XLVII. THE INFLUENCE OF THE GONADS ON PROTEIN METABOLISM.

IV. THE EFFECT OF OVARIECTOMY AND OF INJECTIONS OF GONADAL AND ANTERIOR PITUITARY EXTRACTS ON URINARY CREATININE IN FEMALE RABBITS.

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THE experiments on males detailed in the first three papers of this series [Schrire and Zwarenstein, 1932, 1, 2; 1933] were repeated on females. Creatine and creatinine were determined by Folin's method. The daily output of creatinine remained constant within comparatively narrow limits. The excretion of creatine, on the other hand, varied considerably. In all Tables, Rabbits Nos. 1, 2 and 3 are the normal animals and Nos. 4, 5, 6 and 7 the ovariectomised animals.

Ovariectomy.

In Table I the dates refer to the first of five 24-hour periods in each case. The mean and standard deviation are of the daily creatinine output in mg. given for each series of 24-hour periods. The weights of the animals at the beginning and at the end of the experiments are included.

Table I. *Daily urinary creatinine, mg.*

	Normal			Ovariectomised			
	1	2	3	4	5	6	7
Weight, kg.	2.08	1.96	2.0	2.22	1.96	1.89	1.98
Date	A. Pre-ovariectomy periods.						
25. i. 32	65 ± 4.6	—	76 ± 1.9	72 ± 5.6	77 ± 2.6	74 ± 1.4	66 ± 4.0
8. ii. 32	77 ± 7.4	71 ± 4.1	77 ± 4.0	76 ± 3.6	74 ± 6.0	72 ± 4.2	79 ± 2.1
15. ii. 32	70 ± 3.6	67 ± 1.9	74 ± 3.9	76 ± 3.6	68 ± 5.5	73 ± 6.6	75 ± 2.8
22. ii. 32	75 ± 3.4	70 ± 3.3	77 ± 3.8	75 ± 7.2	68 ± 5.3	74 ± 4.0	75 ± 1.0
Nos. 4, 5, 6, 7 ovariectomised 9. iii. 32.							
B. Post-ovariectomy periods.							
21. iii. 32	—	—	70 ± 4.2	79 ± 3.6	—	67 ± 3.9	76 ± 3.2
18. iv. 32	—	—	71 ± 3.9	82 ± 3.0	—	68 ± 1.0	74 ± 6.4
9. v. 32	—	—	57 ± 4.6	74 ± 2.9	—	66 ± 4.1	64 ± 6.8
6. vi. 32	—	—	56 ± 4.8	76 ± 5.5	—	62 ± 4.5	67 ± 2.8
4. vii. 32	—	—	74 ± 6.3	76 ± 10.5	—	68 ± 3.7	75 ± 8.7
13. viii. 32	—	78 ± 5.6	78 ± 5.1	67 ± 3.1	68 ± 3.9	66 ± 3.7	68 ± 3.4
5. xii. 32	—	90 ± 2.5	86 ± 4.5	108 ± 8.5	99 ± 8.2	98 ± 3.5	89 ± 6.3
19. xii. 32	92 ± 3.7	98 ± 7.0	88 ± 4.8	104 ± 6.2	99 ± 4.5	105 ± 5.1	99 ± 3.5
9. i. 33	84 ± 2.2	92 ± 2.7	87 ± 3.6	102 ± 2.5	104 ± 4.9	99 ± 6.5	103 ± 6.3
Weight, kg.	3.13	3.01	2.32	3.06	2.72	2.50	2.44

The data in Table I are summarised in Table II in the form of percentage increases in creatinine and body weight.

Table II.

	1	2	3	4	5	6	7
Creatinine	22	31	22	40	44	46	35
Weight	50	54	16	38	39	32	33

Inspection of the data shows that five months after ovariectomy the creatinine excretion was still normal. This result is in striking contrast to the effect of castration in males in which a post-castration rise in creatinine begins three to four months after removal of the testes.

Nine months after the date of ovariectomy an increased creatinine excretion occurred in all animals but the rise was significantly larger in the ovariectomised animals. The increases in creatinine output bore no constant relation to the increases in body weights. A comparison of the percentage figures for the normal and ovariectomised animals shows that the increase in creatinine excretion and gain in weight bore an inverse relation to each other for the two series of animals. The post-ovariectomy rise in creatinine was still maintained 12-14 months after the operation (Tables III and IV).

Table III. *Daily urinary creatinine, mg.*

Date	Normal			Ovariectomised			
	1	2	3	4	5	6	7
13. iii. 33	89	87	87	96	105	100	105
14. iii. 33	92	90	91	116	110	96	105
15. iii. 33	87	91	87	108	98	100	104
16. iii. 33	96	90	88	110	105	105	110
17. iii. 33	100	105	—	100	105	110	109
18. iii. 33	88	80	88	84	97	100	85
19. iii. 33	91	88	90	95	100	95	88
20. iii. 33	96	100	91	105	100	91	110
21. iii. 33	83	80	95	112	95	90	100
22. iii. 33	80	95	85	100	85	80	84
23. iii. 33	84	91	91	100	95	100	110
24. iii. 33	90	88	85	110	105	100	112
17. iii. 33	No injection	Ovary extract 10 cc.	No injection	Ovary suspension	Brain suspension	Brain extract 10 cc.	Ovary extract 10 cc.
21. iii. 33	Testis extract 20 cc.	Testis extract 20 cc.	No injection	Brain extract 10 cc.	Ovary extract 10 cc.	Testis extract 20 cc.	Testis extract 20 cc.

Table IV. *Daily urinary creatinine, mg.*

Date	Normal			Ovariectomised			
	1	2	3	4	5	6	7
24. iv. 33	95	90	88	105	116	110	105
25. iv. 33	100	95	81	116	110	110	116
26. iv. 33	88	91	90	116	105	100	116
27. iv. 33	89	88	90	105*	105*	99*	116*
28. iv. 33	91	100	89	95	89	70	110
29. iv. 33	95	95	91	89	95	105	110
30. iv. 33	91	88	85	100	96	100	110
1. v. 33	95	91	84	100	105	105	110

* 27. iv. 33 Nos. 4, 5 and 6 injected with saline suspension of ovaries.
No. 7 injected with saline suspension of brain.

Injection of saline suspensions of ovaries and of ovarian and testicular extracts.

Finely divided ovaries of cats or sheep and pigs were suspended in Locke's solution and injected subcutaneously. Ovarian and testicular extracts were prepared from sheep's or pig's ovaries or testes by the method of Moore, McGee and co-workers [McGee *et al.*, 1928; Moore *et al.*, 1930]. The final extract was taken up in olive oil before injection. Suspensions or extracts of brain were used as controls. 1 ml. of extract represents 93 g. testis, 58 g. ovary and 91 g. brain.

Injection of saline suspension of ovary into ovariectomised animals caused a decreased excretion of creatinine. In animal 6, Table IV, there was a fall to the pre-ovariectomy level. Both ovarian and testicular extracts caused a slight but probably not significant fall when injected into normal animals. Larger decreases occurred on injection into ovariectomised animals. In all cases brain suspension or extract had no effect.

Injection of anterior lobe extracts.

An extract of anterior lobe of sheep pituitary was prepared according to Bellerby's method [1933]. An extract of brain prepared by the same method served as a control. Both extracts were injected subcutaneously.

Table V. *Daily urinary creatinine.*

Date	Normal			Ovariectomised			
	1	2	3	4	5	6	7
10. v. 33	96	88	85	99	105	102	107
11. v. 33	100	110	107	105	105	101	100
12. v. 33	93	111	82	105	105	116	112
13. v. 33	93	87	83	106	96	100	110
14. v. 33	84	86	91	105	100	105	108
15. v. 33	105	91	105	110	100	105	105
16. v. 33	88	91	88	110	100	98	116
17. v. 33	92	85	93	108	105	—	112
22. v. 33	88	84	88	110	116	—	105
23. v. 33	98	93	92	113	117	—	109
24. v. 33	95	110	108	100	105	—	105
25. v. 33	90	108	105	105	108	—	110
26. v. 33	88	88	91	105	116	—	116
27. v. 33	95	81	92	116	105	—	107
10. v. 33	Nos. 1 and 6 injected with 3.5 ml. brain extract.						
	Nos. 2, 3, 4 and 5 injected with 3.5 ml. anterior lobe extract.						
14. v. 33	Nos. 1, 3 and 7 injected with 2.0 ml. anterior lobe extract.						
	Nos. 2 and 4 injected with 2.0 ml. brain extract.						
23. v. 33	Nos. 2, 3, 4 and 5 injected with 4.0 ml. anterior lobe extract.						
	Nos. 1 and 7 injected with 4.0 ml. brain extract.						

As in the same experiment with males, injection of anterior lobe extract into normal rabbits caused an increased excretion of creatinine, while injection into operated animals had no effect on the high creatinine output. Brain extract had no effect either in the normal or in the ovariectomised animals.

DISCUSSION.

The results of ovariectomy and of injection of ovarian extracts affords evidence of the existence of an endocrine relationship between the ovaries and creatinine metabolism. The fact that both ovarian and testicular extracts have the same effect on the high creatinine level of ovariectomised animals suggests that the active principle in the ovarian extract is similar to, if not identical with, the active principle in the testicular extract.

Evidence presented in a previous paper [Schrire and Zwarenstein, 1933] suggests that the persistent increase in creatinine which occurs as the result of

castration is due primarily to hypertrophy of the anterior lobe of the pituitary. The results recorded above (Table V) indicate that the post-ovariectomy increase in creatinine is referable to the same factor.

Two outstanding differences emerge on comparing the effect of gonadectomy on creatinine excretion in the two sexes. The post-castration rise in creatinine commences 3 to 4 months after removal of the testes and in some cases sooner. In females the creatinine level is still normal 5 months after ovariectomy. It is unfortunate that owing to unavoidable circumstances no determinations could be carried out during the 6th–8th post-operative months. When determinations could again be resumed in the 9th post-operative month a definite increase in creatinine output was apparent.

In the ovariectomised animals the absolute individual rises in creatinine output above the values for the pre-periods were of the order of 41 %. Parallel normal controls showed an increase of about 25 % so that the true increase due to ovariectomy was actually about 16 %. Castration of males on the other hand led to a 25–50 % increase in the output of creatinine. Since in both sexes the effect on creatinine is primarily due to a pituitary hypertrophy, this difference is explicable on the assumption that functional hypertrophy of the anterior lobe is more pronounced in males than in females. This suggestion is supported by the observations of Hatai [1913] who found that the pituitary of castrated male rats was about 70 % heavier than that of spayed females, and by the results of Evans and Simpson [1929] who showed that the anterior lobe of the pituitary of gonadectomised male rats was more potent than that of gonadectomised females, as judged by the capacity of implants to produce precocious oestral changes in young females.

The excretion of creatine was too variable to allow of any certain conclusion regarding the effect of ovariectomy.

SUMMARY.

1. Ovariectomy of adult female rabbits leads to a 16 % increase in the excretion of urinary creatinine commencing at least 5 months after the operation. The latent period is longer and the increase smaller than in castrated males.

2. Injection of saline suspension of ovary causes a fall in the high urinary creatinine of ovariectomy. Both ovarian and testicular extracts cause a slight fall when injected into normal animals. Larger and significant decreases occur on injection into ovariectomised animals.

3. Injection of extract of the anterior lobe of the pituitary leads to an increased excretion of urinary creatinine in normal females but has no effect on the high creatinine output of ovariectomised animals.

4. The results reported above afford evidence of an endocrine relationship between the ovaries and creatinine metabolism, this relationship being an indirect one through the anterior lobe of the pituitary.

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