CCXXIV. THE INFLUENCE OF THE GONADS ON PROTEIN METABOLISM.

II. THE EFFECT OF INJECTIONS OF SALINE SUS- PENSIONS OF TESTES AND TESTICULAR EXTRACTS ON URINARY CREATININE AFTER CASTRATION.

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(Received November 1st, 1932.)

IN a previous paper [Schrire and Zwarenstein, 1932] it was shown that castration of adult male rabbits led to a persistent increase in the excretion of urinary creatinine, that grafting of testes into castrated animals caused a decrease to the normal pre-castration level, and that after subsequent removal of the graft the usual castration effect on urinary creatinine was re-established. These results suggest the existence of an endocrine relationship between the testes and creatinine metabolism, but a definite conclusion can be arrived at only when evidence has been presented that the effects of castration can be compensated by injection of testicular suspensions and extracts. The results in regard to the effect of testicular injection on the increased elimination of creatinine after castration form the substance of this paper.

EXPERIMENTAL.

The procedure with regard to feeding, caging *etc.* was the same as reported in the previous communication. Three to four months after removal of the testes the usual castration effect on creatinine appeared. The testes of rabbits or portions of bull testis were finely divided with a sharp scalpel, the macerated liquid mass suspended in Locke's solution and injected subcutaneously by means of a large veterinary syringe and needle. The effects were controlled by injection of brain suspension prepared in the same way. The testicular extracts were prepared from the testes of bulls, pigs and rams according to the method described by Moore, McGee and co-workers [McGee, Juhn and Domm, 1928; Moore, Price and Gallagher, 1930]. The final extract was taken up in olive oil before injection.

Extracts of sheep's brain prepared by the same method or olive oil alone were injected as controls.

1 cc. of olive oil extract represents 94 g. of fresh testis or 57 g. of brain. In the first part of Table I (pre-period and 3 months castration figures) the

Table I. *Daily urinary creatinine.*

Rabbits Nos. 1, 2, 3. Normal animals.

,, 4 and 5. Castrated 14. iii. 32.

,, 6 and 7. Castrated 16. iii. 32.

Date	mg.						
	Normal (1)	Normal (2)	Normal (3)	Castrated (4)	Castrated (5)	Castrated (6)	Castrated (7)
A. Pre-periods and 3 months castration							
Jan. 17	95	63	63	94	60	74	86
Feb. 1	95	75	78	97	68	81	89
„ 29	97	75	81	94	72	79	93
Mar. 7	94	79	86	87	75	79	87
„ 29	91	—	—	89	—	79	74
Apr. 11	92	—	—	87	—	74	81
„ 25	93	—	—	91	—	79	86
May 16	93	—	—	90	—	93	92
June 15	97	—	—	94	—	84	96
B. Injection of saline suspensions of rabbit's testes							
July 11	79	92	81	116	104	107	100
„ 12	90	85	96	111	91	106	106
„ 13	95	85	79	120	106	114	106
„ 14	98	88	88	112	116*	125*	117
„ 15	92	92	86	103	75	93	108
„ 16	81	85	84	115	62	84	110
„ 17	96	85	82	112	110	105	113
C. Injection of saline suspensions of bull's testis and brain							
		(1)	(3)		(5)	(6)	
Sept. 9		92	78		116	98	
„ 10		86	80		112	104	
„ 11		83	74		108	111	
„ 12		92	72		112	106	
„ 13		92	80†		114†	108†	
„ 14		90	92		120	116	
„ 15		96	90		85	125	
„ 16		100	79		105	121	
„ 17		100	Died		124	127	
„ 18		100	—		120	127	
D. Injection of testicular and brain extracts and olive oil							
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
July 23	78	70	85	120	105	105	109
„ 24	80	74	75	109	89	100	109
„ 25	76	80	90	109	104	109	120
„ 26	87	83	92	96	120	83	120
„ 27	93	75	80	96	106	75	109
„ 28	86	75	75	72	120	78	120
„ 29	86	70	51	86	104	100	114
„ 30	85	86	—	98	116	116	127
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
July 25	No injection	Brain 4 cc.	Oil 4 cc.	Testis 4 cc.	No injection	Testis 4 cc.	Brain 4 cc.
„ 26	„	„	„	Testis 6 cc.	„	Testis 6 cc.	„
„ 27	„	„	„	Testis 10 cc.	„	Testis 10 cc.	„
„ 28	„	Oil 4 cc.	Testis 10 cc.	Testis 10 cc.	„	Testis 10 cc.	Oil 4 cc.

* On July 14 Nos. 5 and 6 injected with saline suspension of testis.

† On Sept. 13 No. 5 injected with saline suspension of testis. Nos. 3 and 6 injected with saline suspension of brain.

dates refer to the first of five 24-hour periods in each case. The mean of the daily creatinine output in mg. is given for each series of 24-hour periods. Rabbits Nos. 2, 3 and 5 were allowed to run free for about 4 months when they were returned to the metabolism cages.

DISCUSSION.

The post-castration rise in urinary creatinine commencing 3 to 4 months after removal of the testes confirms the results reported previously. Examination of the data shows further that injection of saline suspensions of rabbit's or bull's testis led after a slight rise to a fall in creatinine to the pre-castration level. On the other hand, injections of brain suspension had no such effect on either the normal or castrated animal. With rabbits' testes the effect persisted for two days, after which the creatinine rose to the castration level. With bull's testis the fall occurred on the second day after injection after which there was again a rapid increase.

Injection of testicular extracts also caused a decrease to the normal pre-castration level. In rabbit No. 4 the final injection on July 28th led to a creatinine output for that day which was below normal. The decrease persisted throughout the injection periods, rising again when injections ceased. Injections of brain extract or of olive oil had no effect. Rabbit No. 3, a normal animal, was injected on July 28th with testicular extract instead of with olive oil and this led to an immediate drop in creatinine excretion.

The decrease in the high creatinine of castration caused by injections of testicular suspensions or extracts and the subsequent post-injection rise are effects which are in complete agreement with those obtained with grafting of testes and subsequent removal of the graft.

The results as a whole thus afford strong evidence in favour of the existence of an endocrine relationship between the testes and creatinine metabolism. This relationship may be a direct one or it may be an indirect one through some other organ of internal secretion. The fact that the castration effect only appears after a latent period of several months suggests that it is the result of a slow, gradual change in another endocrine organ. This hypothesis is supported by the results of recent work on the relation between the gonads and the pituitary and the pituitary and creatinine metabolism. Martins and Rocha [1931] review the evidence in regard to the regulation of pituitary activity by the testes and report their results on parabiotic experiments in rats. They come to the conclusion that as a result of castration a pituitary hypertrophy, associated with the presence of "castration cells" in the anterior lobe, occurs and the organism becomes flooded with anterior pituitary hormones. The gonads normally exert an inhibitory effect on the pituitary gland.

Braier [1931] has shown that hypophysectomised dogs on a complete diet eliminate 24 % less creatinine than the controls. This difference is accentuated

in fasting animals, removal of the pituitary leading to a 35–40 % decrease in creatinine excretion. This effect was obtained as early as 30 days after operation. Evidence in regard to the effect of injections of pituitary extracts on urinary creatinine is lacking but it is reasonable to assume on the basis of these results that increased activity of the pituitary gland associated with hypertrophy of the anterior lobe occurring as the result of castration would lead to an increased elimination of creatinine.

On the assumption that castration causes marked hypertrophy of the anterior lobe of the pituitary in male rabbits the castration effect on creatinine excretion is explicable as being due to the unantagonised hyperactivity of the anterior lobe.

The continuous stimulating effect of the pituitary is rapidly and effectively antagonised by injection of saline suspensions of testes or testicular extracts and more slowly but as effectively by grafting testes into castrated animals. In both cases the creatinine excretion drops to normal. The effect is a transient one with injection but persists as a result of grafting. Removal of the graft almost immediately brings into operation the effect of pituitary hyperactivity and the creatinine rises to the typical castration level; the same effect is seen during the post-injection periods.

SUMMARY.

1. Injection of saline suspensions of testes or of testicular extracts causes the high urinary creatinine of castration to decrease to the normal pre-castration level.
2. It is pointed out that the results reported above together with those in a previous paper afford strong evidence in favour of the existence of an endocrine relationship between the testes and creatinine metabolism.
3. It is suggested that the castration effect is an indirect one through the anterior lobe of the pituitary gland.

We have to thank Dr L. P. Bosman and Mr D. G. Duncan for assistance in the preparation of extracts.

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