CCV. THE EFFECT OF TESTICULAR HORMONE ON NORMAL SEXUALLY MATURE RATS. A METHOD OF BIOLOGICAL ASSAY.

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IN the previous paper [Korenchevsky *et al.*, 1933, 1] a study was made of the influence of a purified preparation of testicular hormone isolated from human urine on young normal rats, about one month old, *i.e.* on rats sexually immature at the beginning of the injections. The injected animals, as compared with the controls, showed depression of the development of the testes in most cases with small doses and in all rats with large doses. The prostate with seminal vesicles and the penis were, in most cases, slightly decreased in weight with small doses, whilst with large doses an increase in weight was obtained and also an acceleration in the involution of the thymus and a slight increase in the growth of the kidney. No large or constant changes were found in the weights of the other organs or in the body weight. In the present paper a description is given of experiments performed on normal adult rats which had reached sexual maturity at the beginning of the injections.

Technique.

Experiments were performed on 37 rats belonging to 9 litters. The ages and weights of the rats are given in Table I. The age at the beginning of the experiment of the first six litters of 29 rats was from 74 to 77 days. The 4 rats of

Table I. *Age and average weight of the rats in each group of each litter.*

No. of litter	No. of rats in litter	Initial age (days)	Final weight of rats			
			Controls	0.5 R.U.	1 R.U.	10 R.U.
1	6	77	330	313	—	329
2	5	75	305	290	—	312
3	3	75	322	—	325	—
4	5	75	361	—	380	—
5	5	75	312	—	350	297
6	5	74	364	—	—	341
7	4	134	341	—	—	344
8	2	261	476	—	—	401
9	2	About 1 year	595	—	—	456

litter 7 were used in the present experiment about 3 weeks after the end of another experiment in which they had been fed on a diet partially deficient in vitamin D. During the intervening three weeks they were fed on a complete diet with the addition of 5 drops of cod-liver oil daily. For this reason the results with this litter, as well as those with the four much older rats of litters 8 and 9, are not included in the averages in Tables III to VI, but are given separately in these

Tables. The results of litters 8 and 9 have been averaged since each litter consisted of only two rats, one control and one injected rat.

A preparation of testicular hormone, isolated from human urine and dissolved in sesame oil, prepared by Messrs Schering Ltd., was used for the injections¹. This preparation was assayed by us, using our method of assay [Korenchevsky *et al.*, 1932] and was found to contain about 50 rat units per cc. In the experiments two small doses of 0.5 R.U. and 1 R.U. and one large dose of 10 R.U. per day were used, injected in half doses twice a day, the period of injection being 21 days in litters 1 to 7 and 10 days in litters 8 and 9. The rest of the technique was the same as that used by us in our previous experiments [Korenchevsky *et al.*, 1933, 1].

Influence on the weight of the rats.

As can be seen from Tables I and II, neither the difference in the final weight nor in the gain in weight was of such a degree or constancy that any definite significance could be attributed to these with the possible exception of the rats

Table II. *Influence on gain in weight.*

Average gain in weight (g.) in each group of each litter after the injection of testicular hormone.

No. of litter	Actual gain				Gain per 100 g. of initial weight			
	Control rats	Rats injected with			Control	Rats injected with		
		0.5 R.U.	1 R.U.	10 R.U.		0.5 R.U.	1 R.U.	10 R.U.
1	55	42	—	56	20	15	—	21
2	43	44	—	48	16	18	—	18
3	40	—	47	—	14	—	17	—
4	49	—	67	—	16	—	21	—
5	22	—	55	25	8	—	19	9
6	53	—	—	43	17	—	—	14
7	35	—	—	31	11	—	—	10
8+9	9	—	—	10	2	—	—	4

injected with 1 R.U. All the rats of this latter group in all three litters gained more than either their controls or the rats of the same litter injected with other doses of the hormone. In litters 3, 4 and 5 the average gain in weight per 100 g. of initial weight of rats was 21.4 %, 31.3 % and 137.5 % more than that of the controls.

Influence on testes.

The results are summarised in Table III. The changes in weight of the testes were small, more particularly when considering the results calculated for the same unit of body weight, being on the average in the three different groups of litters 1 to 6, — 2.7 %, + 5.3 % and — 9.2 % as compared with the average for the control group.

In litters 7 to 9, of older rats, a decrease was noted in the weight of the testes, when calculated per 200 g. of body weight, of 21.1 % and 27.4 %. As can be seen from Table III, a constant although small decrease in the weight of these glands was only obtained with the large dose of 10 R.U., the decrease occurring in all litters except litter 5. A preliminary histological examination of the testes of the injected rats in no case revealed any changes in the seminiferous cells or in spermatogenesis. The Leydig's cells in the testes of rats injected with the large dose of 10 R.U., however, seemed to contain less protoplasm and had smaller nuclei than those of either the control rats or of those injected with 0.5 and 1 R.U. Detailed histological results will be published elsewhere.

¹ We wish to express our thanks to Messrs Schering Ltd., for kindly supplying this preparation.

Table III. *Influence on testes.*

The average weights (g.), actual and calculated per 200 g. of body weight, of testes of normal rats in each group of each litter and their percentage change after the injection of testicular hormone.

		Rats injected with			Percentage change		
No. of litter	Control rats	0.5 R.U.	1 R.U.	10 R.U.	0.5 R.U.	1 R.U.	10 R.U.
A. Actual weights.							
1	2.581	2.408	—	2.173	-6.7	—	-15.8
2	2.952	2.677	—	2.511	-9.3	—	-14.9
3	2.228	—	2.575	—	—	+15.6	—
4	3.215	—	3.211	—	—	-0.1	—
5	2.340	—	2.735	2.292	—	+16.9	-2.1
6	3.076	—	—	2.649	—	—	-13.9
Average	2.732	2.543	2.840	2.406	-8.0	+10.8	-11.7
7	2.022	—	—	1.598	—	—	-21.0
8+9	3.592	—	—	2.111	—	—	-41.2
B. Weights per 200 g. of body weight.							
1	1.565	1.545	—	1.337	-1.3	—	-14.6
2	1.935	1.855	—	1.611	-4.1	—	-16.7
3	1.384	—	1.608	—	—	+16.2	—
4	1.785	—	1.688	—	—	-5.4	—
5	1.500	—	1.577	1.544	—	+5.1	+2.9
6	1.693	—	—	1.554	—	—	-8.2
Average	1.644	1.700	1.624	1.512	-2.7	+5.3	-9.2
7	1.191	—	—	0.940	—	—	-21.1
8+9	1.357	—	—	0.985	—	—	-27.4

Thus with the small doses no definite changes in the testes were obtained, while after the injection of the large dose a slightly depressing effect on the Leydig's cells and in most litters a small decrease, if any, in the weight of the testes were indicated.

Influence on prostate with seminal vesicles and penis.

No definite effect on the weight of these sexual organs (Tables IV and V) was found as the result of the injection of testicular hormone, the variations obtained being small and not constant.

However, it is significant that in most litters a decrease of prostate with seminal vesicles, although slight, was present, indicating that the preparation had no stimulating effect on these organs in adult normal rats.

Influence on thymus.

In all the rats receiving injections of 1 and 10 R.U. the thymus was smaller than in their controls, the average decrease in weight, calculated per 200 g. of body weight, being 23 % and 25.5 % respectively for the six litters of younger rats, while the decrease in litter 7 was 50.4 % and in litters 8 and 9 was 18.6 % (Table V).

Influence on kidneys.

In the previous paper [Korenchevsky *et al.*, 1933, 1] the injection of testicular hormone into young normal rats was shown to be followed by a slight increase in the weight of the kidneys.

In the present experiment this increase was only found consistently in those rats of the first six litters which were injected with 10 R.U. the average increase

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Table IV. *Influence on prostate with seminal vesicles.*

The average weights (g.), actual and calculated per 200 g. of body weight, of the prostate and seminal vesicles of the normal rats in each group of each litter and their percentage change after the injection of testicular hormone.

No. of litter	Control rats	Rats injected with			Percentage change		
		0.5 R.U.	1 R.U.	10 R.U.	0.5 R.U.	1 R.U.	10 R.U.
A. Actual weights.							
1	1.685	1.489	—	1.880	-11.6	—	+11.6
2	1.823	1.298	—	2.088	-28.8	—	+14.5
3	1.482	—	1.476	—	—	- 0.4	—
4	2.173	—	2.041	—	—	- 6.1	—
5	1.859	—	1.744	1.764	—	- 6.2	- 5.1
6	2.386	—	—	1.578	—	—	-33.9
Average	1.901	1.394	1.754	1.828	-20.2	- 4.2	- 3.2
7	2.351	—	—	2.458	—	—	+ 4.6
8+9	3.596	—	—	2.665	—	—	-25.9
B. Weights per 200 g. of body weight.							
1	1.022	0.954	—	1.151	- 6.7	—	+12.6
2	1.195	0.896	—	1.340	-25.0	—	+12.1
3	0.920	—	0.910	—	—	- 1.1	—
4	1.205	—	1.072	—	—	-11.0	—
5	1.191	—	0.994	1.188	—	-16.5	- 0.3
6	1.314	—	—	0.927	—	—	-29.5
Average	1.141	0.925	0.992	1.152	-15.9	- 9.5	- 1.3
7	1.379	—	—	1.433	—	—	+ 3.9
8+9	1.400	—	—	1.252	—	—	-10.6

Table V. *Influence on penis, thymus, kidneys and intra-abdominal fat.*

Average percentage changes in weight (actual and per 200 g. of body weight) of the organs in each group of each litter of the rats injected with testicular hormone, as compared with those of control rats.

No. of litter	Penis			Thymus			Kidneys			Intra-abdominal fat		
	0.5 R.U.	1 R.U.	10 R.U.	0.5 R.U.	1 R.U.	10 R.U.	0.5 R.U.	1 R.U.	10 R.U.	0.5 R.U.	1 R.U.	10 R.U.
A. Actual weights												
1	-14.5	—	-2.5	+18.4	—	-21.0	+0.2	—	+14.4	-5.0	—	+2.2
2	-11.8	—	+9.0	-2.0	—	-11.5	-18.6	—	+6.9	+2.2	—	+1.1
3	—	+4.2	—	—	-23.1	—	—	+17.4	—	—	-27.7	—
4	—	-0.7	—	—	-6.3	—	—	+4.8	—	—	+19.7	—
5	—	-2.1	-1.8	—	-29.3	-48.3	—	+18.5	+14.8	—	+70.7	-19.3
6	—	—	-10.5	—	—	-26.6	—	—	+4.7	—	—	-31.6
Average	-13.2	+0.5	-1.5	+8.2	-19.6	-26.9	-9.2	+13.6	+10.2	-1.4	+20.9	-11.9
7	—	—	+14.6	—	—	-50.2	—	—	+9.4	—	—	+8.4
8+9	—	—	+2.5	—	—	-31.1	—	—	-22.5	—	—	-16.8
B. Weights per 200 g. of body weight.												
1	-9.3	—	-1.6	+24.9	—	-20.1	+5.8	—	+15.1	-1.9	—	+1.7
2	-7.1	—	+6.7	+3.9	—	-13.5	-14.1	—	+4.5	+7.3	—	-1.1
3	—	+3.8	—	—	-23.2	—	—	+16.7	—	—	-28.0	—
4	—	-6.1	—	—	-10.1	—	—	-0.6	—	—	+13.9	—
5	—	-12.0	+3.2	—	-35.6	-45.7	—	+4.8	+20.8	—	+48.9	-15.2
6	—	—	-4.5	—	—	-22.5	—	—	+11.7	—	—	-28.3
Average	-8.2	-4.8	+1.0	+14.4	-23.0	-25.5	-4.2	+7.0	+13.0	+2.7	+11.6	-10.7
7	—	—	+12.5	—	—	-50.4	—	—	+8.7	—	—	+7.9
8+9	—	—	+26.9	—	—	-18.6	—	—	-4.5	—	—	+8.4

being 13 %. The changes in the kidneys of the rats injected with the small doses and in those of the old rats were less constant (Table V).

Influence on other organs.

No definite influence could be seen on the fat deposition (Table V) or on the weights of the adrenals, thyroid, hypophysis, liver, spleen or heart (Table VI).

Table VI. *Influence on adrenals, thyroid, hypophysis, liver, spleen and heart.*

Average percentage changes in weight (actual and per 200 g. of body weight) of organs of the injected rats as compared with those of the control rats.

Organs	No. of litter	Actual weights			Weights per 200 g. of body weight		
		0.5 R.U.	1 R.U.	10 R.U.	0.5 R.U.	1 R.U.	10 R.U.
Adrenals	1 to 6	- 9.1	+ 10.2	- 1.1	- 3.9	+ 4.4	+ 1.8
	7	—	—	—	—	—	- 11.9
	8 + 9	—	—	- 19.6	—	—	- 3.4
Thyroid	1 to 6	- 12.7	+ 7.1	+ 1.7	- 8.0	+ 0.7	+ 4.2
	7	—	—	- 2.8	—	—	- 4.6
	8 + 9	—	—	- 9.1	—	—	+ 11.0
Hypophysis	1 to 6	- 8.0	+ 7.7	+ 7.0	- 1.7	+ 2.5	+ 9.9
	7	—	—	- 6.7	—	—	- 8.1
	8 + 9	—	—	- 2.8	—	—	+ 22.2
Liver	1 to 6	- 5.9	+ 10.5	+ 3.1	- 0.9	+ 6.2	+ 5.3
	7	—	—	+ 4.1	—	—	+ 3.2
	8 + 9	—	—	- 16.5	—	—	+ 3.8
Spleen	1 to 6	- 6.6	+ 6.9	- 2.2	- 0.9	+ 1.4	+ 0.5
	7	—	—	± 0	—	—	- 2.1
	8 + 9	—	—	- 24.1	—	—	- 3.7
Heart	1 to 6	- 7.4	+ 11.4	+ 0.1	- 2.4	+ 5.5	+ 2.8
	7	—	—	- 3.3	—	—	- 3.1
	8 + 9	—	—	- 13.2	—	—	+ 15.3

DISCUSSION.

The change in weight of the organs, used as a method of assay of different substances as applied to normal rats.

In previous papers [Korenchevsky, 1930, 1932, 1, 2] a method was proposed for the accurate estimation of changes in organs as judged by their change in weight, the weighing being made of the organs fixed in Bouin's fixative. The method gives very accurate results provided the other conditions of the method are fulfilled; its accuracy was proved when applying it to the study of the changes produced by castration and cryptorchidism in male rats [Korenchevsky, 1930] and those produced by testicular hormone preparations in castrated and cryptorchid rats (Korenchevsky *et al.*, 1932; 1933, 1, 2, 3].

The data obtained with adult rats, given in Tables III to VI of the present paper, together with the figures obtained on young rats, given in Tables VIII to XII in the previous paper (Korenchevsky *et al.*, 1933, 1, pp. 570-572], show that the method when applied to normal rats also gives results of a satisfactory accuracy. The variations in the average weights in the different groups of the same litter (where the injection was without effect) were small, especially when the weights of the organs were calculated for the same unit of body weight. These variations become smaller, sometimes even insignificant, if the mean of the average weights of the organs of at least three litters is taken. The importance of this latter consideration has been emphasised in our previous papers.

Taking for example the figure for litters 1 to 6 (Table VI of the present paper) it can be seen that of the 18 figures giving the mean variations of the average changes in weight of organs, as compared with that in control rats of 6 litters, none exceeds 9.9 % and in 13 cases they are less than 5 % when calculated per 200 g. of body weight).

The method may, therefore, be considered to be accurate and reliable also with normal animals and might with advantage be applied, not only to the study of various physiological and pathological conditions, but also to the assay of substances having a specific influence on the weight of one or more organs.

On the influence on normal male rats of the preparation of testicular hormone, isolated from urine.

The strength of the testicular hormone used in the present experiments was similar to that of batch I which was injected into young normal rats in the previous experiments [Korenchevsky *et al.*, 1933, 1, pp. 568–573, see litters 6, 7 and 8]. Therefore, although a special assay of batch I was not made, the doses described in the previous paper as 0.25 D, 0.5 D and 2 D were probably about 1.25 R.U., 2.5 R.U. and 10 R.U. respectively. These doses had a depressing effect on the testes of young rats, more particularly, as is shown by the histological examination, on the seminiferous tissue. As judged by changes in the weights of the organs, the small doses of 1.25 R.U. and 2.5 R.U. had also a depressing effect on the prostate with seminal vesicles and penis, while the large dose of 10 R.U. had a stimulating effect.

In the present experiments on adult sexually mature rats only the large dose of 10 R.U. seemed to have any effect of the kind described above, the weight of the testes being slightly decreased in most litters by this dose. Histologically there was a slightly depressing effect on the Leydig's cells but spermatogenesis and the seminiferous tissue seemed normal.

Therefore, injections started before sexual maturity have a definitely depressing effect on the sexual organs, but if started after sexual maturity the effect, if any, is very slight even with large doses.

Comparison of the data of the present experiments with those of the previous experiments [Korenchevsky *et al.*, 1933, 1] shows that the rate of retrogression of the thymus was accelerated both in sexually immature and mature rats, while the slightly stimulating effect of the testicular hormone on the kidneys was not so marked in the mature as in the immature animals.

No definite changes, in either mature or immature animals, were noticed in the weights of the other organs.

SUMMARY.

1. The changes produced by injections of a purified preparation of testicular hormone extracted from urine were studied on 37 normal adult male rats belonging to 9 litters.

2. The physiological variations from the average of the weights of the organs investigated were small.

3. Therefore, by comparing the mean of the average changes in the litters and following exactly the technique and precautions given in the previous papers, the method described could be applied to normal rats for the study of physiological or pathological conditions or for the assay of substances which influence the weight of one or more organs.

4. The effect of injections of the testicular preparation on adult rats was small, as judged by changes in the weight of the organs and the histological examination of the testes, differing in this respect from the quite definite effect

on some of the organs of young rats, since only the large dose of 10 R.U. seemed to have a slightly depressing effect on the weight of the testes and the Leydig's cells.

5. Acceleration in the rate of retrogression of the thymus was noticed in all the rats injected with 1 and 10 R.U.

6. The increase in the weight of the kidneys was very small, being present in most of the groups injected with the large dose.

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