CII. ON THE ASSAY AND THE ABSORPTION OF TESTICULAR HORMONE DISSOLVED IN OIL.

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IN a previous paper [Korenchevsky, Dennison and Kohn-Speyer, 1933] it was stated that the assay of testicular hormone on rats had given accurate results in our experiments, in spite of the fact that, on autopsy, some unabsorbed oil was found in the subcutaneous tissue in the places where the injections had been more recently made; an appreciable amount being found at the site of the last 2 days' injections.

Because of the accurate results obtained in the assay it was suggested that, with the doses used, either the oil is absorbed from the subcutaneous tissue at a constant rate, or the testicular hormone is more readily absorbed from the oil than is the oil itself. If these assumptions were incorrect, very variable results would be expected.

This is of especial importance in the assay of the rat unit, as proposed by us, since the injections have to be made during a period of only 7 days, *i.e.* during a short period, in which, if the rate of absorption varied, much more variable results would be expected than in experiments of long duration. Moreover, if the testicular hormone during the last 2 or 3 days of the experiment is not absorbed from the subcutaneous tissue, it would be advisable for the assay to inject the rats for the first 4 days only, to wait for the following 3 days for a complete absorption of the oily solution from the subcutaneous tissue, and only then to kill the rats after 7 days of experiment, *i.e.* 3 days after the last injection.

For the elucidation of the question, whether the hormone injected in the second half of the assay period is absorbed, and whether perhaps only 4 days' injections are sufficient to give approximately the same result as 7 days', the present experiments were performed. At the same time, we have used the results of these experiments and of some others in order to compare again the standardisation of the hormone preparation by the capon comb-growth method and by our own method.

Technique.

The experiments were performed on 15 rats belonging to 3 litters (Table I). Litters 1 and 2 were each divided into 3 groups of rats: group I, control rats; group II, rats injected daily with 2.5 c.u. of testicular extract on the first 4 days of the experiment and then left without injections during the last 3 days; group III, rats injected with the same daily dose of extract on each of the 7 days of the experiment. The rats of litter 3 were divided into 2 groups only: control rats and those injected for 7 days. All the rats were killed on the 8th day. In other respects the technique used was the same as that suggested for the assay of the rat unit [Korenchevsky *et al.*, 1932]. The diet used was the same as that described in our previous paper [Korenchevsky *et al.*, 1933]. The testicular

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

No. of litter	No. of rats in litter	Age in days			No. of days after castration at beginning of injections	Final weight		
		At castration	When injections started	Final		Control rats g.	4 days injected g.	7 days injected g.
1	6	24	64	71	40	246	252	287
2	5	25	67	74	42	217	262	244
3	4	28	46	53	18	183	—	174

hormone was prepared by Messrs Schering, Ltd.¹ by extraction from urine and was dissolved in sesame oil. It was of a different batch from that used in our previous experiments [Korenchevsky *et al.*, 1932] and the strengths in rat units of these two batches will be compared below (Table III). This hormone preparation had been standardised by Messrs Schering, Ltd., by the method of comb-growth in capons and was stated to contain 2.5 c.u. in 0.1 cc. as in previous batches. Unfortunately, the amount of preparation available was only sufficient for experiments on three litters.

The influence on prostate with seminal vesicles and on penis.

We had at our disposal only two litters, Nos. 1 and 2 (Table I), which were of similar age, castrated at about the same period before sexual maturity and kept for about 41 days after castration, *i.e.* which fulfilled all the requirements for assaying the rat unit as previously defined [Korenchevsky *et al.*, 1932, p. 2106]. The third litter was younger and the rats weighed less, and, although they were castrated at a similar age to that of litters 1 and 2, only 18 days elapsed between the date of castration and the date on which injections were started, instead of from 30 to 50 days as is required for the assay. We, therefore, took the average of the results of the first 2 litters only, using the third litter merely to show that 7 days' injections give altogether different results from those obtained when 4 days' injections are made. However, in spite of the difference in age and in the length of time after castration, the increase in the weight of the prostate with seminal vesicles was very similar to that obtained in the rats of litters 1 and 2 (see Table II), and therefore, even if we use all 3 litters for the average, the percentage increase of these organs calculated per 200 g. of body weight is nearly the same.

Since the rats differed in weight, only the results calculated per 200 g. of body weight are discussed below.

From Table II it is quite clear that if the injections were given for 7 days, the increase in the weight of prostate with seminal vesicles was about 3 times as great, and the increase in the weight of penis was about 4 times as great, as that of the respective organs in the rats injected during the first 4 days only. This indicates that the testicular hormone, injected in doses of 0.1 cc. of oily solution, was absorbed during the whole period of injection, possibly at a definite rate per day. In our previous experiments the effect of the injections appeared to be proportionate not only to the size of the dose but probably also to the number of days of injection [Korenchevsky *et al.*, 1933]. Therefore, taking into consideration the average percentage increase per day of sexual organs after 7 days' injections, the effect of 4 days' injections was much smaller than would

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

Table II. *Influence on prostate with seminal vesicles, penis, adrenals and thymus.*

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

		Actual weights				Weights per 200 g. of body weight				
No. of litter	Con- trol rats	Rats injected		% change		Con- trol rats	Rats injected		% change	
		4 days	7 days	4 days	7 days		4 days	7 days	4 days	7 days
<i>Prostate with seminal vesicles</i>										
1	66	88	162	+33.3	+145.4	54	70	114	+29.6	+111.1
2	53	87	122	+64.2	+130.1	49	66	100	+34.7	+104.1
Average	60	88	142	+48.8	+137.8	52	68	107	+32.2	+107.6
3	64	—	115	—	+79.7	70	—	133	—	+90.0
<i>Penis</i>										
1	84	97	141	+15.5	+67.8	68	77	99	+13.2	+45.6
2	72	100	120	+38.9	+66.7	66	74	98	+12.1	+48.5
Average	78	99	131	+27.2	+67.3	67	76	99	+12.7	+47.1
3	71	—	111	—	+56.4	77	—	128	—	+66.2
<i>Adrenals</i>										
1	100.8	79.6	89.6	-21.0	-11.1	82.0	63.3	62.4	-22.8	-23.9
2	85.4	84.2	72.8	-1.5	-14.7	78.7	64.4	59.1	-18.2	-24.9
Average	93.1	81.9	81.2	-11.3	-12.9	80.4	63.9	60.8	-20.5	-24.4
3	70.5	—	65.0	—	-7.8	77.3	—	75.0	—	-3.0
<i>Thymus</i>										
1	604	478	582	-20.9	-3.6	491	380	405	-22.6	-17.5
2	637	497	597	-21.9	-6.3	587	382	488	-34.9	-16.9
Average	621	488	590	-21.4	-5.0	539	381	447	-28.8	-17.2
3	721	—	548	—	-24.0	786	—	631	—	-19.8

be expected. Such a slight effect is probably explained by the fact that after the cessation of injections the weight of the sexual organs, which was increased by 4 days' injections, started to revert towards the weight of the pre-injection period, owing to the lack of testicular hormone in the organism. Thus, cessation of the injections in castrated animals produced an effect on the sexual organs which is similar to that obtained by removal of the testes in normal animals, and possibly equally rapid.

The influence on adrenals and on thymus.

It was found before that after 7 days' injections, besides the sexual organs, only the thymus and occasionally to a slight extent the adrenals, thyroid and hypophysis of castrated rats showed changes towards the normal condition. In the present experiments, after injection there were noticeable changes in the involution of the thymus and in the decrease in size of the hypertrophied adrenals. Both these changes were, however, slight and irregular and independent of the number of days of injection. In litter 3 changes in the adrenals were negligible.

In all other organs and in the appetite and rate of growth of the rats, no regular changes were observed.

Comparison of assays of the rat unit and capon unit.

For this purpose we have summarised in Table III data obtained in the assay of two batches of testicular hormone. The results obtained in the present experiment with batch B on litters 1 and 2 were compared with those obtained

Table III. *Number of hormone units in two batches of testicular extract as assayed by the authors' method on rats and as assayed by the comb-growth method on capons.*

No. of batch of extract	Average final weight of rats g.	Average age in days		Average percentage increase per 200 g. of body weight				No. of units	
		At castra- tion	Final	Prostate with seminal vesicles		Penis		By capon method	By rat method
				Total	Per 1 c.u.	Total	Per 1 c.u.		
Batch A	193	28	67	88.4	35.4	38.7	15.5	2.5	2.21 $\left(\frac{88.4}{40}\right)$
Batch B	251	25	73	107.6	43.0	47.1	18.8	2.5	2.69 $\left(\frac{107.6}{40}\right)$

in a previous paper [Korenchevsky *et al.*, 1932, Table I, p. 2097 and Table V, p. 2101] on litters 4 and 6 injected with the same daily dose of testicular hormone of batch A.

Each batch was assayed on two litters only, but in spite of the small number of litters used in the assay, the results obtained by both methods agreed satisfactorily. Thus, the strength of both batches, using the capon method, was marked by Schering, Ltd., to be 2.5 c.u. in 0.1 cc. of the preparation, whilst we have found the strength of batch A to be 2.21 R.U. and of batch B to be 2.69 R.U. in 0.1 cc. This is only 11.6 % less and 7.6 % more respectively than the number (2.5) of capon units indicated. Using our method, in some batches, however [see for example, Korenchevsky *et al.*, 1933, p. 563, Table V] the strength of the extract was found to be nearly twice as great as that given by the capon method, whilst in others again it was quite close to the figure indicated. Since, in the latter experiments the period of injection and the time elapsed since castration were longer, they will be discussed elsewhere.

SUMMARY.

1. Experiments were performed on 15 castrated male rats belonging to 3 litters, and the results were compared with those previously published.

2. The effect of 7 days' injection of testicular hormone preparation was about 3 times as great on prostate with seminal vesicles, and about 4 times as great on penis, as that on the respective organs in the litter-mates, injected during the first 4 days only, but killed simultaneously with the former group of rats on the 8th day.

3. In these rats the increase in weight of the sexual organs obtained after 4 days' injections was smaller than would be expected considering the total dose injected and the number of days of injection.

4. It is, therefore, most probable that the weight of the sexual organs, which was increased by 4 days' injections, after cessation of the injections started to return towards the weight of the pre-injection period, owing to the lack of continued supply of hormone in the organism.

5. The above data, together with the accuracy and similarity of the results obtained in the present as well as in previous experiments, indicate that in rats the hormone was absorbed from oily solution (injected in doses of 0.1 cc.) during the whole period of injection at some definite constant rate of absorption.

6. A comparison of the content of rat units and of capon units in two different batches of testicular hormone gave similar figures, showing that the rat unit was nearly equal to the capon unit in these extracts.

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REFERENCES.

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