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Comparison of Atrophy and Glycogen Storage in Some Muscles After Castration and Denervation.* (22978)

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Castration in adult male rats causes atrophy and loss of glycogen in perineal muscles (including levator ani) and testosterone restores the muscles to normal(1,2). Denervation of skeletal muscle (gastrocnemius) also causes loss of glycogen, coincident with atrophy(3,4). The present study is concerned with a comparison of atrophic and glycogen changes which follow castration and denervation in the male hormone sensitive levator ani and cremaster muscles. The effect of some hormones on glycogen deposition in denervated muscles is also presented.

Methods. Adult male rats of matched body weights were employed, except where indicated, and all operations were performed using ether anesthesia. The genito-femoral nerve to the cremaster was evulsed just posterior to the ilio-lumbar vessels. The nerves forming the pudendal plexus on each side of animal were destroyed to denervate the perineal muscles. A section of the posterior division of the femoral nerve was removed to denervate the rectus femoris muscle. Wherever it was obviously feasible, the right muscle was denervated and the left one used as control, otherwise separate groups of rats were used. In experiments with hormone treatment, testosterone propionate (1 mg doses) and cortisone acetate (2 mg doses) were administered daily for 3 days prior to autopsy.† A 24-hour fast preceded the autopsies and muscles were removed from the anesthetized (nembutal) rats. Only the levator ani of the perineal muscle group was used because of its discrete nature. Varying periods of time elapsed after the operations and before autopsy. Weighing and glycogen determinations were made as previously described(5).

Results. The data in Table I show weight changes in the rectus femoris, cremaster and levator ani muscles following castration and denervation. Fifteen days after denervating the right rectus femoris, the muscle weighed 48% less than the control. Previous observations have shown no appreciable weight loss in this muscle after castration and no data are recorded.

The weight of the levator ani 15 days after either denervation or castration was less than the controls but denervation caused a greater loss in weight. In contrast, there was no significant weight loss in the cremaster 15 days after either denervation or castration.

On the assumption that a time factor might be involved in the latter instance, another group of rats was castrated and autopsied 32 days later. A significant weight loss in the cremaster now occurred. For the denervation experiment, young male rats (120-140 g body weight) were employed and 42 days elapsed after denervation of the right cremaster before autopsy. In addition, reference controls were obtained on day of operation by weighing cremaster muscles from a similar group of young males. The weights of the cremaster muscle 42 days after denervation were significantly less than the paired controls. How-

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† Hormones used in these experiments were generously given by Schering Corp., Bloomfield, N. J.

TABLE I. Comparison of Muscle Weights following Denervation and Castration.

Exp. (No. of rats)	Period, days	Wt in mg ± S.E. Exp.	Control	% loss
		(Rectus femoris)		
Denervate (6)	15	402 ± 23	769 ± 44	48
		(Cremaster)		
Denervate (7)	15	423 ± 27*	486 ± 38	13
" (10)	42	313 ± 20†	394 ± 14	21
Castrate (10)	15	509 ± 28*	541 ± 21	6
" (12)	32	271 ± 15	426 ± 14	36
		(Levator ani)		
Denervate (11)	15	96 ± 6	197 ± 10	51
Castrate (10)	15	145 ± 12	211 ± 19	31

* Difference of means not significant; others $P < .02$.

† Avg initial wt of cremaster muscles (6 rats) at time of operation; right = 126 ± 8, left = 124 ± 8 mg.

ever, a comparison of weights of the reference control and the denervated muscles showed that the latter increased from an initial weight of 126 ± 8 mg to 313 ± 20 mg. Growth was greater in the contralateral muscle with intact nerves over the same period.

Although it has been shown that glycogen in the denervated muscle is always less than in the control whether rats are killed in a fed state or after a fast(4), the experiment was repeated using the rectus femoris and cremaster muscles. Six rats in each group were employed and the period of denervation was for 15 days. The percentage loss in glycogen from control levels in the rectus femoris in the fed and fasting state was 41% and 31% respectively. For the cremaster, it was 38% and 35% respectively. Although it appears to make little difference on a relative basis whether the animals are fed or starved, subsequent glycogen studies were made on the fasting animal.

After periods of 5, 15 and 42 days following denervation or 15 days after castration, the glycogen levels of the cremaster were all significantly lower than the controls (Table II). In the levator ani, castration and denervation also decreased glycogen concentration and, in both muscles, the effect of denervation was greater than castration at comparable periods.

The ability of testosterone and cortisone to elevate glycogen in the rectus femoris and cremaster, denervated for 16 days, was studied. Because of their greater responsiveness to these steroids, the leg muscles of female rats were employed and cortisone was not used in castrated males since this hormone has no effect on the cremaster(6). Table III shows that both hormones increased glycogen levels in the denervated leg muscles but these steroids were 2-3 times more effective when the nerves were intact. Testosterone produced a substantial increase in the denervated cremaster of castrated males but not as great as in the intact muscle.

TABLE II. Muscle Glycogen Levels following Denervation and Castration.

Exp. (No. of rats)	Period, days	Glycogen, mg % ± S.E. Exp.*	Control
		(Cremaster)	
Denervate (6)	5	359 ± 14	524 ± 10
" (7)	15	333 ± 11	476 ± 16
" (10)	42	365 ± 15	477 ± 27
Castrate (10)	15	423 ± 20	507 ± 13
		(Levator ani)	
Denervate (11)	15	155 ± 9	387 ± 13
Castrate (12)	15	304 ± 17	396 ± 9

* In all experiments, difference of means significant, $P < .01$.

Since distention or stretch of the abdominal wall muscles of pregnant rats is sufficient to decrease their glycogen level(7), the possible effect of distention of the cremaster due to the physical presence of the testis was investigated. Ten rats were made unilaterally cryptorchid and 25 days later both cremaster muscles were assayed for glycogen. The glycogen of the muscle on the cryptorchid side

TABLE III. Hormone Effect on Glycogen Deposition in Denervated and Intact Muscles.

Treatment (No. of rats)	Glycogen, mg % ± S.E. Denervated musc. Inj.	Denervated musc. Control	Intact musc. Inj.	Intact musc. Control
	(Rectus femoris)			
T† (24)	292±14	221± 6	542±15	380±17
C (24)	281±15*	233±12	535±20	385±17
	(Cremaster)			
T (24)	459±27	322±11	648±33	450±11

* Difference of means, statistically $P = .02$; others $P < .001$.

† T = Testosterone; C = Cortisone.

was 505 ± 19 mg% and the control, 485 ± 21 mg %, showing no effect due to presence of testis.

Discussion. Although the cremaster and levator ani are male hormone sensitive muscles, the effect of denervation on weight loss is more pronounced in the latter. The data do not permit a similar comparison for castration effects but based on previous results, the relative weight loss about 1 month after castration is 61% for the levator ani(8) versus 36% for the cremaster. No difference in rate of denervation atrophy in 4 leg muscles of the rat is reported(9), but the laryngeal muscles regress faster than either the diaphragm or the external anal sphincter(10.11). It is not clear why there is a difference in rate of denervation atrophy among these skeletal muscles. In the levator ani, denervation causes a greater atrophy than castration within a comparable period of time but the experiments as designed, can only suggest that denervation is less effective than castration in the cremaster.

Growth of the cremaster continues after denervation in the young rat but at a rate slower than normal. Sections of 2 denervated muscles stained for nerve fibres gave no evidence of nerve regeneration by 42 days.‡ Part of the continued growth may be attributed to the male hormone. Some workers (12) state that testosterone increases the rate of denervation atrophy of the gastrocnemius in young males and others(13) report no significant effect of this hormone on the weight of the denervated anterior tibial muscle.

The decrease in glycogen concentration in the cremaster following denervation or castration precedes the loss in weight. In the levator ani, however, both the loss of glycogen and weight is observed to occur together. At comparable periods of time, the decrease in glycogen is greater after denervation than after castration in both muscles. The loss of glycogen after denervation occurs with great rapidity(4) and it is suggested that a reduced glycogen level offers an early criterion of the success of muscle denervation, *i.e.*, as in the cremaster.

‡ Dr. M. Singer kindly prepared these slides.

Testosterone and cortisone elevate glycogen in the denervated rectus femoris although the effect of cortisone is not great. The effect of testosterone on the denervated cremaster is very pronounced. Adrenal cortical extract did not alter the glycogen in denervated muscles of the adrenalectomized rat(14) but insulin elevated muscle glycogen provided glucose was given 3 hours after the hormone(4). Thus some hormones can stimulate glycogen deposition in denervated skeletal muscle; the effect of the nerve is quantitative, not conditioning.

Summary. Comparison of the weight and glycogen losses in the levator ani and cremaster muscles of the rat after castration and denervation show that (1) denervation atrophy is greater in the levator ani than in the cremaster, (2) denervation atrophy is greater than castration atrophy in the levator ani, (3) the loss of glycogen in both muscles after denervation is greater than after castration. Growth of the denervated cremaster continues in the young animal. Testosterone and cortisone can induce glycogen deposition in some denervated skeletal muscles; the effect of the nerves on this response is quantitative rather than conditioning.

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