CLV. CASTRATED RATS FOR THE ASSAY OF TESTICULAR HORMONE.

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IN a previous paper¹ [Korenchevsky, 1932] the increase in weight of the prostate with seminal vesicles of injected castrated rats by at least 40 % as compared with those of control uninjected rats was proposed as a quick and sensitive method of assay of testicular hormone.

Since, after castration, the weights of the penis and thyroid also decrease, whilst the hypophysis, adrenals and body fat increase in weight, the suggestion was made that, for a test of the complete activity of the testicular hormone, it was necessary to obtain a return towards normal of these organs.

The importance for accurate assay of using litter-mate castrated rats was stressed. In this case the litters were to be divided into three groups, (1) controls uninjected, (2) rats injected with a standard preparation, or a previously prepared preparation, (3) rats injected with the new preparation of hormone. The chief difficulty in the assay is that, up to the present time, such a standard preparation is not available. Even if it were available it would with difficulty be kept at a constant strength owing to the instability of the testicular hormone, due chiefly to oxidation. In any case it would be necessary to test the standard preparation from time to time.

The question, therefore, arises under what conditions the castrated rats of different litters will give the nearest comparable results in assay.

In the previous paper was noted the importance of using rats in litters from the same stock, reared on the same diet, of the same age, at the same date after castration, and kept during the experiment on the same complete synthetic diet.

The present communication contains a study of the post-castration changes of the organs mentioned above in different litters of male rats, in relation to sexual maturity at the time of castration of the rats and to the number of days elapsing between castration and the examination of the rats.

¹ On p. 420 of this paper, the weights of adrenals, thyroids and hypophysis must be divided by 10, the decimal points having been inadvertently omitted.

PRESENT INVESTIGATION.

Eighty-eight castrated rats belonging to 48 litters (40 litters in pairs and 8 litters singly) have been arranged in Tables I and II according to sexual maturity at the time of castration and the interval in days between castration and the time at which the rats were killed. These rats were used as the controls of several other experiments. All the rats belonged to the Lister Institute stock and were reared on the same diet.

The 50th day after birth was chosen as the age of sexual maturity. In the Lister Institute, sexual maturity, as judged by fertility, has been exceptionally noted as early as the 38th day after birth, and frequently, but not generally, at the 50th to 56th day (unpublished results of Miss H. H. Smith). The 50th day was, therefore, taken as the earliest frequent date of sexual maturity. This is in agreement with most observations on different stocks. Allen [1918], using rats from the Wistar Institute, found the first ripe spermatozoa in testes between the 36th and 40th day; Andersen [1932] gives the age of sexual maturity as about 60 days as judged by the finding of motile spermatozoa in the epididymis. The rats, however, about 50 days old sometimes had large numbers of non-motile spermatozoa. Hewer [1916] alone gives a later date for sexual maturity in his stock, namely, at about 70 days old.

It is clear from Table I that each of the four groups of rats castrated in an immature state, arranged in relation to the number of days after castration, represents a particular age with comparatively small deviations from the

Table I. *The number and age of the castrated male rats in the different groups and their average ages (with the deviations from the average in brackets).*

		No. of days after castration when killed							
		20-30		31-70		71-90		136-182	
	No. of Rats	Age in Days	No. of Rats	Age in Days	No. of Rats	Age in Days	No. of Rats	Age in Days	
Castrated when sexually immature	8	58 (+11) (-4)	13	95 (+18) (-15)	6	111 (+12) (-10)	11	203 (+13) (-28)	
Castrated when mature	22	88 (+12) (-11)	19	137 (+64) (-36)	8	184 (+12) (-16)	1	286	

average age for each group. In the rats castrated after maturity these deviations are greater in the second group. As would be expected, the longer after castration the rats were killed, the older they were.

The weights of the organs were calculated per 200 g. of body weight for each rat. When the litter was represented by a pair of rats the average weight of each organ was found for this litter. From these weights a general average weight of the organs for each group of litters was made and tabulated, together with the maximum deviations from the general average.

Thus the greatest fluctuations in each group between the different litters are shown for each organ. In addition the variations between the different

groups in relation to sexual maturity at castration and the number of days elapsing between castration and the killing of the rats are shown.

In relation to the thymus and penis the following facts must be taken into consideration. The thymus was not dissected in the earlier experiments and, therefore, the averages are obtained for only 28 rats belonging to 14 litters, that is from a small number of observations.

With regard to the penis the technique of dissection of this organ was changed, and in some of the first litters, which unfortunately were not noted, the penis was dissected together with the enlargement at the root by which it is attached to the pelvic bones. Later it was found that the weighing of the penis is much more accurate if the section was made just above these enlargements¹. The weights by the second method are, therefore, smaller than by the former method. Hence the most reliable data with regard to the sexual organs are those for prostate with seminal vesicles. It is also necessary to note that the fourth group of "mature" litters was represented by one litter only and, therefore, no conclusions can be drawn for this group.

Sexual organs.

From Table II it is clear that the period at which castration is performed, *i.e.* before or after sexual maturity, is of great importance with regard to the

Table II. *The average weights of the organs and retroperitoneal fat per 200 g. of body weight of castrated male rats belonging to different litters in relation to the sexual maturity at the time of castration and the number of days after castration at death (with the maximum percentage deviation from the average in brackets).*

Organs	Condition of rats at castration	Days elapsed between castration and killing			
		20-29	30-67	70-89	136-182
Prostate and seminal vesicles (mg.)	Immature	103 (10.7)	60 (26.7)	55 (18.2)	60 (23.3)
	Mature	154 (24.0)	152 (38.1)	180 (16.1)	199 —
Penis (mg.)	Immature	85 (20.0)	90 (52.2)	59 (27.1)	65 (83.1)
	Mature	110 (36.3)	183 (59.5)	221 (10.0)	224 —
Thymus (mg.)	Immature	635 (12.8)	495 (6.3)	390 —	— —
	Mature	447 (29.3)	256 —	— —	— —
Adrenals (mg.)	Immature	66.6 (10.7)	47.4 (27.2)	41.1 (12.9)	42.4 (20.7)
	Mature	46.1 (21.0)	40.0 (41.2)	38.3 (6.5)	43.9 —
Thyroids (mg.)	Immature	22.0 (20.4)	19.8 (42.9)	19.2 (18.2)	17.9 (38.5)
	Mature	20.5 (20.0)	17.8 (47.4)	19.6 (6.6)	15.4 —
Hypophysis (mg.)	Immature	11.8 (11.9)	9.0 (25.5)	8.9 (5.6)	8.6 (11.6)
	Mature	8.7 (25.3)	8.6 (66.3)	8.2 (26.8)	7.1 —
Retroperitoneal fat (g.)	Immature	2.79 (19.3)	6.65 (47.3)	8.00 (19.6)	10.88 (25.9)
	Mature	6.87 (47.0)	7.22 (58.3)	7.34 (29.2)	4.89 —

degree of atrophic changes in the sexual organs. If the rats are spayed before maturity the atrophic changes are greater than if the operation is performed after maturity. At the same time the weight of the prostate with seminal

¹ It has now been found that weighing the dissected penis is more accurate after previous fixation in Bouin's fixative than without fixation (*i.e.* the penis should be treated in the same way as the other organs).

vesicles per 200 g. of body weight remains constant from the end of the first month after castration, being practically the same at six months after castration. During the first month after castration the weight is a little greater.

The fluctuations between the different litters of the same group are not very large, the maximum deviation not exceeding 27 %, and the average deviations being 8.5, 15.0, 10.9 and 15.0 % for the four groups respectively.

If the rats are castrated after sexual maturity, the fluctuations are similar, being again greatest in the second group (31–70 days after castration); the largest deviation was about 38 %, the average deviations being 9.4, 19.3 and 9.4 % for the three first groups respectively.

The somewhat greater fluctuations in the weight of the penis may be due to the use of two methods of dissecting the penis, as mentioned above.

Forty-eight litters yield too small a number of observations from which to select adequate material for the study of the influence of age at castration within the two large divisions grouped as before and after sexual maturity. However, eight litters can be chosen to indicate this latter influence since all were killed at about the same time after castration (from 64 to 69 days after the operation).

1. *Before sexual maturity.* The average weights of the prostates with seminal vesicles of three litters castrated 22–25 days after birth were 46, 50 and 60 mg. per 200 g. of body weight respectively, whilst the average weights of these organs were 76 mg. in each of two litters castrated at 49 days after birth, *i.e.* at a date close to maturity.

2. *After sexual maturity.* These organs in two litters castrated at 64 and 74 days after birth weighed 112 and 126 mg. per 200 g. of body weight, whilst in the litter castrated at 134 days old the weight was 210 mg.

Taking into consideration the fact that maturity may vary from the 36th to the 70th day, as mentioned above, and that the 50th day was taken only as a frequent early date of sexual maturity, some of the rats within this period might actually be operated on before, others during and others after, maturity. Therefore, on the average within this period of age the changes observed may be nearly similar.

The thymus in rats, as in other mammals, reaches its greatest weight at sexual maturity and from then the normal process of involution begins. Castration prolongs the period of growth of the thymus and inhibits involution. In our rats the weight of the thymus per 200 g. of body weight was greater in those castrated before, than in those castrated after, sexual maturity (Table II), which is in agreement with the fact mentioned above.

Retroperitoneal fat increased in amount in the “immature” group with increase in the number of days elapsing between castration and the date of killing of the rats. This difference was not so distinctly marked in the “mature” group.

In the rats killed 20–30 days after castration the weight of fat was greater in the “mature” group (6.87 g.) than in the “immature” group (2.79 g.), which may be due to the greater average age of the rats in the former group.

Adrenals, thyroids and hypophysis. The largest weights per 200 g. of body weight of the adrenals, and to a less degree of the thyroids and hypophysis, of castrated rats were observed in the "immature" group about 20–30 days after castration, gradually decreasing with the increase in number of days after castration. This difference was less marked in "mature" litters. The last group of the latter contained one litter only and the figures must, therefore, be taken with reservation.

The average figures obtained are in good agreement with those previously published [Korenchevsky, 1930, Tables 1–8].

DISCUSSION.

Taking into consideration all the data relating to the changes in weights of organs calculated for the same unit of body weight the following facts are noticeable.

The greatest differences were observed (a) between the litters castrated before sexual maturity and those castrated after, and (b) between the litters killed during the first month after castration and those killed $4\frac{1}{2}$ months and later after castration.

About $2\frac{1}{2}$ months after operation the changes obtained became more or less stationary (prostate, penis, adrenals) or continued to develop more slowly (fat, thyroids, hypophysis) so that the similarity was found to be greatest in the litters killed $2\frac{1}{2}$ –6 months after castration.

The variations in nearly all the organs were greatest in the litters killed 31–70 days after castration, probably owing to uneven rates of development of the changes towards the final state during this period.

Thus the changes produced by castration may be expected to be most nearly similar in different litters if the rats are operated on at the same number of days after birth.

Since the time of operation, before or after sexual maturity, influences profoundly the changes obtained, and since the exact date of maturity in different litters and stocks varies from the 36th to the 70th day, it is advisable to castrate the rats before the 30th day if immature age is chosen for operation, or after the 70th day if sexually mature rats are wanted for operation.

It is also necessary to choose a definite period after castration for beginning the experiment.

Taking into consideration all the limitations mentioned above it must be admitted that in rats belonging to different litters the variations in the weights of the organs investigated were not great provided the rats belonged to the same group.

The inter-relationship between sexual maturity and castration is probably due to the fact that castration before maturity (i) hinders the development and growth of sexual and some other organs, and (ii) produces changes in the already developed organs (*e.g.* atrophy of the sexual organs). After maturity only this latter effect of castration operates.

SUMMARY.

1. Calculated per the same unit of body weight the weights of retroperitoneal fat, sexual and endocrine organs of 88 castrated male rats belonging to 48 different litters were compared.

2. On the average the variations observed between different litters were not great provided the litters belonged to the same group in relation to sexual maturity at the date of castration (before or after sexual maturity) and in relation to the time elapsing after castration.

3. Because of the uncertainty of the exact age of sexual maturity in rats belonging to different litters and stocks, it is advisable to operate on rats before the 30th day after birth if the operations are to be made on immature rats, and after the 70th day if sexually mature animals are to be castrated.

4. In order to obtain accurate and comparable results all the other precautions described in the previous paper must be taken.

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