrone 6γ +		Testosterone		Oestrone 6γ +	
	Normal	Spayed		167γ	700γ	Test. prop. 167γ	Test. prop. 700γ	167γ	700γ	Test. 167γ	Test. 700γ
Uterus (horns + cervix) (mg.)	360	41	162	185	240	313	236	58	138	235	290
	—	36	157	186	—	273	—	45	113	—	—
Cervix of uterus (mg.)	111	20	63	73	100	155	111	25	71	94	104
	—	15	58	86	—	116	—	16	48	—	—
Vagina (mg.)	232	142	223	322	349	313	339	184	221	267	284
	—	119	196	249	—	313	—	139	194	—	—
Female preputial glands (mg.)	108	74	80	206	306	219	277	140	165	148	207
	—	77	67	225	—	192	—	167	145	—	—
Adrenals (mg.)	73	80	85	58	67	67	64	71	57	83	79
	—	83	67	49	—	56	—	53	64	—	—
Hypophysis (mg.)	14.6	14.1	14.5	13.7	13.3	14.7	13.7	11.2	14.0	12.2	12.5
	—	12.8	13.1	11.1	—	10.4	—	9.9	10.6	—	—
Thymus (mg.)	292	503	353	349	138	219	110	388	319	345	283
	—	792	507	317	—	361	—	437	409	—	—
Liver (g.)	8.0	9.5	7.0	10.2	8.9	7.4	7.5	8.9	8.0	8.6	8.7
	—	8.3	7.0	7.8	—	7.3	—	7.3	8.4	—	—
Kidneys (g.)	1.53	1.60	1.50	1.88	1.80	1.80	1.72	1.50	1.61	1.71	1.80
	—	1.52	1.36	1.55	—	1.44	—	1.43	1.65	—	—
Heart (mg.)	688	763	678	864	796	708	763	712	846	746	850
	—	717	581	635	—	588	—	574	740	—	—
Fat (g.)	11.7	20.3	9.0	14.4	7.2	11.1	5.8	18.0	14.4	20	20
	—	8.0	7.4	6.3	—	6.1	—	3.3	6.1	—	—
Final body weight (g.)	206	279	199	271	237	226	220	277	280	270	278
	—	211	188	182	—	170	—	155	204	—	—
Gain in body weight (g.)	40	70	15	42	44	25	23	37	5	34	22
	—	92	77	69	—	57	—	96	92	—	—
No. of rats in group	4	6	7	5	4	4	5	4	8	6	2

Note. For rats injected with testosterone propionate, first line represents weights of organs of rats 82 days old, second line those of rats 61 days old. For rats injected with testosterone, first line represents rats 106 days old, second line rats 56 days old.

hormones injected alone and in combination with oestrone. In other respects this experiment was arranged in the same way as the previous experiment on male rats. In Table II two lines of data are given for each organ of the propionate-injected rats and their controls (cols. I-VII), the upper line for rats which were on the average 82 days old and the lower line for those 61 days old when killed.

The respective lines in cols. VIII–XI give the data for the testosterone-injected rats, the average ages of which were 106 days (upper line) and 56 days (lower line). The control normal and spayed rats (cols. I and II), litter-mates of the testosterone propionate-injected rats (cols. III–VII), can therefore only be used for making an approximate comparison with the rats of the testosterone experiment (cols. VIII–XI). The true control data for these latter rats are given in Table V, cols. I and II, in our previous paper [Korenchevsky *et al.* 1936, p. 567]. These data however do not in most cases differ much from those of the controls of the present experiment.

The changes in the weight of the organs per unit of body weight will only be mentioned in those cases where the results per unit of body weight differ from those obtained from the actual weights.

Sexual organs. Since we always remove part of each horn with the ovary at ovariectomy in order to ensure the completeness of the operation, the weight of the “uterus” (horn and cervix) of the spayed rats in our experiments is always less by the weight of the parts of the horns removed, than it would otherwise be. Taking this into consideration the following conclusions may be drawn from the data given in Table II: (1) with the doses used, testosterone propionate caused a greater restoration to the normal weight of the atrophied uterus than did oestrone (cols. III–V); (2) in combination with oestrone the propionate (cols. VI and VII), as judged by the weight of the cervix, caused a return of the uterus to the normal weight; (3) although, as compared with the same doses of propionate, pure testosterone alone (cols. VIII and IX) or in combination with oestrone (cols. X and XI) was less effective, the large dose of testosterone (700 γ) with oestrone (6 γ) brought about nearly complete recovery of the weight of the uterus (col. XI); (4) the propionate both alone and in combination with oestrone produced a vagina, which was abnormally large for rats of that age, and preputial glands the increase in weight of which was even more abnormal (cols. II–VII). Though testosterone had a similar effect on the preputial glands (cols. VIII–XI), only with 700 γ of testosterone in combination with oestrone (col. XI) was a vagina (284 mg.) obtained, which was slightly above the normal (254 mg. at this age); (5) oestrone alone, in the doses used (6 γ per day 4 times a week), did not bring about a return of any of the sexual organs to the normal weight, the nearest approach to normal occurring in the case of the vagina; (6) a hypertrophy of the peripheral end of the urethra into the clitoris-like structure, as described in our previous paper [Korenchevsky *et al.* 1937], also occurred under the influence of testosterone and testosterone propionate, being more pronounced after the propionate injections.

Adrenals. Whilst the effects of ovariectomy and of oestrone injections (cols. I–III) were not definite, there was a considerable decrease in the size of the adrenals after the injection of the propionate both alone and in combination with oestrone. Testosterone alone, but not when combined with oestrone, had a similar effect, i.e. oestrone is able to depress this activity of testosterone.

Thymus. The rate of involution of the thymus, which is decreased by ovariectomy, was restored to (small doses) or exceeded (large doses, cols. V and VII) normal after the injection of the propionate either alone or in combination with oestrone. Oestrone alone or testosterone alone and in combination with oestrone had less effect.

Other organs. No definite changes were obtained in the weights of the thyroids, hypophysis, liver, kidneys, spleen and heart, especially when the weights per unit of body weight were considered, e.g. the increase in the actual weight of the liver of ovariectomized rats (col. II) as compared with normal rats (col. I) or the

decrease in that of the oestrone-injected rats was not seen when the weights were calculated per unit of body weight.

Fat deposition and body weight. As in our previous experiments [Korenchevsky *et al.* 1936; Korenchevsky & Dennison, 1936, 3], the body weights of ovariectomized rats and their gains in body weight were greater than those of the normal litter-mates (cols. I and II), though the increased fat deposition was seen only in the group of rats ovariectomized for a longer period (first line, in Table II). 6 γ of oestrone checked the growth and deposition of fat, which effect varied in the individual rats.

Testosterone propionate alone, and more particularly in combination with oestrone, brought about a decrease in the fat deposition and in the gain in body weight as compared with the uninjected spayed litter-mates (col. II) to a degree which approximated to or was below normal. It is thus evident that these hormones influence the metabolism; this effect however must be studied in special experiments.

Note on the histological changes induced by testosterone propionate in the sexual organs of spayed females. These changes will be described in detail elsewhere. After the injection of 700 γ of testosterone propionate alone or in combination with oestrone, the changes in the uterus and vagina were generally similar to those which we had observed after the injection of testosterone [Korenchevsky & Dennison, 1936, 2] or of androstenediol [Korenchevsky & Dennison, 1936, 1], except that after the propionate injections the uterine mucosa had the lace-like appearance of that seen in the pregnancy uterus. However, although the appearances of both the vagina and uterus were strongly reminiscent of those of these organs in pregnancy, there were at the same time some definite and abnormal differences. No cornification similar to that seen in the vaginal epithelium during oestrus or after oestrone injections was observed after testosterone propionate injections either alone or in combination with oestrone. On the contrary the vaginal epithelium was seen in various stages of mucification.

DISCUSSION

In our previous experiments we made a study of the effects on male and female rats of different male and female sexual hormones, both those isolated from urine [Korenchevsky *et al.* 1933] and of the following pure crystalline hormones: androsterone [Korenchevsky, 1935; Korenchevsky & Dennison, 1935, 2; 1936, 1; Korenchevsky *et al.* 1935, 2], androstenediol (at the time designated by us "androsteronediol") [Korenchevsky & Dennison 1935, 3; 1936, 1; Korenchevsky *et al.* 1935, 2]; the water-soluble esters of androsterone and androstenediol [Korenchevsky *et al.* 1935, 1, 2]; testosterone [Korenchevsky, 1936; Korenchevsky & Dennison, 1936, 2; Korenchevsky *et al.* 1936]; *trans*dehydroandrosterone [Korenchevsky & Dennison, 1936, 3]; androstenedione and androstenediol [Korenchevsky *et al.* 1937]; oestrone alone [Korenchevsky & Dennison, 1934, 1] or in combination with the hormones mentioned above [see references above and also Korenchevsky & Dennison, 1934, 2; 1935, 1, 4]. Some of these results have been obtained independently or confirmed by other workers in this country and abroad. Other workers, however, have chiefly studied the effect on the male sexual organs and, when the effect on the female sexual organs has been investigated, this has been done almost exclusively by the method of smears from the vagina ("vaginal cornification test"), though the effect of oestrone has been studied more fully. We made a study not only of the effect on the sexual organs both in male and female rats, but also of that on several other organs. In the case of female rats we have been forced to the conclusion that investigation by the method of smears alone, more particularly in short-duration experiments, is unreliable.

Castrated males. From the experimental evidence, given in the papers mentioned above, on the effects of those male hormones and of their derivatives, of which we have made a study, we can say that the atrophied sexual organs of rats castrated a few days after weaning and injected for the first time not earlier than 30 days after castration and for a period of at least 3 weeks, returned to the normal condition with androstanediol (700 γ daily [Korenchevsky *et al.* 1935, 2]) and with testosterone propionate (500 γ or more, present paper). All the other hormones, including testosterone, in spite of the large doses used, failed to bring about a complete recovery of the atrophied sexual organs to the normal condition though there was some return towards this condition. *trans*Dehydroandrosterone was the weakest of these hormones. The tendency to bring about the development of abnormally large preputial glands is a common feature of the male sexual hormones (except of *trans*dehydroandrosterone), when large doses are used. Other common properties, also shown in the present experiments by testosterone propionate, are the reduction in the size and weight of the hypertrophied "castration adrenals", the restoration of the rate of involution of the thymus, which is delayed by castration, the increase, in most cases to or towards the normal weight of the liver, kidneys and heart. The changes in the weight of the liver, of the kidneys and more especially of the heart only occur in most of the rats and often are not marked in degree, so that the nature of these can only be accurately defined when the histological investigation is completed. Changes in the thymus may be small or even absent when weak compounds are injected, such as *trans*dehydroandrosterone or androstenediol. In most castrated rats the gain in body weight, which is less after castration, improved with small and medium doses of the sexual hormones but was often decreased by large doses. The present experiments with testosterone propionate demonstrate this clearly. Since the deposition of fat did not increase simultaneously, and even sometimes decreased, this increase in the gain in body weight must be ascribed to the building of other than fat tissues, i.e. to the stimulation of the true anabolic processes of metabolism, while large doses possibly increase the catabolic processes.

Contrary to expectation there was no definite return to normal of the hypertrophied "castration hypophysis" either on treatment with testosterone or with testosterone propionate. Some degree of recovery however was obtained with oestrone, androsterone and androstanediol [Crooke & Korenchevsky, 1935; Korenchevsky *et al.* 1935, 2, p. 2539]. A co-operative effect of male hormones with oestrone and with oestradiol was also seen, especially when small doses were used.

Ratio ($\times 100$) of percentage increase in actual weight of prostate to that of seminal vesicles after injection of testosterone propionate as compared with the ratio obtained with testosterone or that observed during natural growth of the organs in normal rats

The significance of this ratio was emphasized and explained in our previous papers [e.g. Korenchevsky *et al.* 1936; 1937].

Table III

Daily dose of the hormones γ	Testosterone propionate	Testosterone
167	24	37
500	22	28
1400	17	29

From Table III it can be seen that (1) with both testosterone propionate and testosterone this ratio approximates closely to the "normal ratio" which from our observations is on the average about 25; (2) the low ratio with the injections of 1400 γ is explained by the very large increase in the weight of the seminal vesicles; (3) taking into consideration that large doses of androstanediol (700 γ) gave the ratio 24, and that the ratios obtained with the other hormones were considerably higher [Korenchevsky & Dennison, 1936, 3, Table IV, p. 1519], it may be concluded that a normal qualitative development of the prostate and seminal vesicles was obtained with androstanediol, testosterone and testosterone propionate.

Spayed females. With none of the male sexual hormones, of which we have so far made a study, were we able to obtain histological evidence from our long-duration experiments, of the so-called "oestrogenic" properties of these hormones, i.e. cornification of the vaginal epithelium, though this reaction has been obtained with various compounds by some workers [e.g. Butenandt, 1936; Deanesly & Parkes, 1936] in experiments of short duration. On the other hand from the evidence of changes in the weight of the female sexual organs of spayed rats and from histological investigation we have been able to show that all the male sexual hormones which we have investigated stimulated the development of the atrophied sexual organs to different degrees. The male hormones investigated may be arranged in descending order of activity as "female sexual stimulators" as follows:

- (1) testosterone propionate,
- (2) testosterone,
- (3) androstanediol,
- (4) androstenediol,
- (5) androstenedione,
- (6) *transdehydroandrosterone*,
- (7) androsterone.

However, for the accurate comparative assay of female activity, especially of androstenediol, androstenedione and *transdehydroandrosterone*, special assay experiments must be carried out.

Another important result, which was obtained in our experiments, was the fact that there is co-operative activity between the male and female hormones (with the possible exception of *transdehydroandrosterone* and androstenediol).

The male hormones investigated decreased the weight of the adrenals and thymus in females as well as in males, but the changes in the other organs, when they occurred, were small and present only in the majority of the animals. Thus an increase in the weight of the liver was seen after the injection of androsterone, androstanediol, *transdehydroandrosterone* and possibly after androstenedione, while a decrease in the weight of the hypophysis and a slight increase in the weight of the kidneys was observed after androsterone and androstanediol injections. The gain in body weight also improved after injections of small doses of these hormones. On the other hand testosterone propionate in the doses used depressed the gain in body weight, as was also the case in all our experiments with oestrone.

SUMMARY

1. The effect of testosterone propionate injected alone or in combination with oestrone was studied in 31 male and 35 female rats and compared with that obtained after the injection of testosterone and of other male sexual hormones which had been investigated previously.

Castrated males

2. Testosterone propionate injected into castrated males produces the following definite effects on the weights of the organs changed by castration: complete recovery to normal of the atrophied sexual organs; a decrease in the weight of the "castration" adrenals, and of the thymus, the involution of which is delayed by castration; a slight increase in the weight of the kidneys and liver, which organs decrease in weight after castration; an improvement with small doses in the gain in body weight (anabolic effect), and a lasting decrease (catabolic effect) with large doses.

3. The activity of testosterone propionate was found to be much greater than that of testosterone and the changes produced are to some extent (e.g. sexual organs) or completely (e.g. adrenals) maintained 9 days after the last injection.

Spayed females

4. The method of vaginal smears (vaginal cornification test) when used alone is unreliable and insufficient for the explanation of changes induced by the sexual hormones in the female sexual organs, while "the method of weights", advocated by us, supplemented by histological investigation of the results obtained in prolonged experiments, gives satisfactory and accurate results.

5. The terms "oestrogenic" or "oestrogens" can be applied only to those sexual hormones or compounds which produce not only specific changes in the vagina (in rodents, cornification of epithelium) but also specific changes in the uterus (at least distension of the uterus).

6. The hormones or compounds giving only a positive vaginal cornification test in rodents or some other single feature of oestrus might be termed "pseudo-oestrogenic" or "pseudo-oestrogens" or "vaginal keratinizing" or "cornifying" compounds to distinguish them from the "oestrogenic" compounds producing complete oestrus.

7. Although all the male hormones, which we investigated, have a stimulating action on the development of the atrophied female sexual organs, testosterone propionate was found to be the most active, having even more effect than oestrone in the doses used.

8. Testosterone propionate increases the weight of the uterus (as judged by weight of cervix) nearly to the normal when injected alone, and to normal when injected in combination with oestrone, produces an abnormally large vagina and female preputial glands, both when injected alone or in combination with oestrone, decreases the weight of the adrenals, greatly increases the rate of involution of the thymus and decreases the fat deposition and gain in body weight.

9. The histological changes in the uterus and vagina are similar to those which we obtained with androstanediol or with testosterone, but are more reminiscent of the changes observed during pregnancy in normal rats, though some abnormal features are seen.

10. The peripheral end of the female urethra under the influence of testosterone or testosterone propionate injections develops into a structure having the appearance of a clitoris.

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REFERENCES

- Butenandt (1936). *Naturwissenschaften*, **24**, 15.
Crooke & Korenchevsky (1935). *Proc. R. Soc. Med.* **28**, 72.
Deanesly & Parkes (1936). *Brit. med. J.* i, 257.
Korenchevsky (1932). *Biochem. J.* **26**, 413 and 1300.
—— (1935). *Nature, Lond.*, **135**, 434; **136**, 185.
—— (1936). *Nature, Lond.*, **137**, 494.
—— & Dennison (1934, 1). *Biochem. J.* **28**, 1474.
—— — (1934, 2). *Biochem. J.* **28**, 1486.
—— — (1935, 1). *J. Path. Bact.* **41**, 323.
—— — (1935, 2). *Biochem. J.* **29**, 1720.
—— — (1935, 3). *Biochem. J.* **29**, 2122.
—— — (1935, 4). *Proc. R. Soc. Med.* **28**, 71.
—— — (1936, 1). *J. Path. Bact.* **42**, 91.
—— — (1936, 2). *J. Path. Bact.* **43**, 345.
—— — (1936, 3). *Biochem. J.* **30**, 1514.
—— — & Brovsin (1936). *Biochem. J.* **30**, 558.
—— — & Eldridge (1937). *Biochem. J.* **31**, 467.
—— — & Kohn-Speyer (1932). *Biochem. J.* **26**, 2097.
—— — — (1933). *Biochem. J.* **27**, 557 and 778.
—— — & Levy Simpson (1935, 1). *Biochem. J.* **29**, 2131.
—— — — (1935, 2). *Biochem. J.* **29**, 2534.
Miescher, Wettstein & Tschopp (1936, 1). *Schweiz. med. Wschr.* **66**, 310.
—— — — (1936, 2). *Biochem. J.* **30**, 1970.
Parkes (1936). *Lancet*, ii, 674.