Non-Suitability of Levator Ani Method as an Index of Anabolic Effect of Steroids. (23025)

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In continuation of our studies on the mechanism of testosterone induced nitrogen retention(1,2), we proposed to investigate further the effect of some recently introduced steroids with allegedly increased anabolic and decreased androgenic properties. For the screening of such compounds Eisenberg and Gordan(3) suggested a bioassay method in which weight changes of the m. levator ani (m.l.a.) of male rats are determined after injection of androgens. According to these authors the observed weight changes indicate "myotropic," *i.e.* anabolic effect of the compounds. From our observations on size and growth of this muscle under various conditions it appeared that weight changes of the m.l.a. might be a sex-linked function, the growth effect of the steroids representing an androgenic rather than a general myotropic effect. It was therefore questionable whether the m.l.a. method could be used as an indicator of the anabolic effect of steroid compounds. The present study deals with the validity of this method.

Methods. Male Wistar rats were kept in individual cages. The animals received Purina Chow *ad lib.* except when used in protein depletion and carbohydrate and fat repletion experiments. The composition of the *protein depletion* diet was as follows: Corn Starch 75.5%, Sucrose 10%, Butter Oil 8%, Corn Oil 3%, U.S.P. Salt Mixture #2 3%, Cod Liver Oil 0.5%. The high *carbohydrate repletion* diet contained: Casein 15%, Sucrose 8%, Corn Starch 72%, Cod Liver Oil 0.5%, Corn Oil 2%, U.S.P. Salt Mixture #2 2.5%. The *high fat repletion* diet contained: Casein 23%, Sucrose 12.5%, Butter Oil 50%, Cod Liver Oil 0.7%, Corn Oil 1%, U.S.P. Salt Mixture #2 3.9%, Celluflour 8.9%. The usual vitamin supplements were added to all 3 diets to bring them to the level indicated earlier(1). Bilateral castration was performed under light ether anesthesia. At termination of experiments the rats were killed by a blow on the head. The m.l.a. was dissected according to the technic of Hershberger, Shipley and Meyer(4), and the weight of this muscle, of the seminal vesicles, and of other organs was determined immediately with an accuracy of .1 mg. For water content determination the muscle was dried to constant weight at 80° C in an oven for 24 hours. The N-content of the dry m.l.a. was determined according to Folin-Nessler(5) after digestion in 10% H_2SO_4.

Results. In the first group of experiments we investigated the effect of a protein-free diet. The results, condensed in Table I (Group I), show that animals on protein-free diets lost body weight. The m.l.a., seminal vesicles, and kidneys participated in this general loss in body weight and were smaller than in animals on a full diet.

In further experiments (Group II), the effects of testosterone propionate (Lilly) (T.P.) on protein depleted animals were investigated. Every other day, the animals received 2.5 mg T.P. in sesame oil injected subcutaneously. Rats maintained on stock diets and protein depleted rats which received only sesame oil injections served as controls. The results indicated, in confirmation of earlier experiments(1), that the loss of body weight on protein-free diets was not affected by T.P. injections. In contrast, weight of m.l.a., seminal vesicles, and kidneys increased considerably.

The same effect could also be demonstrated in protein depleted rats which had been castrated a few days before the start of depletion. The determination of water and of nitrogen content of the m.l.a. showed further (Table I, Group II) that an increase of organ

* Supported by grant from Dairy Industry Advisory Board of State of California.

TABLE I. Effect of Testosterone Propionate on Organ and Muscle Weight of Normal and Castrated Male Rats on Protein-Free Diets.

Group	No. of rats	Condition	Depletion period (days)	Diet	Treatment	Body wt (g)		Wt (mg) of:							N. cont. of dry lev. ani in mg %
						Initial	Final	Lev. ani	Water cont. lev. ani (%)	Sem. ves.	Kidney	Liver	Testic.	Diaphragm	
I	4	Normal	12	Stock		119 (± 13)	182 (± 29)	70.4 (± 30.0)	78.5 (± .91)	143.1 (± 59)	1756 (± 202)	9505 (± 1310)	2430 (± 212)		
	4		12	Prot. free		118 (± 5)	83 (± 2.7)	13.2 (± 2.9)	74.5 (± 1.4)	28.7 (± 6.5)	833 (± 53)	4050 (± 448)	1685 (± 272)		
II	5	"	15	Prot. free	15 mg T.P.*	170 (± 6)	137 (± 11)	94.5 (± 22.9)	78.5 (± .7)	1103.0 (± 356)	1307 (± 94)	5340 (± 605)	2590 (± 129)	354.2 (± 46.2)	15.5 (± 1.2)
	5		15	"		170 (± 6)	131 (± 6)	33.1 (± 10.9)	76.5 (± 1.2)	102.2 (± 22.8)	1137.5 (± 65.3)	5590 (± 515)	2355 (± 179)	387.0 (± 52.3)	14.8 (± .9)
III	5	Castrated	12	Prot. free	15 mg T.P.*	99 (± 7)	71 (± 6.1)	51.9 (± 6.7)	78.6 (± .42)	501.8 (± 43.5)	948.5 (± 76.5)	2781 (± 420)			
	5		12	"		98 (± 9)	72 (± 5.8)	7.6 (± 2.5)	77.8 (± 2.1)	17.8 (± 5.2)	783.7 (± 44.5)	2891 (± 274)			

* 2.5 mg every second day. Values in parentheses are stand. dev. from the mean.

weight after testosterone treatment was not due to water accumulation but represented a real increase of organ protein.

To ascertain how T.P. affects the growth of another muscle, we also determined (Group II) the weight of diaphragm-muscle. This muscle was selected because it could be easily dissected. The results indicated that, in contrast to the increase in m.l.a. weight, the weight of the diaphragm muscle was not affected by T.P. injections.

We then sought to determine why the weight loss of the m.l.a. on protein-free diets was relatively much larger than the loss of total body weight (Table II). One possible explanation of this phenomenon is that in rats on protein deficient diets the secretion of gonadotropic hormones decreases(6), and as a consequence the stimulus for androgen production is diminished. To investigate this possibility, normal and castrated rats were protein-depleted and during 11 days on a protein-free diet they were injected subcutaneously with 2 to 4 rat units daily of pituitary gonadotropin (Squibb). It was found (see Groups II and III, Table II) that the weight of m.l.a., of the penis, and of seminal vesicles of normal gonadotropin-treated increased considerably over weights of these tissues in control animals (Group IIIA) despite the protein depletion. In protein depleted castrated rats (Group IV), injection of gonadotropic hormone proved to be ineffective, thus suggesting that this hormone elicited its effect through the testes. To eliminate the possibility that traces of growth hormone present in gonadotropin might be responsible for the described effect, another group of animals (Group V) was treated with 10 rat units daily of Antuitrin Growth (Parke Davis). Neither total body weight nor the organ weights investigated were influenced by injection of somatotropic hormone on a protein-free diet. It should be mentioned here that an increase of the m.l.a. by injection of growth hormone had been found earlier in animals on *normal diets*(3).

Table III contains the results of experiments in which the effect of Norethandrolone on protein-depleted castrated rats was inves-

TABLE II. Effect of Growth Hormone and Pituitary Gonadotropin on Normal and Castrated Male Rats on a Protein-Free Diet for Eleven Days.

Group	No. of rats	Condition	Treatment	Body wt (g) Initial	Body wt (g) Final	Wt (mg) of: Lev. ani	Sem. ves.	Penis	Kidney	Liver	Diaphragm
I	5	Castrated ♂ controls		137 ± 13	107 ± 13	16.5 ± 5.7	37 ± 12	94 ± 9	1002 ± 193	4580 ± 1130	350
II	4	Normal	20 rat units gonad.*	133 ± 6	112 ± 1	52.5 ± 8.1	680 ± 88	166 ± 14	1168 ± 106	4195 ± 173	
III	4	"	40 " "	134 ± 3	101 ± 4	57.5 ± 8.4	750 ± 229	179 ± 10	1079 ± 59	4006 ± 630	322 ± 17
III-a	5	"		140 ± 2	115 ± 4	21.6 ± 1.5	65 ± 15	110 ± 8	995 ± 65	5145 ± 450	348 ± 25
IV	4	Castrated ♂	20 " "	131 ± 3	107 ± 2	15.7 ± 1.6	30 ± 5	95 ± 5	1030 ± 220	4000 ± 550	
V	4	*Idem*	100 rat units growth h.	140 ± 4	108 ± 5	14.1 ± 1.5	54 ± 12.7	99 ± 12	1168 ± 83	5505 ± 387	372 ± 15

* Gonadotropin.

tigated in comparison with T.P. Norethandrolone has been recently introduced under the trade name of Nilevar (Searle) and, according to the manufacturer, has a high anabolic, but low androgenic, activity. For these experiments the rats were castrated at the age of 31 days and kept on stock diet for an additional 25 days. They were then protein depleted for 24 days, at the end of which time they were sacrificed. Beginning on the eleventh day of protein depletion diet, the hormones were injected daily at 2 different dose levels. The results show that Norethandrolone increased the organ weight of protein depleted castrated rats in the same way as did T.P. Body weight was not affected by the injection of these hormones.

The concluding experiments were designed to determine whether the weight loss of the m.l.a. and the seminal vesicles, which was observed during protein depletion, was reversible, *i.e.* whether these tissues could be restored to normal size after feeding the animals protein repletion diets. Rats on a depletion diet for 15 days lost on the average 22% of their weight. During the repletion period one group of rats received the high fat and the other the high carbohydrate diet in isocaloric quantities containing equal amounts of protein. The animals on high fat repletion diet were restricted to a daily intake of 7 g and the carbohydrate animals to a daily intake of 11 g, both quantities containing 44 calories and 1.6 g protein. All the offered food was consumed by the animals. Suboptimal levels of protein were fed to make more apparent any difference in the sparing effect of the other components such as carbohydrate or fat.

Table IV shows that during feeding of the protein repletion diets for 13 days the body weight increased rapidly and the weight of the investigated organs such as the m.l.a., seminal vesicles, kidney, and liver was restored to normal values. The high fat and the high carbohydrate animals responded very similarly, only the weight of the m.l.a. was significantly higher in fat-fed animals. The reasons for this difference are under further investigation.

TABLE III. Effect of Testosterone Propionate and Norethandrolone* on Organ and Muscle Weights of Castrated Protein Depleted Male Rats.

Group†	Treatment	Weight in mg				
		Lev. ani	Sem. ves.	Kidney	Liver	Penis
I	T. P., 12 mg	46.2	488	1100	4435	173
II	Nilevar "	37.1	408	1045	5365	150
III	T. P., 2.4 mg	44.3	270	1083	3873	153
IV	Nilevar "	33.1	126	1023	4313	138
V	Controls	4.2	18	988	4820	50

* Nilevar (Searle) supplied by courtesy of Searle & Co.
† Avg body wt of rats after depletion—111 (± 9) g.

Discussion. The preceding results show that T.P. and Norethandrolone induced growth of the m.l.a. and of some other accessory sex organs in normal and in castrated male rats, even on protein-free diets. Such deficient diets cannot support somatic growth; therefore, the animals lost weight. The fact that the growth of certain target organs can be stimulated by hormones even on such an unfavorable dietary regime must be attributed to the androgenic effect. These results also suggest that on depletion diet the proteins necessary for the hormone stimulated growth of the m.l.a. and other sex organs must be obtained from other tissues, possibly from the liver or the skeletal muscle. These findings indicate that the hormone-induced anabolism in the m.l.a. is associated with protein catabolism in other organs. Further work involving a greater number of animals would be required to identify the tissues from which the protein was shifted to the sex organs.

The fact that gonadotropic hormone and growth hormone did not affect weight of the accessory sex organs or of the m.l.a. of castrated male rats during protein depletion further indicates the specificity of the hormone effect on growth of the m.l.a.

Our conclusions that size and weight of the m.l.a. are a sex-linked function are supported by earlier observations. For example, the weight of this muscle in females is only a small fraction of that observed in adult males. After castration in males the m.l.a. loses about 60 to 70% of its original weight within 10 to 14 days. Loss of the skeletal muscle during this time is, however, usually insignificant.

Our experiments do not explain why the relative activity of the various androgenic steroids differs according to the organ selected for comparison, *i.e.* why some of them have a greater effect on the weight of the seminal vesicles, while others have a greater effect on the m.l.a. Such differences have been observed in the past, *e.g.* testosterone is ten times more active than androsterone when tested on the seminal vesicles but only 2 to 5 times as active when the comparison is based on the increase in prostate weight. On the basis of such observations and of our results, we must assume that the threshold levels, the speed of absorption and destruction and similar factors, rather than differences in

TABLE IV. Effect of a High Fat and High CHO Repletion Diets on Muscle and Organ Weight of Rats Fed at an Isocaloric Level.

Diet	Body wt (mg)			No. of rats	Lev. ani	% water in lev. ani	Sem. ves.	Liver	Kidney
	Initial	Depleted	Repleted						
High CHO	182 (± 11)	140 (± 12)	177 (± 7)	6	45.6 (± 8.6)	75.3 (± 0.8)	281 (± 51)	6003 (± 401)	1348 (± 96)
High fat	180 (± 13)	144 (± 14)	183 (± 10)	6	67.7 (± 13.9) (p = .01)	76.5 (± 1.3)	219 (± 82)	6468 (± 480)	1373 (± 90)
Controls (at end of depletion period)				5	33.1 (± 10.9)	76.5 (± 1.2)	102.2 (± 22.8)	5590 (± 515)	1137 (± 65.3)

androgenic and anabolic activities of the compounds, must be responsible for the differences in the effect on various organs.

Summary. Injection of androgens such as testosterone propionate or Norethandrolone in normal and castrated male rats promotes growth of the m. levator ani even on protein-free diets. Based on these and other experiments, it is assumed that the growth of this muscle is a sex-linked function. It is, therefore, concluded that the hormone-induced growth of this muscle is not an appropriate index for the general "myotropic," *i.e.* anabolic effects of steroid compounds.

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Received December 18, 1956. P.S.E.B.M., 1957, v94.

Early Development of Glycogen Infiltration in Duct Epithelium of Dog Pancreas after Growth Hormone Administration. (23026)

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The original hypothesis concerning hydropic change in the pancreas advanced by Allen(1) attempted to achieve a common pathogenetic denominator for vacuolization of B cells and duct cells. B cell hydropic degeneration was considered to be due to "exhaustion of an internal secretory function" while that occurring in duct epithelium was thought to be due to "exhaustion of a proliferative function." Most subsequent workers(2,3,4) emphasized B cell hydropic degeneration as a characteristic lesion of the pancreas in experimental diabetes, resulting from "exhaustion" with the duct epithelial vacuolization as a more or less unexplained concomitant. The pathogenetic implication of the duct epithelial lesion is usually not mentioned and the exhaustion theory accepted uncritically. Toreson(5) demonstrated that hydropic degeneration in the pancreas in diabetes consists actually of the deposition of glycogen. It was suggested that the glycogen accumulation might represent merely one component of the widespread pathologic glycogen deposits found in diabetes or that it might be indicative of proliferative activity of duct epithelium with deranged islet regeneration. More recently it was shown that in cortisone treated rabbits the glycogen infiltration of duct epithelium occurs prior to and even in the absence of that in B cells(6). This observation is at variance with the exhaustion hypothesis of hydropic change unless the B cell and duct epithelium lesions are thought of as being distinctly separate and of different etiologies. However, the known facts concerning their development would negate this latter idea. It was therefore theorized that the pancreatic glycogen deposition was similar to that observed in other organs in diabetes. It was considered of importance to determine whether the time sequence observed in the cortisone-treated rabbit also occurs in other forms of experimental diabetes. The present study was designed therefore to investigate whether ductular glycogen infiltration occurs prior to or in the absence of that in B cells in the growth hormone treated dog.

Material and methods. The study was carried out on 27 mongrel dogs of either sex weighing between 12-15 kg. Nineteen of