CLVI. THE RESPONSE OF CASTRATED MALE RATS TO THE INJECTION OF TESTICULAR HORMONE.

By VLADIMIR KORENCHEVSKY, MARJORIE DENNISON and ROSA SCHALIT.

From the Lister Institute, London.

(*Received July 4th, 1932.*)

A method of testing the activity and strength of testicular hormone on castrated rats of the same litter was previously described and conditions were suggested for applying the same method of assay of testicular hormone to rats belonging to different litters [Korenchevsky, 1932, 1]. For this latter purpose it is most important to ascertain (1) how castrated rats belonging to different litters will respond to the same dose of testicular hormone, (2) what conditions must be observed to obtain comparable and accurate results.

In a previous paper [Korenchevsky, 1932, 2] it was shown that the weights of the sexual and endocrine organs and of retroperitoneal fat, when calculated per 200 g. of body weight, did not vary greatly in different castrated litters, bred and kept in the same conditions provided they were operated on at the same period in relation to sexual maturity, *i.e.* before or after puberty, and also that the litters were killed at the same period after castration.

In this paper a study is made of the influence of these two factors on the changes in rats injected with testicular preparations, in order to ascertain under what conditions comparable results for the assay of the hormone when using different litters may be obtained.

The response of the rats of the same litter to different doses was also compared in a few experiments so as to obtain an indication of the sensitivity of the test to differences in the dose.

Technique.

Experiments were performed on 67 rats belonging to 17 litters, all of which came from the stock of the Lister Institute and were reared and kept on the same diet. During the experiments a special paste was given, and all the other details of technique for the assay of testicular hormone were the same as those described elsewhere [Korenchevsky, 1932, 1]. The amount of paste consumed daily was recorded. Castration was performed at different ages in different litters. All the data mentioned above, together with the changes in body weight of the rats, are summarised in Table I.

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

No. of litter	No. of rats in litter	Age in days: At castration	Age in days: At beginning of injections	No. of days after castration injections started	Change in wt. per 200 g. of initial wt. of rat: Control rats g.	Change in wt. per 200 g. of initial wt. of rat: Injected rats g.	Paste intake per day per 200 g. of body wt.: Control rats g.	Paste intake per day per 200 g. of body wt.: Injected rats g.
1	4	72	82	10	+50	+75	27	28
2	3	70	80	10	+35	+38	20	17
3	4	57	67	10	+92	+84	29	26
4	4	57	67	10	+74	+61	27	25
Average	4	64	74	10	+63	+64	26	24
5	3	25	81	56	+ 7	+14	20	22
6	4	23	79	56	+20	+27	22	20
Average	4	24	80	56	+14	+20	21	21
7	4	63	82	19	+ 4	+25	18	21
8	5	71	90	19	+16	+26	20	21
9	4	57	76	19	+28	+15	25	19
Average	4	64	83	19	+16	+22	21	20
10	5	74	103	56	+ 2	+ 5	16	14
11	3	23	85	62	+44	+16	23	19
12	3	67	93	26	+51	+14	23	16
13	3	44	116	72	+ 7	+ 1	16	11
14	2	150	279	129	− 2	− 7	14	10
15	2	39	168	128	+ 3	− 1	13	14
16	3	76	148	72	+ 4	− 1	15	11

The testicular extracts Nos. 126 and 130 were prepared by the "dry" method previously described [Korenchevsky, Schalit and Graetz, 1932] and dissolved in sesame oil. The extracts Nos. 4, 89 and 92 were prepared by Koch and Gallagher's method [1929] as far as the stage of extraction from hexane solution with 70 % alcohol. Extract 111, prepared by the same method, was purified to the last stage. These last four extracts were emulsified in 1 % gum acacia. All the extracts were sterilised for 30 minutes on three consecutive days in the steamer at 100° and kept in sealed ampoules in the cold store at 0°. No induration or inflammation was in any case produced by the injections but when the extract was dissolved in sesame oil some amount of unabsorbed oil was always found at autopsy in the subcutaneous tissue at the sites of more recent injections.

The influence of the injections in different litters.

These results are summarised in Table II. The experiments on litters 1 to 10 were designed to show this influence. Those on litters 11 to 16 were preliminary experiments for the purpose of testing the strength of a testicular preparation. In these experiments the difference in the response of different litters became evident. In addition to the organs given in Table II the other endocrine organs and the retroperitoneal fat were weighed. These are not included in the Table, since the amount of the testicular preparation injected

Table II. *The influence of the injection of testicular hormone on the weights of the sexual organs and thymus, calculated per 200 g. of body weight, of the rats in each group of each litter.*

Extract injected and length of period of injections	No. of litter	Prostate and seminal vesicles (mg.) Control rats	Injected rats	Increase %	Penis (mg.) Control rats	Injected rats	Increase %	Thymus (mg.) Control rats	Injected rats	Decrease %
No. 126	1	137	206	50·4	85	110	29·4	382	341	10·7
20 mg.	2	160	280	75·0	88	114	29·6	339	328	3·2
10 days	3	150	253	68·7	100	118	18·0	471	384	18·4
	4	153	274	79·1	97	124	27·8	467	441	5·6
	Average	150	253	68·3	93	117	26·2	415	374	9·5
No. 130	5	60	145	141·6	43	99	130·2	525	415	21·0
12 mg.	6	50	129	158·0	46	95	106·5	464	335	27·8
10 days										
	Average	55	137	149·8	45	97	118·4	495	375	24·4
No. 130	7	157	298	89·8	126	136	7·9	516	325	37·0
12 mg.	8	157	300	91·1	105	129	22·9	359	277	22·8
10 days	9	121	267	120·6	87	108	24·1	460	323	29·8
	Average	145	288	100·5	106	124	18·3	445	308	29·9
No. 130	10	126	221	75·4	104	123	18·3	256	185	27·7
12 mg.										
10 days										
No. 4	11	46	114	147·8	—	—	—	—	—	—
20 mg.	12	184	260	41·3	—	—	—	—	—	—
10 days										
No. 89	13	65	146	124·6	—	—	—	—	—	—
20 mg.	14	199	291	46·2	—	—	—	—	—	—
7 days										
No. 92	15	50	100	100·0	—	—	—	—	—	—
20 mg.	16	110	171	55·5	—	—	—	—	—	—
6 days										

and the length of the period of injections were not sufficient to produce marked changes. During these experiments dissection and investigation of the changes in the thymus were begun. It was found that, like the sexual organs, the thymus is also sensitive to the small amounts of testicular preparations used for the test.

Experiments with testicular extract 126.

The same amount of extract 126 (20 mg. per day per rat) was injected daily for 10 days into two rats of each of the litters Nos. 1 to 4. The remaining litter-mates served as the controls. As can be seen from Table I, the rats of all the litters were castrated at the age of sexual maturity; the injections were made for 10 days and the rats killed 20 days after castration.

The weight of the prostate with seminal vesicles increased in the injected rats, as compared with the controls, on the average by 68·3 %, the deviations from this average not exceeding 26·2 %. The maximum difference in the increases was between the litters 1 and 4, being about 57 % greater in the latter litter than in the former.

The response of the penis to the injections was smaller than that of the

prostate and seminal vesicles, the increase being on the average 26·2 %, with a maximum deviation from the average of 31·3 %.

The response of the thymus was still less, its reduction averaging 9·5 %, with a maximum deviation of 93·6 %.

Experiments with extract 130.

Three groups of litters were injected with extract 130, using 12 mg. per rat daily for 10 days. Litters 5 and 6 belonged to the first group of rats castrated before sexual maturity at the age of 23 and 25 days. Litters 7, 8 and 9 formed the second group of rats castrated after maturity and injected 19 days after castration. The third group, containing only litter 10, was also operated on after maturity, but the injections were made much later, namely, 56 days after castration.

The increase in the prostate and seminal vesicles on injection was much greater in the first group (litters 5 and 6) than in the second and third groups (litters 7–10), as was also the case with regard to the increase in weight of the penis. The decrease in the thymus was on the average nearly the same in all three groups.

This experiment indicates quite clearly that, with regard to the increase in weight of the sexual organs on injection with the hormone, the reaction is much greater in rats castrated before, than in rats castrated after, sexual maturity.

The results are influenced more by the date of operation in relation to maturity than by the length of time between the operation and the beginning of the injections.

Thus the increases in weight of the prostate with seminal vesicles in litters 7 and 8 are very close to that of litter 10, being 89·8 %, 91·11 % and 75·4 % respectively. The maximum difference between litters 8 and 10 was about 21 %, whilst litter 9, castrated at 57 days, *i.e.* close to the date of maturity (perhaps not even having reached maturity), showed a greater deviation from litters 7 and 8, to which group litter 9 belongs, the weight of prostate with seminal vesicles being 34·3 % greater in litter 9 than in litter 7. The maximum deviation from the average, however, of the prostate with seminal vesicles in the second group was 20 %.

Experiments with extracts Nos. 4, 89 and 92.

Six litters were used for these experiments Nos. 11 to 16. Each of the extracts was injected into two litters, one castrated before and the other after sexual maturity (Table II). Although in some litters there were only two rats, one injected and the other serving as the control, the results are identical with those of litters 5–10, *i.e.* in the rats castrated before maturity the increase in the prostate with seminal vesicles on injection was much greater than in those castrated after maturity.

The injection of different doses into rats of the same litter.

These experiments were performed on three litters, Nos. 5, 7 and 17, and the results are summarised in Table III. The results of the injection of litters

Table III. *Percentage changes in the weights of organs after the injection of different doses of testicular extract into groups of rats belonging to the same litter, the food intake per day per 200 g. of body weight and the change in body weight per 200 g. of initial weight.*

		Prostate and seminal vesicles			Penis			Thymus		
			Rats given large dose			Rats given large dose			Rats given large dose	
No. of litter	No. of rats in litter	Rats given small dose	Observed increase	Expected increase	Rats given small dose	Observed increase	Expected increase	Rats given small dose	Observed decrease	Expected decrease
5	6	+142	+263	+213	+130	+191	+195	-21	-27	-31
7	6	+ 90	+129	+135	+ 8	+ 8	+ 12	-37	-43	-56
17	6	+ 39	+ 76	+ 77	+ 1	+ 19	+ 2	—	—	—

		Paste intake per day per 200 g. body weight			Gain in body weight per 200 g. of initial weight			
No. of litter	No. of rats in litter	Control rats	Rats given small dose	Rats given large dose	Control rats	Rats given small dose	Rats given large dose	Extract and daily doses injected
5	6	21	22	21	+ 7	+14	+25	No. 130 12 mg. 18 mg.
7	6	18	21	22	+ 4	+25	+33	No. 130 12 mg. 18 mg.
17	6	21	18	18	+31	+16	+17	No. 111 4 mg. 8 mg.

5 and 7 with the small dose will also be found in Table II), since this small dose and the test preparation, No. 130, were the same as were used for litters 5 to 10. The larger dose of this extract injected was 18 mg. per day.

Litter 17 was castrated at 65 days old, and injections were started at 81 days old, or 16 days after castration, the period of injection being 12 days. Extract 111 was injected into 2 rats of this litter in the daily dose of 8 mg. per rat, the two remaining rats serving as uninjected controls.

In Table III the first two columns for each organ give the changes % actually observed after the injection of two different doses; an increase in the case of prostate with seminal vesicles, a decrease in the case of thymus. In the third column is given the expected increase or decrease with the larger dose, assuming that the increase in the change would be in direct proportion to the increase in the dose. Thus, in litter 17 (see Table IV) the average weight of the prostate with seminal vesicles of the control rats was 191 mg. After the injection of 4 mg. per day per rat the average weight of the prostate with seminal vesicles of the two injected rats was 265 mg., an increase of 74 mg.

Therefore the double dose would be expected to produce an increase of 148 mg. though so close an agreement with the actual result of 146 mg. was probably fortuitous.

In litter 7, however, the expected and actual increases are again very close; though in litter 5 they are more divergent, the actual increase being 263 % while the expected increase was only 213 %.

It is necessary to add that this close agreement is only true for the comparison of the averages of each group. Variations are always present between the individual rats of the group, as was shown in a previous paper [Korenchevsky, 1932, 1].

For instance, in these two litters 7 and 17, giving the striking results mentioned above, the weights of the prostate with seminal vesicles, when calculated per 200 g. of body weight, in the individual rats of the three groups, were as follows:

Table IV.

Weights in mg.

		Litter 7	Litter 17
Two rats of control group		165	193
		149	189
	Average	157	191
Two rats injected with small dose of hormone		314	280
		281	250
	Average	298	265
Two rats injected with large dose of hormone		409	298
		308	376
	Average	359	337

This shows the importance of using at least two rats for a group, in which case the reliability of the average obtained from the data given even by only two rats, as has already been shown in a previous paper [Korenchevsky, 1932, 1], is usually satisfactory, provided that more than one litter is used for the conclusions.

Discussion.

Although the number of experiments performed was not large, very similar results were obtained in all cases. At the same time the corroboration of the results by a large number of experiments on the stocks of rats of different laboratories is necessary.

Some facts, however, became evident. Thus, in castrated rats the prostate and seminal vesicles, weighed together, are the most sensitive organs for the assay of testicular hormone.

The response of these organs is much greater, *i.e.* they are much more sensitive, if the rats are castrated before than after sexual maturity. It seems advisable, therefore, to use rats castrated before sexual maturity for the assay.

Since the exact date of maturity is uncertain it is necessary to castrate the rats before the 30th day after birth and to use only such castrated rats for the tests.

The data are insufficient to enable us to decide what time should elapse after castration before the rats are used for assay.

As has been stated before, however [Korenchevsky, 1932, 2], from the beginning of the second month to 6 months after castration, when this was performed before maturity, the weights of the atrophied prostate and seminal vesicles, calculated for the same unit of body weight, were found to be practically constant. For the other organs, however, more nearly constant results are not obtained until $2\frac{1}{2}$ months after castration. Should it be found that rats, within this period after castration, respond to the same degree to the injections, this period would perhaps be most suitable. In those experiments in which injections of different doses of testicular hormone were made, on the average a remarkably exact correlation was noted, in the form of a direct proportion between the increase of the dose and in the increase in the weight of the atrophied prostate with seminal vesicles. If this is found to be corroborated by more numerous experiments, it will be possible to calculate sufficiently nearly, from any dose injected in a preliminary experiment, the dose necessary for the assay injections. In this case the standardisation of the hormone will be easier than by the comb-growth method, in which only small doses give reliable comparable results.

It seems at the present moment, on the basis of the experiments described in this and the papers mentioned above, that a method of assay on rats belonging to different litters and a rat unit of testicular hormone can be suggested. The following definition is proposed only as a foundation for testing the method experimentally for simplicity, reliability and degree of exactness and for comparison with other methods.

As a rat unit, we suggest the minimum daily dose of testicular hormone injected during 7 consecutive days into at least 3 litters of rats castrated before the 30th day after birth, which will produce, on the average, an increase of 40 % in the weight of the prostate with seminal vesicles as compared with the weight of these organs in the uninjected litter mates.

For the assay the emulsification (or solution if possible) of testicular hormone in watery solvents will be preferable, since when the testicular hormone is injected dissolved in oil uncertain amounts of unabsorbed oil were always found in the subcutaneous tissue at the places of more recent injections. This probably indicates that some of the hormone injected during the assay was not absorbed. The amount of this unabsorbed hormone in oil may vary and become a source of error. In our experiments an attempt to decrease this error was made by increasing the period of injections to 10 days, thus allowing time for the complete absorption of the extract injected during approximately the first 7 days, *i.e.* the greater part of the injected material.

It is clear from Table II that although the increase in weight of the penis and decrease in weight of the thymus confirm the activity of the extract, and therefore, it is advisable to weigh them also, the response is not sensitive and the variations observed are sometimes large as compared with those of the

prostate with seminal vesicles. They are not, therefore, suitable for a quantitative assay.

In Tables I and III the figures showing the increase in weight and the appetite of the injected as compared with the uninjected rats are given. Marked simultaneous decreases of these in the injected rats usually indicate the presence of depressing substances in the preparation. This was noted for instance in litters 11 to 17, which were injected with the first extracts we prepared.

Loss in weight without a diminished, or even with an improved, appetite may indicate an increased metabolism.

In litters 1, 5, 6, 7 and 8 (Tables I and III) better growth was obtained after the testicular injections, especially when larger doses were injected (Table III).

A large reduction in the appetite of the injected rats also checks the normal rate of increase of the weight of prostate and seminal vesicles.

Summary.

1. Using 67 rats belonging to 17 litters a study has been made of the response of rats belonging to different litters to the same dose of testicular extract, and also of the response of rats to different doses of the same testicular preparation.

2. The conditions necessary for the assay of testicular hormone suggested in previous papers were corroborated.

3. In castrated rats the most sensitive organs suitable for the assay of testicular hormone are the prostate with seminal vesicles.

4. Although the penis and thymus respond to the injections simultaneously with the prostate and seminal vesicles, the response is less in degree, is more variable and is less sensitive.

5. The increase on injection in the weight of the atrophied prostate with seminal vesicles is much greater in rats castrated before, than in rats castrated after, sexual maturity.

6. In rats belonging to different litters, injected at a similar period after castration with the same dose of the same testicular extract, the variations in the increase in weight of the prostate with seminal vesicles were not large provided the litters were operated on at the same time in relation to the date of sexual maturity, *i.e.* before or after maturity.

7. In three experiments a very close correlation of direct proportion between the increase of the dose of the injected hormone and the degree of the average increase of prostate with seminal vesicles was obtained.

8. On the basis of the results mentioned in this and other papers a suggestion has been made of a possible method of assay of testicular hormone preparations on rats belonging to different litters.

A grant from the Medical Research Council and the hospitality of the Lister Institute have enabled us to carry out this work and to them our thanks are due.

REFERENCES.

Koch and Gallagher (1929). *J. Biol. Chem.* **84**, 495.
Korenchevsky (1932, 1). *Biochem. J.* **26**, 413.
—— (1932, 2). *Biochem. J.* **26**, 1300.
—— Schalit and Graetz (1932). *Biochem. J.* **26**, 423.