CVI. THE EFFECT OF CRYPTORCHIDISM AND OF CASTRATION ON THE CHEMICAL COMPOSITION OF RATS.

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THIS investigation not only aimed at ascertaining whether cryptorchidism and castration produce any change in the chemical composition of the tissues of the rat, but was also a trial of a simple method of obtaining approximate, but accurate, information about the general chemical composition of the bodies of such small animals as mice, rats or guinea-pigs. It was mainly desired, in this method, to avoid a long and difficult treatment of the whole body of the animal for analysis. In the present paper only the amount of water, solids, nitrogen and fat in the tissues of the rats was investigated.

HISTORICAL.

Reference has been made in previous papers [Korenchevsky, 1914, 1925, 1928, 1930; Korenchevsky and Carr, 1925; Korenchevsky and Schultess-Young, 1928] to the whole literature on castration and cryptorchidism. It is perplexingly contradictory. The fact which is most definitely established is that in most castrated animals and human beings an increase in the body-fat is observed when the testes are removed or are atrophied. Korenchevsky's [1930] experiments on rats confirmed this quite definitely, as well as showing that most cryptorchid animals also become obese, although to a lesser degree than the castrates.

As far as we know there is only one investigation on this subject, by Lüthje [1902, 1903], who studied the chemical composition of whole castrated animals, namely, of two castrated dogs as compared with that of their normal litter mates. Lüthje's technique was perfect, but unfortunately the number of animals used in the experiments, owing to the difficulty of technique, was insufficient for any conclusions. Nevertheless, we refer to these experiments at length, since, considered in connection with our experiments, Lüthje's results will assist in the drawing of our conclusions. The following interpretation of Lüthje's results is ours and was not suggested by him. Lüthje's figures have been recalculated by us as percentages of the final body-weight of the animals and as fat-free body-weight; and are shown in Table I.

Table I. *Percentage content of solids, fat and nitrogen in two castrated dogs as compared with that of normal litter mates.*

	Final weight of dog in g.	Solids %	Fat %	Nitrogen %	Nitrogen per 100 g. fat-free body-weight
Normal ♂	17,650	46.5	9.2	3.01	3.31
Castrated ♂	16,600	47.5	8.6	3.14	3.44
Normal ♀	11,400	45.3	9.15	2.93	3.22
Castrated ♀	12,620	48.1	12.8	2.81	3.23

As was stated in our experiments on dogs [Korenchevsky, 1925], only some of these animals became obese after castration. Similarly in Lüthje's experiments one dog (male) became a "thin" castrate after castration, whilst the bitch was a typical "fat" castrate. Consequently, the results of chemical investigation were different, the general nitrogen content being slightly greater (by 4.3 %) in the "thin" castrate, whilst in the castrated bitch it was slightly less (by 4.1 %), than in the respective control animals. This decrease of nitrogen content in the obese bitch was not evident when the nitrogen content was recalculated per 100 g. of fat-free body-weight. This decrease of nitrogen in the "fat" castrate is therefore only apparent and due to the increased proportion of fat in the body and not to an impoverishment in protein substances of the castrated organism. There was, moreover, a slight increase in the nitrogen of the "thin" castrated male dog. It was shown in a previous paper [Korenchevsky, 1925] that, after castration, the deposition of nitrogen in the body of most castrated animals increases. This increase, indicated by metabolism experiments, agrees well with an increased nitrogen content of the body of the "thin" castrate found in Lüthje's experiment (Table I). An increase in the solids in the bodies of both castrated dogs (see Table I) can be explained by the deposition either of fat, or of nitrogen, or of both.

PRESENT EXPERIMENTS.

The experiments were performed on 46 rats, belonging to 9 litters (see Table II). Each litter was divided into a group of control rats and groups of cryptorchid and castrated rats. The cryptorchids of some litters were divided into one-sided cryptorchids, in which only one testicle was made cryptorchid, and into both-sided cryptorchids in which both testicles were made cryptorchid. Altogether 18 control rats, 5 one-sided cryptorchids, 16 both-sided cryptorchids and 7 castrates were used. All these rats have been described elsewhere [Korenchevsky, 1930] and all the details of the general technique employed are there given, as well as a description of the influence of cryptorchidism and castration on growth, fat deposition, sexual and endocrine organs. In the present investigation, it was decided to use the hind legs for analysis, since they are suitable as a general indication of the chemical composition of the principal tissues of the body, namely, skin (with fur), muscles and skeleton. Two controlling chemical analyses are obtainable, so that the results obtained on one

Table II. *The percentage content of nitrogen in each dry hind leg of each rat.*

No. of litter	Normals		Cryptorchids: one-sided		Cryptorchids: both-sided		Castrates	
	No. of rat	Nitrogen content	No. of rat	Nitrogen content	No. of rat	Nitrogen content	No. of rat	Nitrogen content
5	829	10.21	835	9.88	831	9.58	834	8.92
		10.25			9.63			8.88
	830	9.90			832	10.24		
		9.89			10.14			
Average		10.06		10.18		9.90		8.90
6	836	9.80	838	9.32	839	9.28		
		9.75			8.84		8.96	
	837	9.61			840	—		
		9.58						
841	9.83							
	9.69							
Average		9.71		9.08		9.12		
7	842	—	843	10.01	844	9.50		
		10.26			9.53			
	845	10.09			846	10.17		
		10.11			10.04			
Average		10.10		10.14		9.81		
8	863	9.60	865	9.45	866	9.07	868	9.20
		9.65			8.91		9.30	
	864	10.01						
		9.86				867	8.77	
						8.39		
Average		9.78		9.59		8.99		8.92
9	869	9.04	872	9.99	871	9.55	873	8.94
		9.22			9.47		8.82	
	870	9.57				874	9.42	
		9.74			9.62			
Average		9.39		9.82		9.51		9.20
22	847	9.78			849	9.35	851	9.12
		9.75				9.29		8.94
	848	9.49					852	9.59
		9.43					9.63	
Average		9.61				9.32		9.32
23	856	9.85			854	10.10		
		9.87				9.87		
					855	9.84		
						9.91		
					857	9.73		
						9.70		
Average		9.86				9.86		
24	858	—			861	9.73		
						9.75		
	859	11.23			862	9.62		
		10.54				9.87		
Average		10.89				9.74		
25	875	9.68			880	9.28		
		9.53						
	877	10.04			881	10.13		
		10.11				10.18		
Average		9.84				9.86		
Average of averages of litters		9.92		9.76		9.57		9.09

leg can be used to check the results obtained with the other. It was decided to investigate the amount of water, solids and nitrogen in the legs, but it is obvious that any other necessary chemical analyses could also be made. For the approximate estimation of the deposition of fat in the body, a still simpler method, which was used by us in the previous paper [Korenchevsky, 1930], was chosen. In this method, the weight of the retroperineal and testicular fat, which can be quickly and easily separated from the body by means of a scalpel, was taken for the comparative estimation of the degree of deposition of fat in the animal. The comparative changes in fat deposition in different animals can be checked in each animal by the separate weighing of the testicular and retroperineal fats. Elsewhere [Korenchevsky, 1930], only the total amount of intra-abdominal fat (*i.e.* the retroperineal plus testicular) is given. Therefore in the present paper we give, in Table III, the weights of retroperineal fat and of

Table III. *Average weight of retroperineal and testicular fat in groups of normal, cryptorchid and castrated rats.*

No. of litter	Normal rats		One-sided cryptorchids		Both-sided cryptorchids		Castrates. Retro-perineal fat
	Retro-perineal fat	Testi-cular fat	Retro-perineal fat	Testi-cular fat	Retro-perineal fat	Testi-cular fat	
5	6.27	5.70	8.60	8.24	4.13	6.68	12.91
6	6.41	6.97	8.59	9.77	10.02	7.98	—
7	6.96	6.54	6.81	8.49	5.22	9.21	—
8	6.47	8.40	8.28	9.97	8.07	7.95	11.41
9	8.68	7.18	6.02	6.78	8.89	9.91	9.36
22	6.97	5.98	—	—	8.15	9.96	9.67
23	5.27	5.79	—	—	7.35	4.98	—
24	4.32	5.35	—	—	7.74	8.48	—
25	5.77	4.94	—	—	6.66	8.95	—
Average	6.35	6.32	7.66	8.65	7.36	8.23	10.84

testicular fat separately, so that both data can be compared. The weight of fat is calculated in the table as g. per 200 g. body-weight of the rat. The separation of the legs from the body was performed in the following way: the hind leg was bent at the fibula-tibia-femoral junction by the left hand of the investigator and the blades of the scissors placed round and just a little above this junction, in such a manner that the lower blade of the scissors was placed in the angle made by bending the leg at the junction; then the leg was cut off, without producing tension of the skin and muscles and the cut made neat with the scissors. It was possible, without any special training, to sever the legs so accurately, that the dry weight of the right leg of the animal differed from that of the left leg of the same animal, on the average, by only $\pm 2.6\%$ (minimum ± 0.2 , maximum $\pm 9.1\%$). The legs were placed in weighing bottles and weighed, first fresh and then after desiccation at about 103° . The nitrogen was estimated by the Kjeldahl method in each leg separately. In order to save space only one table, No. II, is given in detail. In all the other tables the changes obtained in groups of litters, and not those in individual rats, are given.

FAT DEPOSITION.

From a comparison of the weights of retroperineal and testicular fat in Table III, it is possible to say that in five of the groups of cryptorchid rats, of litters nos. 5, 7, 8 and 23, there is a lack of agreement in the changes obtained between testicular and retroperineal fats, namely that, whilst the testicular fat, as compared with that of the normal control rats, was increased, the retroperineal fat was decreased or showed no change, or *vice versa*. This indicates that, whilst it is only in the majority of cases that the increase, or decrease, in fat deposition is evenly distributed between the retroperineal and testicular regions, in some rats fat is deposited in one or other of these regions preferentially. It is therefore necessary, in animals with normal testes, to use the sum of the weights of the testicular and retroperineal fats to obtain an indication of the deposition of fat in the body. However, when a sufficiently large number of animals is used for the conclusions, the average figures give similar results in both cases. A comparison of the average figures of Table III shows that, on the average, the weights of both the retroperineal and testicular fats were increased in cryptorchid animals, as compared with those of normal control animals. The amount of fat in castrated animals was also increased and in all cases to a greater degree than in cryptorchid animals. For further conclusions based on the total intra-abdominal fat and on a larger number of animals we refer to the previous paper [Korenchevsky, 1930].

NITROGEN CONTENT.

In Table II is given the percentage content of nitrogen in each dry hind leg of each rat. The average for each group of each litter and a general average for all the rats of the group are also given. It can be seen, by comparing the nitrogen content of the two legs of the same rat, that in the 42 animals investigated, the fluctuations, from the average for the two legs of the same rat, was about or less than $\pm 0.5\%$ in 21 rats, from ± 0.5 to $\pm 1\%$ in 10 rats, from $\pm 1.1\%$ to $\pm 1.75\%$ in 7 rats and ± 2 to $\pm 3.2\%$ in 4 rats.

These results indicate that the method employed was quite satisfactory, giving data sufficiently accurate for the comparison of the different animals, with a probable error not greater than 1.8% in most cases and in no case investigated more than 3.2% .

On a general average, it was found that the legs of one-sided cryptorchids contained 1.6% , of both-sided cryptorchids 3.5% and of castrates 8.4% less nitrogen than the legs of their normal controls. Consequently it is necessary to note that the castrated rats were more obese than the cryptorchids and both of them were more obese than the normal rats. We consider therefore that we are justified in explaining this small decrease in the nitrogen content in the same way as we did for Lüthje's dogs (p. 955); that it is unlikely that it can indicate a decrease in the amount of nitrogenous substances in the bodies of cryptorchid and castrated rats; that the decrease is apparent and is due to

the increased proportion of fat in the cryptorchid and castrated animals. This is in agreement with the cases where the nitrogen content was even slightly increased, as was the case in the one-sided cryptorchids of groups 5, 7 and 9 and in the both-sided cryptorchids of group 9. In all these groups the amount of solids (Table IV) was less, in spite of the increased amount of fat in most of the groups. Therefore an even higher nitrogen content is suggested in these cases.

Table IV. *The average percentage content of solids and water in groups of normal, cryptorchid and castrated rats.*

No. of litter	Normal rats		One-sided cryptorchids		Both-sided cryptorchids		Castrates	
	Solids	Water	Solids	Water	Solids	Water	Solids	Water
5	38.1	62.0	37.4	62.6	38.9	61.1	40.8	59.2
6	39.1	60.9	40.2	59.8	41.4	58.7	—	—
7	38.9	61.1	37.7	62.3	39.6	60.4	—	—
8	39.4	60.6	39.7	60.3	41.7	58.3	41.8	58.2
9	40.6	59.5	39.8	60.2	40.0	60.0	40.2	59.8
22	39.6	60.5	—	—	40.4	59.6	39.8	60.2
23	39.1	60.9	—	—	39.7	60.3	—	—
24	37.1	63.0	—	—	40.1	59.9	—	—
25	39.2	60.8	—	—	40.1	59.9	—	—
Average	39.0	61.0	39.0	61.0	40.2	59.8	40.7	59.4

SOLIDS AND WATER CONTENT.

From Table IV it is clear that the solids content of the legs was slightly higher, and the water content slightly less, in both-sided cryptorchids and in castrates, than in the normal control rats. On the average, there was no change in one-sided cryptorchids.

Although this increase in the content of solids was only small (by 3.1 % in cryptorchids and 4.4 % in castrates) nevertheless its occurrence in nearly all the groups investigated indicates that it is not of an accidental character. The increased deposition of fat is probably only partly responsible for this increase, since in one-sided cryptorchids the degree of obesity was about the same as in both-sided cryptorchids, but the increase in solids was absent in the former group.

Taking into consideration the increased deposition of nitrogen in most castrated animals [Korenchevsky, 1925], we suggest the same explanation as we have given for Lüthje's results, namely, that in castrated and both-sided cryptorchid animals an increase in the content of solids is to be explained by an increased deposition of fat and probably also of nitrogenous substances in the body.

SUMMARY.

1. A method was used for the investigation of the chemical composition of small animals, which gives approximate but accurate data.
2. This method was applied for ascertaining the content of water, solids, nitrogen and fat in castrated and one-sided and both-sided cryptorchid male

rats, as compared with that of normal litter mate animals. Altogether 46 rats were investigated.

3. The amount of intra-abdominal fat was increased in both cryptorchid and castrated animals, but to a greater degree in the latter.

4. The nitrogen content was slightly decreased in both-sided cryptorchid and in castrated rats.

5. This decrease, however, is chiefly due to the increased proportion of fat in the bodies of cryptorchid and castrated rats, and therefore is not due to the impoverishment of the bodies of these animals in nitrogenous substances.

6. A slight increase in the content of solid matter was found in both-sided cryptorchid and in castrated rats. This increase can be explained by an increase in the deposition of fat and probably also of nitrogenous substances.

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