Relationship of Protein Intake to Protein Anabolic Activity of Testosterone Propionate.* (19277)

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Androgens stimulate an increase in body weight and a positive nitrogen balance in various species(1,2). Preliminary studies in the rat(1a,2) indicated that these phenomena could not be enhanced by an increase in the protein intake or by supplementation with cystine. Further data are presented.

TABLE I. Composition of Diets.*

	I	II	III
Casein	16.7	27.9	46.4
Sugar	61.2	50	31.5
Hydrogenated vegetable oil	7.4	7.4	7.4
Wesson's salts	3.7	3.7	3.7
Celluflour	1.8	1.8	1.8
Yeast	9.2	9.2	9.2
N, %	2.95	4.39	6.80
Protein (N X 6.25), %	18.4	27.5	42.5

* Each rat received 3 times per week 2 drops of cod liver oil and one drop of 34% tocopherol concentrate from wheat germ oil† diluted 10 fold with Wesson oil.

† Provided by Distillation Products Incorporated through the courtesy of Dr. Philip L. Harris.

Method. The rats were purchased from the Carworth Farms and weighed 240 to 300 g. They were castrated at 300 to 350 g body weight, brought to body weight and nitrogen equilibrium while eating the 18% protein diet and transferred to their respective diets (Table I) at 60 days prior to the first experiment. The testosterone propionate,‡ a 10 mg per ml solution in sesame oil, was injected subcutaneously at the same time each day for 21 days. The experimental and analytical procedures have been described(3).

Results. The characteristics of the changes in body weight and nitrogen balance during and subsequent to the injection of the androgen were not altered by the changes in protein intake(3).

The increases in protein intake had no

TABLE II. Protein Anabolic Activity of Testosterone Propionate in Castrated Rat at Different Levels of Protein Intake.

Protein in diet, %	Rat No.	Body wt		Urine, pre-inj., mg/day	Nitrogen	
		Pre-injection, g	Max. inc., g		Max. retained/day,* mg/day	Total retained, mg/21 days
			T. P. at .25 mg/day			
18	5	343	13 ± 1.5†	248	48 ± 6†	758 ± 121†
28	4	356	12 ± 1.8	353	50 ± 12	958 ± 160
43	5	353	11 ± 1.7	570	62 ± 8	1048 ± 108
			T. P. at .50 mg/day			
28	5	348	12 ± 1.7	352	51 ± 8	740 ± 137
43	5	321	15 ± 1.6	593	57 ± 5	820 ± 115

* Average of 2nd, 3rd, and 4th periods (7 days) when N retention was maximum.

† Standard error of the mean $= \dfrac{\sqrt{\dfrac{X^2 - \bar{X}^2}{N}}}{\sqrt{N-1}}$

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effect on the body weight stimulating effect of the androgen (Table II). The maximum daily nitrogen retention and total nitrogen retained also were not significantly changed ($P < 0.05$). Furthermore, an increase in the dose of the androgen (Table II) also did not produce any greater anabolic effects at either the 30 or 50% levels of protein intake.

Discussion. Once an adequate protein intake is provided a further increase in the protein intake of the rat by the replacement of part of the carbohydrate of the diet by an isocaloric amount of protein (casein) does not enhance the over all protein anabolic activity of testosterone propionate. Stimulation by androgen of the retention of nitrogen or growth processes apparently is regulated by inherent properties of the various cells.

Summary. The protein anabolic activity of testosterone propionate in adult male castrated rats was not altered by increasing the protein content of the diet from 18 to 28 and 43% by replacement of carbohydrate.

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New Inhibitors of Enzymatic Proteolysis.*† (19278)

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Earlier reports from this laboratory(1,2) described the inhibition of pepsin, trypsin, papain, and cathepsins by several carbonyl group reagents. Since then, additional inhibitors of proteolytic processes have been found. The new inhibitors (guanidine, arginine, ethylenediamine, lysine, and others) show certain structural similarities to the compounds tested before, but they are not carbonyl group reagents.

Most of the new inhibitors were effective only when egg-white served as substrate and would not have been discovered if the current preference for synthetic substrates had been strictly adhered to. The observation that certain inhibitors of the proteolysis of natural substrates fail to interfere with the enzymatic hydrolysis of synthetic substrates, demonstrates that results obtained with well-defined synthetic systems are not necessarily applicable to more complex and more natural systems.

A. Experiments with egg-white as substrate. The results of these experiments are listed in Table I. Tests with a few aldehyde reagents and with pyrophosphate were carried out simultaneously for purposes of comparison. The substrate was prepared as described before(1) and the progress of hydrolysis was followed photoelectrically(1).

B. Experiments with casein as substrate. (a) Substrate for tests with pepsin—same as described before(1). *(b) Substrate for tests with trypsin and chymotrypsin*—a mixture of 2 g casein and 200 ml phosphate buffer (*M*/15; pH 8.30) was heated in boiling water for 15 minutes. After cooling, 20% NaOH was added dropwise until pH 8.4 was reached. *(c) Incubation mixtures*—to 4 ml substrate was added 1 ml water or 1 ml inhibitor solution, which had been adjusted to the pH of the incubation mixture with HCl or NaOH. The mixture was kept at 37°C for 10 minutes.

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