hand, the adeno, Coxsackie, echo, polio and Coe viruses tested, were resistant to chloroform.

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Effect of Testosterone Propionate on Tissue Protein Synthesis in the Castrated Male Rat.* (26460)

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The fact that nitrogen retention occurs after administration of androgenic steroids to gonadectomized animals is well established (1). This nitrogen retention has been equated with the overall synthesis of total body protein, hence the employment of "anabolic" steroids in attempts to increase overall retention of nitrogen. However, this hormonal induced nitrogen retention has been shown to occur in the castrated rat even during consumption of a protein deficient diet (2) and a positive nitrogen balance could be maintained in spite of a loss of body weight. Furthermore rate of body weight loss was not influenced by the nitrogen retained.

No definite information is available regarding the location of the nitrogen stored by the body during androgenic treatment. The amount of nitrogen retained is greater than can be accounted for by growth of the sex organs. It would be of interest to localize the sites of protein synthesis which are stimulated by testosterone.

For this purpose we decided to utilize the rate of labeling of tissue proteins of castrated male rats receiving a tracer dose of 1-C^{14}-glycine as a measure of the anabolic activity of the tissues under consideration.

Materials and methods. Male Wistar rats were bilaterally castrated under light ether anesthesia when they were 2 months old. Fifteen days after gonadectomy they were implanted subcutaneously with sterile polyvinyl sponges (Ivalon), to study the effects of the hormone on rate of synthesis of a newly formed protein such as collagen.

TABLE I. Effect of Testosterone Propionate on Uptake of C^{14}-Glycine into Protein and Non-Protein Fractions of Tissues in Gonadectomized Male Rats.*

Treatment	Serum	Sem. ves.	Kidney	Heart	Liver	Diaphr.	Perin. musc.	Brain
T.P.	60 ± 8† (1.9)‡	57 ±11 (8.9)	40 ± 9 (6.3)	15 ± 2 (2.1)	29 ± 4 (3.5)	9 ± 2 (2.9)	15 ± 4 (13.2)	4 ±.5 (1.0)
Control	72 ± 5 (2.3)	16 ± 5 (5.8)	39 ±10 (7.3)	23 ± 1 (2.7)	39 ± 4 (4.6)	11 ± 3 (4.0)	5 ± 1 (5.5)	3 ±.5 (.6)

* Sacrificed 5 hr after inj. of C^{14}-glycine.
† Tissue protein specific activity counts/min./mg protein, and stand. error.
‡ Non-protein fraction radioactivity counts/min./mg tissue protein.

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TABLE II. Effect of Testosterone Propionate on Specific Activity of Protein and Non-Protein Fractions of Tissues and Polyvinyl Sponges of Castrated Male Rats at Different Time Intervals after Injection of C^{14}-Glycine.

Time after inj., hr	Treatment	Serum	Sem. ves.	Kidney	Heart	Liver	Diaphr.	Perin. musc.	Gluteus medius	Brain	Skin	Subcut. sponge
10	T.P.	55*(1.8)†	80 (6.9)	41 (4.5)	21 (2.5)	43 (3.5)	14 (2.0)	21 (5.8)	9 (4.2)	3 (.6)	49	53
	Controls	55 (2.4)	19 (3.3)	45 (5.0)	17 (2.1)	43 (6.1)	16 (2.2)	9 (2.2)	9 (4.1)	3 (.7)	40	46
20	T.P.	39 (.6)	52 (4.3)	35 (2.4)	14 (3.7)	27 (2.4)	11 (1.3)	22 (4.5)	10 (2.5)	3 (.5)	47	51
	Controls	41 (.7)	11 (2.4)	32 (2.2)	15 (1.8)	24 (1.7)	14 (1.4)	7 (1.2)	9 (2.4)	2 (.5)	40	48
40	T.P.	32 (.5)	72 (3.8)	37 (1.5)	23 (1.7)	24 (1.2)	15 (1.7)	34 (3.1)	11 (2.6)	2 (.5)	50	51
	Controls	31 (.5)	21 (2.6)	30 (1.6)	16 (1.5)	26 (.9)	23 (1.4)	9 (1.1)	11 (1.6)	2 (.4)	43	39
60	T.P.	25 (.3)	42 (1.3)	27 (1.3)	21 (1.5)	28 (1.1)	13 (1.2)	25 (3.4)	13 (1.6)	2 (.5)	48	45
	Controls	25 (.5)	19 (.1)	23 (1.2)	19 (1.2)	27 (1.2)	11 (1.0)	12 (.8)	11 (1.1)	1 (.3)	35	42

* Specific activity of tissue protein = counts/min./mg protein.
† Non-protein fraction = counts/min./mg tissue total proteins.

Two weeks later they were injected intramuscularly for 3 days with 2.5 mg/day of Testosterone Propionate in 0.25 ml of sesame oil. Simultaneously with the last dose of hormone (or sesame oil in the controls), all animals received intramuscularly 5 μC of 1-C^{14}-glycine (4.5 mC/mM) in normal saline. They were then sacrificed by overexposure to ether after removing blood samples by cardiac puncture. The organs under investigation were removed and aliquots were homogenized at high speed in a Potter-Elvehjem glass homogenizer using 4% TCA as homogenizing fluid. After centrifugation the non-protein fraction was measured for radioactivity. The protein component was washed following the procedure of Siekevitz(3), and finally plated on aluminum planchets and counted with a gas-flow ultra thin window Geiger-Muller tube. Corrections were made for self-absorption. Skin was previously shaved, then dehydrated and defatted by shaking during two 10-hour periods with acetone and during 24 hours with ether. It was then dried in an oven to constant weight. Subcutaneously implanted sponges were carefully dissected free of surrounding tissue. Both skin and sponges were gelatinized by autoclaving with water at 15 p.s.i. for 8 hours. Collagenous protein was plated and its activity measured as described above.

Results and discussion. Table I shows the distribution of radioactivity 5 hours after injection of the tracer amino acid in the protein and non-protein fractions of different tissues. The highest specific activity is found in the circulating serum proteins of the control animals. The control rats show also a larger uptake of radioactivity by the liver protein fraction. This observation would seem to correlate with *in vitro* experiments where a slight inhibition of protein synthesis occurred in livers of testosterone treated mice(4) and rats(5) and also with the loss of liver mass which occurs in rats receiving this hormone(6).

The organs most significantly stimulated in their protein synthesis were the sex linked ones, namely seminal vesicles and perineal complex muscles.

Table II summarizes the effect of testos-

terone administration on distribution of radioactivity after different time intervals. Again the principal sites of hormonal stimulation were at the level of the sex linked organs. However, some stimulation of protein synthesis of borderline significance appeared to take place in the skin of the hormone treated animals. The high protein synthesizing activity which takes place in the perineal muscle is of interest. This muscle complex includes the levator ani muscle which is widely utilized as a site for measuring myotrophic activity (namely anabolic activity). The behavior of this muscle differs from that of the other muscles under study (diaphragm and gluteus medius) and points to the undesirability of arbitrary selection of an organ for measuring anabolic properties of compounds. It had previously been shown that hypertrophy of the levator ani muscle occurred as a consequence of hormone administration even during the feeding of protein free diets(7).

The results obtained with skin and implanted sponges indicating an increased synthesis of collagen as a result of hormone administration are preliminary. Further experiments will explore the extent and significance of this increased labeling. This observation could be a consequence of an increased turnover of pre-existing collagen molecules induced by testosterone treatment. Boucek *et al.*(8) observed that the rate of labeling of newly formed collagen after injection of radioactive lysine was greater in the male than in the female, although the net synthesis was approximately equal for both sexes.

The value recorded for the radioactivity present in the non-protein fractions seems to be increased in all the tissues which show a stimulated protein synthesis. Supporting the conclusions of Frieden *et al.*(4), studying *in vitro* uptake of glycine by mouse kidney slices, where it was postulated that testosterone owes at least part of its activity to its efficiency in facilitating intracellular accumulation of amino acids as a preliminary step to protein synthesis.

Summary. Injection of testosterone propionate to castrated male rats stimulated protein synthesis at the level of the sex linked organs, skin, and in subcutaneously implanted polyvinyl sponge. Other tissues studied showed no effect of testosterone on rate of protein labeling after receiving 1-C^{14}-glycine. The unique behavior of the perineal muscle in contrast to other skeletal muscles points to its unsuitability for myotrophic assay of anabolic hormones. The tissues where anabolic activity was stimulated also showed a concomitant increase of radioactivity in their non-protein fractions.

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