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Effect of Dosage on the Morphogenetic Actions of Testosterone.

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The literature concerning many of the morphogenetic actions of steroid hormones in general and testosterone in particular is rich in contradictions. In the course of recent investigations performed in this laboratory it became increasingly more evident that much of the confusion concerning these actions is due to differences in the dosage in which the steroids were administered. While some morphogenetic actions are proportional to the dose, others are not; in fact the same compound may either stimulate or inhibit the development of an organ depending on the dose used. Space does not permit a complete discussion of the voluminous relevant literature here, so that the reader is referred for reviews to more detailed papers on cognate subjects.[1, 2, 3]

In an effort to obtain a clearer picture of the effect of dosage on the morphogenetic action of testosterone we performed the following experiment on 36 five-week-old male albino rats which were divided into 6 groups of 6. The rats in 5 of these groups were injected once daily with a very fine suspension of testosterone in 1 ml of physiological NaCl solution during a period of 20 days. In order to obtain good absorption the material was injected subcutaneously and intraperitoneally, the site of administration being varied on alternate days. An aqueous suspension was given, instead of the usual solution in oil, in order to avoid the administration of the enormous quantities of oil which would have been necessary to dissolve the amount of testosterone given in some of the groups. Biopsy at the end of the experiment indicated that, except for traces in the groups receiving the highest doses, the hormone was well absorbed from the site of injection. On the 21st day all animals were killed, their organs dissected and weighed after complete fixation in Heidenhein's "Susa" solution. Table I summarizes our results. Each figure represents the average of 6 animals. The significance of the apparent differences between the treated and control groups was evaluated by "Student's method for small samples"[4] and is expressed

[1] Selye, H., and Friedman, S., *Endocrinol.*, 1941, **28**, 129.

[2] Selye, H., and Friedman, S., *Endocrinol.*, 1940, **27**, 857.

[3] Selye, H., *J. Am. Med. Assn.*, 1940, **115**, 2246.

[4] Fisher, R. A., *Statistical Methods for Research Workers*, 6th ed., Edinburgh, 1936, p. 128.

TABLE I.
Effect of Dosage on Morphogenetic Actions of Testosterone.

Organ	Saline	Testosterone dose 0.1 mg	0.5 mg	1.0 mg	10 mg	25 mg
Initial body wt (g)	80	70	81	75	85	73
Weight gain (g)	+69	+75	+64	+61	*+42*	*+30*
Pituitary wt (mg)	5.5	5.3	5.1	*4.4*	*4.2*	*4.0*
Thyroid wt (mg)	14.0	12.0	*12.0*	*12.0*	*11.0*	*11.0*
Adrenal wt (mg)	31.0	31.0	*26.0*	*25.0*	*37.0*	*43.0*
Thymus wt (mg)	280	353	288	226	*49.0*	*15.0*
Testis wt (g)	2.13	*1.46*	*1.29*	*1.22*	1.74	1.92
Seminal vesicle wt (mg)	125	86	*231*	*467*	*1116*	*1004*
Prostate wt (mg)	301	247	357	*464*	*837*	*655*
Epididymis wt (mg)	279	205	289	292	*392*	*462*
Kidney wt (g)	1.45	1.48	1.44	1.37	1.56	1.40

in terms of probability estimated by graphic interpolation in Fisher's table of t. In accordance with the usual convention, differences between series cannot be accepted as significant when P is greater than 0.05. All values in which the apparent deviation from the normal control level proved to be statistically significant in this sense, are indicated in italics in the table.

As seen from the table testosterone caused a decrease in the size of the *pituitary*. This became increasingly more pronounced as the dose of the hormone was raised. The hypophyseal atrophy was statistically significant at the 1 mg per day dose level, although this amount of testosterone did not cause any significant inhibition of body growth. We may conclude from this that the pituitary-depressing action of the hormone[5, 6] is specific and not merely a repercussion of the general inhibition of body growth. Histologically the most prominent feature of the atrophic glands is their poverty in eosinophil cells, combined with a relative increase in the number of small chromophobes. The *thyroid* showed a significant decrease in weight with as little as 0.5 mg of testosterone daily, that is long before a general body growth depressing dose had been reached, so that here again the decrease in thyroid size cannot be regarded merely as the result of general growth inhibition. Histological examination shows that this decrease in size is accompanied by definite atrophy of the follicular epithelium, which, like the decrease in the weight of the gland, is roughly proportional to the dose in which testosterone was given. The fact that suitable doses of testosterone cause thyroid atrophy in the rat has already been emphasized in a previous publication.[5] The hormone has also been

[5] Selye, H., *Canad. Med. Assn. J.*, 1940, **42**, 113.
[6] Selye, H., *J. Endocrinol.*, 1939, **1**, 208.

shown to cause thyroid atrophy in the mouse.[6]* The *adrenals* show a statistically significant decrease in weight at the 0.5 and 1.0 mg levels, while larger doses result in an equally significant increase in weight. It is obvious that here the result is not simply proportional to the dose in which the hormone is given but that there is a definite optimum range for the production of atrophy. This may explain the contradictory findings published on this subject by other investigators and recently reviewed by us.[3] It also confirms our earlier reports on the production of adrenal atrophy with androgens in the rat[7, 5] and mouse.[6] Histological examination showed the atrophy to be due to a more or less uniform decrease in the size of the cells in the various layers of the adrenal cortex. In this respect the response differed from that obtained in extremely young rats in which testosterone causes predominantly glomerulosa atrophy.[8] It also differs from the involution caused by hypophysectomy which affects the reticularis more markedly than the other zones. In almost all the animals in which a decrease in adrenal weight was obtained the chromaffine cells of the medulla did not participate to any extent in the involution, but the ganglion cells in the medulla often show a peculiar transformation characterized by the appearance of large vacuoles inside the cytoplasm. The nerve cells affected in this manner were strikingly similar to the "signet ring" or "castration cells" which appear in the anterior lobe of the rat after spaying. The increase in adrenal weight caused by high doses of testosterone is mainly due to cortical enlargement. It should be emphasized, however, that this adrenal hypertrophy occurs only with doses which are definitely damaging as shown by the decrease in body weight. It is quite possible, therefore, that the hypertrophy of the cortical cells should merely be regarded as part of the reaction to non-specific damage, a reaction which is always accompanied by adrenal cortical enlargement.[9] The *thymus* shows progressively increasing atrophy which runs closely parallel with the dose of testosterone given. Histologically this decrease in weight proves to be due mainly to disappearance of thymocytes. The *testis* shows the most pronounced atrophy with a dose of 1 mg per day, but

* Attention is called to an error in the printing of the latter communication in which the explanatory remarks illustrating the microphotographs of the testosterone-treated and control thyroids were exchanged. This gives the impression that testosterone stimulates thyroid growth although the tables in that paper, giving each individual thyroid weight, definitely proved the contrary to be true.

7 McEuen, C. S., Selye, H., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 390.

8 Selye, H., *Anat. Rec.*, 1940, **76**, 145.

9 Selye, H., *Endocrinol.*, 1937, **21**, 169.

as little as 0.1 mg suffices to cause a statistically significant testis involution. However, doses of 10 mg a day no longer cause a statistically significant decrease in testis weight while the animals treated with 25 mg per day had testes which were almost back to normal, a fact which is all the more remarkable since the body weight in the 25 mg group was only 103 g on the average, as compared with 149 g in the controls. This observation gives strong support to the view[1] that large doses of androgens may exert a direct stimulating action on the testis. Histologically the interstitial cells showed progressive atrophy with increasing dosage irrespective of testis weight, but the germinal epithelium revealed marked atrophy only in the 0.1-1.0 mg range. The apparently slightly subnormal size of the *accessory sex organs* (seminal vesicles, prostate and epididymis) in the 0.1 mg group did not prove to be statistically significant and may perhaps be due to the somewhat smaller average size of the animals in this group. Otherwise the increase in the size of these organs became more and more pronounced as the dose was increased. Contrary to expectations there was no statistically significant increase in *kidney* weight in any of the groups of this series, although our previous observations[10, 6, 5, 11] which have amply been confirmed in other laboratories[12-15] definitely indicate that androgens have a renotropic action. The reason for this failure to obtain a more pronounced enlargement in kidney size is probably that, as we have repeatedly emphasized, the rat is not very sensitive to the renotropic action of steroids. Furthermore, we used intact males in this experiment and in these the kidney size is relatively large as compared with that of females or spayed animals of either sex, so that a further increase in size is difficult to obtain. It will be noticed, however, that in the 10 mg group the average kidney size was above normal although the average body weight was below that of the controls and histological study of this material confirmed the existence of a definite, though mild degree, of tubular hypertrophy.

Summary. Young male albino rats were divided into groups treated with daily doses of testosterone ranging from 0.1 to 25 mg per day. These experiments showed that the body weight as well

10 Selye, H., *J. Urol.*, 1939, **42**, 637.

11 Selye, H., *Anat. Rec.*, 1941, in press.

12 Korenchevsky, V., and Ross, M. A., *Brit. Med. J.*, 1940, **1**, 645.

13 Ludden, J. B., and Krueger, E., *J. Clin. Invest.*, 1940, **19**, 780 P.

14 Paschkis, K. E., Shay, H., Gershon-Cohen, J., and Fels, S. S., *Am. J. Physiol.*, 1940, **129**, 191.

15 Pfeiffer, C. A., Emmel, V. M., and Gardner, W. U., *Yale J. Biol. and Med.*, 1940, **12**, 493.

as the weight of the pituitary, thyroid and thymus undergo a gradual decrease as the hormone dosage is increased. The seminal vesicle, prostate and epididymis increase in size with increasing dosage. On the other hand, the inhibitory action of testosterone on the adrenal and testis has a definite optimum at the 0.5 to 1.0 mg daily dose level, inasmuch as smaller doses cause less pronounced atrophy and larger amounts may actually stimulate the growth of these glands. The histological changes which accompany these variations in gross weight have been described. We conclude that many of the apparent contradictions concerning the morphogenetic actions of androgens—and perhaps also of other steroid hormones—are due to the fact that depending on the dose given, these compounds may exert diametrically opposite actions on certain organs.

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Riboflavin Content of Blood and Muscle in Normal and in Malnourished Humans.*

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A number of workers have reported significant decreases in the riboflavin content of tissues from experimental animals maintained on a riboflavin deficient diet.[1-6] The resultant conclusions, however, cannot safely be applied to man.

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1 Axelrod, A. E., Sober, H. A., and Elvehjem, C. A., *J. Biol. Chem.*, 1940, **134**, 749.

2 Axelrod, A. E., Lipton, M. A., and Elvehjem, C. A., *Am. J. Physiol.*, in press.

3 Kuhn, R., Kaltschmitt, H., and Wagner-Jauregg, T., *Z. physiol. Chem.*, 1935, **232**, 36.

4 Groen, J., and Schuyl, J. W., *Arch. néerl. physiol.*, 1938, **23**, 271.

5 Carlsson, E. V., and Sherman, H. C., *J. Nutrition*, 1938, **15**, 57.

6 Fraser, H. F., Topping, N. H., and Isbell, H., *Pub. Health Rep., U. S. P. H. S.*, 1940, **55**, 280.