CXVII. THE INFLUENCE OF THE GONADS ON PROTEIN METABOLISM

V. THE EFFECT OF TESTICULAR, URINARY AND ANTERIOR PITUITARY PREPARATIONS ON THE URINARY CREATININE OF NORMAL AND CASTRATED RABBITS

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In the first three papers of this series [Schrire & Zwarenstein, 1932, 1, 2; 1933] it was shown that castration of adult male rabbits led to a persistent increase in the excretion of urinary creatinine, whilst injection of testicular extracts caused the high post-castration creatinine level to return to normal and that extracts of anterior pituitary caused increased excretion of creatinine in normal rabbits but had no effect on castrated rabbits.

In this paper a more detailed analysis of the pituitary-gonadal control of endogenous protein metabolism is made.

EXPERIMENTAL

Caging and feeding of animals and collection and analysis of urines were as described in the first paper of this series. Testicular extracts were prepared from ram's testes according to Moore, McGee and co-workers [McGee *et al.* 1928; Moore *et al.* 1930]. The final extract was taken up in olive oil before injection. Extracts of brain and liver prepared by the same method were injected as controls. Pituitary extracts were prepared from sheep anterior lobe according to Bellerby [1933] and extracts of brain and liver were used as controls. Saline suspensions of testes and brain were prepared from ram's tissues as described previously [Schrire & Zwarenstein, 1932, 2]. The urine extract was prepared from adult male urine according to Funk *et al.* [1930].

The various extracts and suspensions were injected subcutaneously into (a) normals, (b) recent castrates and (c) long-standing castrates. During a latent period of 1-4 months after castration the creatinine excretion remains normal and the animals are called recent castrates. Long-standing castrates are animals in which the usual post-castration rise has established itself. In all cases this type of animal was injected 6 months after castration.

Crystalline male hormones dissolved in nut oil were injected subcutaneously into normal animals only.

RESULTS

Consistent results were obtained in a large series of experiments in all cases. The results shown in the following tables are representative. The pre-period figures represent the averages of 30-35 daily estimations.

(a) *Injection of testicular extract*

Table I. *Daily urinary creatinine, mg.*

	Normal	Long-standing castrates
Pre-period	101	97
Day 1	100	96
2	123	73
3	108	72
4	105	82
5	100	87
6	100	95
7	129	90
8	109	95
9	103	70
10	102	76

Normal: Day 2, 4 ml. and day 7, 5 ml. lipoid extract of testes injected.
Castrate: Animal castrated 6 months previously. Pre-castration creatinine level 70 mg.
Day 2, 5 ml. and day 9, 3 ml. lipoid extract of testes injected.

		Recent castrate			
Pre-period	Normal	Time after castration			
		3 weeks	5½ weeks	11 weeks	17 weeks
Day 1	90	87	90	96	92
2		106	99	96	96
3		90	93	93	96
4		87	89	96	98
Injection on day 2		3 ml.	2.5 ml.	3.3 ml.	3 ml.

No effect was obtained on injecting 1-1.5 ml. (300-450 mg. lipoid) into normal animals but significant increases were obtained on 46 occasions on injection of 2-5 ml. The rise occurs on the day of injection after which there is

Fig. 1. Dose response curve.

a rapid return to normal. In recent castrates injections of testicular extract lead to increased creatinine elimination only during the first 6 weeks after castration,

and only after larger doses than are required to give increases of the same magnitude in normal animals. Later there is a refractory period during which injection has no effect; in this phase the daily creatinine excretion tends to be rather less constant than in normal animals, in recent castrates during the first phase and in long-standing castrates. The injection of testicular extracts into long-standing castrates invariably lead to a fall to the normal precastration level. Control injections of brain or liver extracts have no effect.

The results in normal animals indicated that the dose per unit weight of animal bore a constant relationship to the response. In a new series of experiments 8 adult male rabbits varying in weight from 1.57 to 2.51 kg. were injected with 2, 3 and 4 ml. of testicular extract. The graph (Fig. 1) shows that a definite quantitative relationship exists between dose and response.

(b) *Injection of saline suspension of testes*

Table II. *Daily urinary creatinine, mg.*

Time after castration ...	Normal	Recent castrates		Long-standing castrates
		3 weeks	10 weeks	6 months
Pre-period	72	60	75	86
Day 1	77	64	82	90
2	73	60	75	90
3	70	52	80	87
4	67	40	68	69
5	51	40	67	70
6	53	59	64	78
7	65	58	80	90

All animals were injected with 20 ml. saline suspension of testes (1 ml. = 0.33 g. testis tissue). Normal and recent castrates were injected on day 2, the long-standing castrate on day 3. The long-standing castrate had a pre-castration output of 63 mg.

Injection of saline suspension of testes into normal animals produces a fall in urinary creatinine of 23–33% for a dose of 20 ml. The diminished output begins on the 2nd to the 4th day after injection and usually reaches its lowest level on the 5th or 6th day after injection.

Recent castrates (animals castrated 3 or 10 weeks previously) give results similar to but less pronounced than those in normal animals. Long-standing castrates respond to the injection of saline suspension with a fall in the creatinine excretion to the pre-castration level on the day following the injection. The pre-castration output is maintained for 3–4 days after which there is a gradual increase to the previous high post-castration level.

Control injections with saline or with saline suspension of brain had no effect on the creatinine output.

(c) *Injection of urine extract*

In addition to extracts prepared in the laboratory, proviron (Schering-Kahlbaum) was also injected. The first batch of proviron used was prepared from male urine and is designated proviron 1. Proviron 2 obtained about 18 months later is androsterone benzoate. Only normal animals were injected.

Table III, cols. 1 and 2, shows typical responses to urinary extracts, viz. a fall below normal 24–48 hr. after injection followed by a transient rise above normal. Such a biphasic response was not obtained with androsterone benzoate (Table III, col. 3) which produced the normal male hormone effect of increased creatinine output.

Table III. *Daily urinary creatinine, mg.*

	Normal		
	1	2	3
Pre-period	96	62	73
Day 1	88	61	65
2	64	65	82
3	54	52	103
4	129	62	88
5	90	75	75
6	91	87	72
7	—	79	—
8	—	65	—

All animals were injected on day 2.

No. 1 injected with 12 ml. urine extract (1 ml. = 200 ml. urine).

No. 2 injected with 2 ml. proviron 1 (a urine extract).

No. 3 injected with 2 ml. proviron 2 (1 ml. = 5 mg. androsterone benzoate).

(d) *Injection of androsterone, testosterone and their esters*

The required amounts of androsterone, testosterone and testosterone propionate were dissolved in nut oil. In addition, the Schering-Kahlbaum products proviron (androsterone benzoate) and testoviron (testosterone propionate) were also injected. Only adult normal male rabbits were used (Table IV).

Table IV. *Daily urinary creatinine, mg.*

	Normal			
	1	2	3	4
Pre-period	46	74	53	68
Day 1	45	68	50	74
2	47	77	49	85
3	50	90	69	85
4	70	98	60	82
5	62	91	61	76
6	56	67	60	70
7	50	—	50	61

No. 1 was injected with 15 mg. androsterone on day 2.

No. 2 was injected with 14.5 mg. androsterone benzoate on day 2.

No. 3 was injected with 22 mg. testosterone on day 3.

No. 4 was injected with 20 mg. testosterone propionate on day 2.

Injections of 5 or 10 mg. of androsterone had no effect; 15 and 20 mg. produced a 30–40 % increase on the 2nd day after injection, lasting 2–3 days. Androsterone benzoate gave similar results, the rise occurring on the day following injection and persisting for several days.

Amounts of testosterone up to 15 mg. had no effect; with 20 mg. however increases were obtained on the day of injection and were followed by a relatively rapid return to normal, a result similar to that obtained with testicular extract.

Testosterone propionate in doses of 5 to 20 mg. produced increased elimination of creatinine on the day of injection or on the following day. With 10–20 mg. the high level was maintained for 3–4 days after which there was a gradual return to normal. Testoviron gave similar results.

These results show that testosterone and the two esters exercise their effects sooner than androsterone. Furthermore, the actions of the esters are more prolonged than those of the pure hormones, a result which is in agreement with Parkes [1936] and Miescher *et al.* [1936].

(e) *Injection of anterior pituitary extracts*

Extracts prepared from fresh sheep anterior lobes, ambinon (Organon) and antuitrin (Parke Davis) were injected into normal animals and into long-standing castrates. Significant increases in creatinine output were obtained on injection into normal animals but there was no effect on the high creatinine level of long-standing castrates (Table V). These results confirm our previous findings [Schrire & Zwarenstein, 1933].

Table V. *Daily urinary creatinine, mg.*

Time after injection ...	Normal	Recent castrates		Long-standing castrates 6 months
		4 weeks	10 weeks	
Pre-period	68	65	62	108
Day 1	70	66	60	108
2	75	65	61	112
3	97	83	78	115
4	85	67	66	107
5	74	65	60	111
6	—	60	64	113

All animals were injected on day 2 with 3 ml. anterior pituitary extract.

In addition, anterior pituitary extracts and antuitrin were injected into animals castrated 4 and 10 weeks previously. In both types the creatinine output was still normal at the time of injection, and injection of anterior pituitary extract led to significant increases in urinary creatinine. Antuitrin gave the same result. As with normal animals in the recent castrates the rise occurred on the day following injection and there was rapid return to normal. Other experiments showed that the longer the animal had been castrated the less was the effect of injection. The maximum effects were obtained in normal animals and in the recent castrates, type (a).

All experiments were controlled by injections of brain extract which had no effect.

DISCUSSION

In previous papers it was suggested that the effect of castration on urinary creatinine is due to the hypertrophy and increased functional activity of the anterior pituitary. This results in an increased output of creatinine. When this has reached a maximum, after a latent period of some months, a constant high level in creatinine output is maintained. As pointed out by Schrire & Zwarenstein [1933], cessation of pituitary hypertrophy is not the sole limiting factor since injections of anterior lobe extracts have no further effect on creatinine excretion in long-standing castrates. The data presented above confirm this finding and show in addition that the decrease in the effect of anterior pituitary injections after castration is continuous. The limiting factor and the factor responsible for the gradual decrease in effect observed on injection of pituitary extracts must probably be ascribed to some action of a pituitary hormone on the formation of creatine from its precursors or on the transformation of creatine into creatinine. Shapiro & Zwarenstein [1936] found in fact that hypophysectomy decreased and injections of anterior lobe extract increased the muscle creatine content of *Xenopus laevis* (the South African clawed frog).

The different effects of injecting testicular extract into the various types of animals suggest that the response depends on the state of the pituitary. In normal animals the full effect is obtained, i.e. maximum stimulation of an

unaltered anterior lobe. In the 4-week castrate the stimulating effect is less than in the normal whilst in the 10-week castrate there is no effect. On the completely hypertrophied pituitary (long-standing castrate) the effect is inhibitory.

Androsterone and testosterone also stimulate the normal pituitary. The concept that such stimulation may occur is supported by recent work [cf. Marrian & Butler, 1937] which shows that oestrogens may stimulate the production of gonadotropic hormone and also the adrenotropic activity of the pituitary. The gonad-pituitary relationship in some cases may be more complicated as indicated by the biphasic effect of urine extracts and of oestradiol (unpublished experiments) on urinary creatinine and also by the fall followed by a rise in the metabolic rate of rats as the result of the action of oestrin on the thyrotropic activity of the anterior pituitary [Sherwood & Bowers, 1936].

Saline suspensions of testes on the other hand diminished the urinary creatinine in both normal and castrate animals. Changes in the responsiveness of the anterior pituitary after castration may account for quantitative differences in the degree of inhibition. The different results obtained with lipid extracts and with saline suspensions of testes suggest the presence of two different active principles in the testes, as has been suggested by Lower *et al.* [1935]. McCullagh & Walsh [1935] have actually prepared water-soluble extracts of testes which inhibit the anterior pituitary and cause atrophy of the accessory sex organs of normal male rats.

The complicated biphasic effect produced by urine extract is probably due to the fact that the extract contains a mixture of hormones. It has been shown that male urine extracts contain not only androsterone and dehydroandrosterone but probably also oestrogens. Oestradiol benzoate causes a fall followed by a rise in the urinary creatinine when injected into adult male rabbits.

SUMMARY

1. Injection of a lipid extract of testes into normal adult male rabbits and into animals castrated less than 6 weeks previously increases urinary creatinine. Injection into older castrates not yet showing a post-castration rise in creatinine has no effect, whilst injection into long-standing castrates (6 months after castration) leads to a fall to the pre-castration level.

2. A quantitative relationship exists between dose and response.

3. Injections of saline suspensions of testes into normal animals, recent castrates and long-standing castrates result in a fall in urinary creatinine.

4. The administration of testosterone and androsterone increases creatinine excretion in normal animals. The effect is prolonged if the hormones are injected in the form of esters.

5. The injection of extracts of adult male urine gives a biphasic effect—a fall below normal followed on the next day by a rise above normal and then a return to the normal level the day after. This is probably due to the presence of both androgens and oestrogens in the extract.

6. Injection of anterior pituitary extracts increases urinary creatinine in normal animals and in recent castrates but has no effect on the high creatinine output of long-standing castrates.

7. The results suggest the presence of two different active principles in the testes. The response to injections of testicular extracts varies according to whether the anterior pituitary is normal or has undergone changes due to castration.

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