CLXXX. THE INFLUENCE OF THE GONADS ON PROTEIN METABOLISM.

III. (a) THE EFFECT OF INJECTIONS OF ANTERIOR PITUITARY EXTRACTS ON URINARY CREATI- NINE IN NORMAL AND CASTRATED RABBITS. (b) THE TOLERANCE OF NORMAL AND CAS- TRATED ANIMALS TO INJECTED CREATINE.

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(Received June 16th, 1933.)

THE evidence for the existence of an endocrine relationship between the testes and creatinine metabolism was presented in the first two papers of this series [Schrire and Zwarenstein, 1932, 1, 2]. This conclusion is supported by the observations of Vedel, Peuch and Lang [1926] who describe the case of a man of 56 who had been castrated and showed regression of the secondary sexual characters. His creatine and creatinine excretion was subsequently investigated. No creatine was excreted but the amount of creatinine in the urine was 2.8 g. per litre. According to Folin [1905] the concentration of creatinine in normal human urine varies from 0.61 to 2.0 g. per litre with an average of 1.08. In the second paper cited above it was suggested that the increased elimination of creatinine after castration was primarily due to hyperactivity of the anterior lobe of the pituitary, consequently injections of anterior lobe extracts should cause an increase in the excretion of creatinine. In this paper evidence is presented in support of the hypothesis. As a contribution to the more detailed analysis of the problem the effect of injections of creatine into normal and castrated animals was also investigated.

EXPERIMENTAL.

Details in regard to caging, feeding, collection and analysis of urine were given in the first paper of this series. In Table I the dates refer to the first of a number of 24-hour periods. The mean and the standard deviation of the daily

Table I. *Daily urinary creatinine, mg.*

Date	Normal (1)	Castrated (2)	Castrated (3)	Normal (4)	No. of 24-hour periods
A. Pre-periods:					
12. iv. 32	92 $\pm$ 5.2	76 $\pm$ 5.8	89 $\pm$ 3.2	—	9
7. v. 32	93 $\pm$ 5.5	93 $\pm$ 3.5	91 $\pm$ 2.4	—	5
17. vi. 32	97 $\pm$ 3.1	87 $\pm$ 4.3	79 $\pm$ 1.0	86 $\pm$ 4.1	4
Nos. 2 and 3 castrated 21. vi. 32.					
B. Post-castration periods:					
12. xii. 32	96 $\pm$ 7.4	120 $\pm$ 1.9	123 $\pm$ 11.6	84 $\pm$ 8.3	5
27. xii. 32	98 $\pm$ 2.5	116 $\pm$ 9.1	119 $\pm$ 10.0	91 $\pm$ 3.2	4
16. i. 33	95 $\pm$ 3.7	111 $\pm$ 2.6	122 $\pm$ 5.2	98 $\pm$ 7.0	4

creatinine output in mg. are given for each series of 24-hour periods. In all Tables rabbits Nos. 1 and 4 are the normal animals and Nos. 2 and 3 the castrated animals.

(a) INJECTION OF ANTERIOR LOBE EXTRACTS.

Parke-Davis antuitrin was used and also an extract of sheep anterior lobe prepared according to Bellerby's method [1933]. As a control an extract of brain prepared by the same method was injected. These were all injected subcutaneously.

Table II. *Daily urinary creatinine, mg.*

Date	Normal (1)	Castrated (2)	Castrated (3)	Normal (4)
16. i. 33	92	109	120	100
17. i. 33	96	109	114	92
18. i. 33	92	*109	126	*92
19. i. 33	101	115	127	109
20. i. 33	105	120	134	114
21. i. 33	100	109	120	93
22. i. 33	101	120	126	85
23. i. 33	100	†120	†126	†92
24. i. 33	92	120	126	112
25. i. 33	86	109	114	92
26. i. 33	100	120	120	92
27. i. 33	100	114	133	90

* On Jan. 18 No. 2 injected with 2 cc. brain extract, No. 4 with 2 cc. anterior lobe extract.

† On Jan. 23 No. 2 injected with 2 cc. anterior lobe extract, No. 3 with 2 cc. brain extract and No. 4 with 2 cc. antuitrin.

Table III. *Daily urinary creatinine, mg.*

Date	Normal (1)	Castrated (2)	Castrated (3)	Normal (4)
28. ii. 33	101	120	114	84
1. iii. 33	100	120	109	86
2. iii. 33	100	*109	*130	*90
3. iii. 33	104	120	123	110
4. iii. 33	98	110	109	80
5. iii. 33	96	109	121	96
6. iii. 33	93	†114	126	†80
7. iii. 33	100	109	120	106
8. iii. 33	104	120	130	109
9. iii. 33	92	109	120	92
10. iii. 33	93	109	130	88

* On March 2 Nos. 2, 3 and 4 each injected with 3 cc. antuitrin.

† On March 6 Nos. 2 and 4 each injected with 3 cc. antuitrin.

Table IV. *Daily urinary creatinine, mg.*

Date	Normal (1)	Castrated (2)	Castrated (3)	Normal (4)
10. iv. 33	96	120	133	100
11. iv. 33	96	126	140	88
12. iv. 33	*92	*120	*136	*96
13. iv. 33	125	134	140	96
14. iv. 33	100	122	125	96
15. iv. 33	100	133	126	96
16. iv. 33	†100	†126	133	†98
17. iv. 33	133	120	133	126
18. iv. 33	114	122	126	105
19. iv. 33	100	120	126	88
20. iv. 33	100	120	135	96

* On April 12 Nos. 1 and 3 each injected with 3 cc. anterior lobe extract; Nos. 2 and 4 each injected with 3 cc. brain extract.

† On April 16 Nos. 1, 2 and 4 each injected with 3.5 cc. anterior lobe extract.

The following conclusions can be drawn from the data presented above.

(a) Injection of antuitrin or of anterior lobe extract into normal animals caused an increased excretion of creatinine. The figures suggest that the increase bore a constant relation to the dosage. This is seen in the following table. The figures in brackets refer to the number of the animal. The average weight of rabbit No. 1 during the injection experiments detailed above was 2.74 kg. and of rabbit No. 4, 2.34 kg.

	Antuitrin		Anterior lobe extract		
Amount injected	2 cc.	3 cc.	2 cc.	3 cc.	3.5 cc.
Percentage increases	22 (4)	26 (4) 28 (4)	22 (4)	30 (1)	33 (1) 30 (4)

In all cases the rise occurred on the day following injection, persisted in a few cases during the next day and in all cases rapidly returned to the normal level.

(b) Injection of antuitrin or of anterior lobe extract had no effect on the high creatinine level in the castrated animals.

(c) Brain extract had no effect either in the normal or in the castrated animals.

Discussion.

Braier [1931] showed that there was a marked diminution in the creatinine excretion of hypophysectomised dogs. Relatively this effect comes on much sooner than the increased excretion of creatinine after castration, which indicates that the effect of castration is due primarily to the pituitary. The castration effect is retarded because it depends upon the gradual hypertrophy of the anterior lobe of the pituitary. The work of numerous investigators has established the fact that in many species castration leads to an increase in the weight of the anterior lobe [Moore and Price, 1932; Stein, 1933, who also give reviews of the literature]. Reese and McQueen-Williams [1932], have shown that the morphological changes in the anterior lobe of the male rat due to removal of the testes can be prevented by administration of extracts containing the active principle of the male gonads. The results of parabiotic experiments show that the hypertrophy is not due to defective utilisation and storage of hormones but involves increased production and secretion. Brauer [1929] found that anterior lobe hypertrophy after gonadectomy also occurs in rabbits although there seems to be some doubt whether females show any appreciable hypertrophy [Wolfe, 1932].

The depressing effect of the gonad secretions on the anterior lobe of the pituitary, only incompletely shown by the normal animal, is unmasked when the gonads are removed, and the effect of the removal of the inhibitory influence becomes more and more apparent as the anterior lobe gradually hypertrophies. This process is reflected in a gradual increase in the elimination of creatinine in the urine.

These facts together with those reported above afford strong evidence of a direct endocrine relationship between the anterior lobe of the pituitary and creatinine metabolism. The persistent increase in creatinine excretion which occurs as the result of castration implies the continued new formation of creatine and its transformation to creatinine. A steady level is reached after several months. This may be partly explained by the fact that hypertrophy of the anterior lobe reaches a maximum. Evans and Simpson [1929] noted in rats that the gonad-stimulating capacity of the pituitary slowly increased up to the 8th post-operative week, after which there was little increase, and Schenk [1927]

found that the cell-type changes in rats occur progressively until a level state is reached some time between the 4th and 7th post-operative months. On injection of anterior lobe extracts into castrated animals no further increase in the excretion of creatinine was obtained, and this indicates that cessation of pituitary hypertrophy is not the sole limiting factor in the production of a maximum steady level of creatinine output. It would be premature at this stage to attempt any detailed interpretation of the facts. Relevant data in regard to the creatine content of the muscles of normal and castrated animals and the effect of repeated daily injections of anterior lobe extracts are lacking.

(b) INJECTION OF CREATINE.

2.5 g. of creatine were injected subcutaneously into normal and castrated rabbits and the creatine excretions estimated in the urine. The animals used in this experiment were the same as those used in the injection experiments reported in the first part of this paper. In Tables V and VI the figures in brackets refer to the amounts of creatine excreted in mg. No creatine was excreted by the control animal or by the other animals on the days preceding the injection.

Table V. *Daily urinary creatine and creatinine, mg.*

Date	Normal (1)	Castrated (2)	Castrated (3)	Normal (4)
27. iii. 33	100	120	136	92
28. iii. 33	100	120	132	91
29. iii. 33	98	114	120	96
30. iii. 33	102	125	135	92
31. iii. 33	109 (291)	126 (474)	141 (659)	92
1. iv. 33	109 (21)	135 (65)	126 (74)	88
2. iv. 33	96 (10)	120 (21)	126 (25)	92
3. iv. 33	100	126 (24)	133 (12)	92
4. iv. 33	100	120 (17)	133 (5)	96
5. iv. 33	105	126	133 (17)	92
6. iv. 33	100	120	135	92
7. iv. 33	105	123	126	96

On March 30 Nos. 1, 2 and 3 each injected with 2 g. creatine.

Table VI. *Daily urinary creatine and creatinine, mg.*

Date	Normal (1)	Castrated (2)	Castrated (3)	Normal (4)
2. v. 33	100	126	133	96
3. v. 33	100 (1144)	127 (1739)	120 (1746)	100 (1300)
4. v. 33	100 (71)	120 (413)	126 (310)	100 (108)
5. v. 33	92 (31)	120 (40)	130 (41)	105 (19)
6. v. 33	91	118 (18)	135 (23)	93 (6)
7. v. 33	92	130 (20)	126 (25)	96
8. v. 33	100	133	130	100
9. v. 33	90	120	124	95

On May 2 Nos. 1, 2 and 3 each injected with 5 g. creatine; No. 4 injected with 3.8 g.

The results of Tables V and VI are summarised below:

	Normal	Cas- trated	Cas- trated	Normal	Cas- trated	Cas- trated	Normal
Creatine injected (g.)	2	2	2	5	5	5	3.8
Total creatine excreted (mg.)	322	601	792	1245	2230	2145	1433
Creatine retained (mg.)	1678	1399	1208	3755	2770	2865	2367
Percentage excreted	16.1	30.0	40.0	25.0	44.6	42.9	37.7

It is evident that normal and castrated animals behave differently towards injected creatine. In all cases the normal animal excreted less creatine and retained more than the castrated, *i.e.*, the normal animals have a high and the castrated a low tolerance to exogenous creatine. The castrated animal excreted a larger proportion of the injected creatine and also took longer to do so. The excretion of creatinine was not affected.

These results are in essential agreement with those of Remen [1932]. He found that intravenous injection of 500 mg. creatine into normal adult men between the ages of 20 and 53 is followed by no greater excretion of creatine in urine than before injection. Men of 70–90 years, a eunuch 43 years old and one man of 68 castrated for carcinoma excreted most of the injected creatine. One case of hypophysial obesity, in which the normal excretion of creatine was high, reacted to injected creatine like normal adult men. The old men with failing sex function, the eunuch and the castrate thus showed a decreased tolerance typical of infants and children and, as shown above, of castrated rabbits. Remen concluded that the male sex glands have a regulatory influence on the metabolism of creatine. Lasch [1932] arrived at the same conclusion on the basis of similar experimental results. The evidence presented in the first part of this communication indicates that the difference between normal and castrated animals in regard to creatine-creatinine metabolism is due primarily to hyperactivity of the anterior lobe of the pituitary.

SUMMARY.

1. Injection of antuitrin or of anterior lobe extracts of the pituitary leads to an increased excretion of urinary creatinine in normal rabbits but has no effect on the high creatinine output of castrated animals.
2. The evidence presented affords evidence of a direct endocrine relationship between the anterior lobe of the pituitary and creatinine metabolism.
3. Normal rabbits show a high, and castrated animals a low, tolerance to subcutaneous injections of large amounts of creatine.

The authors wish to thank Messrs Parke, Davis and Co., London, for a supply of antuitrin.

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