

Androgen-Induced Growth of the Os Penis of Hypophysectomized-Gonadectomized Rats.*

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It was concluded from earlier experiments carried out by other investigators in this laboratory¹ that under certain circumstances testosterone propionate promoted growth of cartilage and bone in the proximal tibial epiphysis of hypophysectomized rats. In the report of that work it was suggested that explanations for apparent discrepancies noted in the literature dealing with the effects of androgens on bone growth may be found in such variables as age, sex, dosage, length of treatment, etc. In the experiment outlined below a new variable has been introduced in that the bone chosen for study, the os penis, is peculiarly situated in a region that might be expected to be affected profoundly by the deprivation or varying supply of androgenic substances. Using normal and castrated rats with intact pituitaries Turner *et al.*² showed that testosterone propionate accelerated the development of this bone as judged by skiagrams.

Experimental. It was previously determined³ that rats of the Long-Evans strain gonadectomized on day 26 and injected daily thereafter for approximately 3 weeks with 0.01 mg of testosterone propionate would show growth of the penis and splitting of the balano-preputial epithelium comparable to that of the normal animal. This dose was therefore assumed to approximate the daily average equivalent of androgen to which the normal animal was exposed during that period of life. As may be seen, then, from Table I some rats hypophysectomized and gonad-

ectomized on day 26 were immediately started on this daily dose of testosterone propionate[†] while others received 0.1 mg. Control animals also doubly-operated on day 26 received 0.1 cc of sesame oil, the volume of diluent used for the hormone.

Terminal normal controls were obtained from the same litters as the experimental rats and kept on the same regime of diet and care. Control tissues from 21- and 26-day-old normal rats as well as from full grown animals were also studied. In this preliminary experiment the rats were allowed to eat *ad lib.*, but it is planned to control the important variable of food-intake in a future experiment in this series. Animals were weighed on days 5, 10, 15, and 21 of the experiment and it was obvious from the appearance of the food cups and the weight gains that the operated animals ate well, although never as much as the normal controls. On the other hand, the normal rats were more active and possessed better body tone.

At necropsy the penis trimmed of skin, and a patella from each animal were fixed in Bouin's fluid, and the operative sites were checked for completeness. After 2 days in Bouin's fluid the os penis becomes decalcified as judged by skiagram, but the tissues were also exposed to 5 parts of concentrated HCl (by volume) to 95 parts of the graded alcohols (50-100%) over another 2-day period. At the 80% alcohol stage the penis was uniformly cut in such a way that the final block of tissue consisted of the distal segment and only the bend of the proximal segment. After the excess fluid was removed from these blocks of tissues they were weighed (see Table I). The penis was cut in dorso-ventral

* Aided by grants from the Research Board of the University of California.

¹ Simpson, M. E., Marx, W., Becks, H., and Evans, H. M., *Endocrinol.*, 1944, **35**, 309.

² Turner, H. H., Lachmann, E., and Hellbaum, A. A., *Endocrinol.*, 1941, **29**, 425.

³ Lyons, W. R., Berlin, I., and Friedlander, S., *Endocrinol.*, 1942, **31**, 659.

[†] Generously supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J.

TABLE I.
Effect of Testosterone Propionate on the Body Weight, Penis Weight and Length of Os Penis in Rats Castrated and Hypophysectomized on Day 26 and Injected for 21 Days.

No. of rats	Daily dose test prop. mg	Age in days	Avg. wt gain g	Avg. wt distal penis, mg	Avg. length os penis, mm
3†		21			2.73 ± 0.11
3†		26			3.33 ± 0.15
7		47	9	23.5 ± 1.8 (5)*	3.20 ± 0.18 (6)
5	.01	47	5.6	57.3 ± 4.5	3.52 ± 0.14 (4)
8	.1	47	21.2	102.3 ± 4.3 (5)	3.80 ± 0.12
7†		47	103.6	81.5 ± 19.3 (5)	4.1 ± 0.2 (5)

± = Standard deviation.

* Figures in brackets indicate the number of rats (when less than the total) used in a calculation, the penes of those excluded being used for different purposes.

† Normal unoperated controls.

planes in a way that permitted one to obtain representative sections through the greatest length and the central part of the os. Measurements of the bone were made with a calibrated oculo-micrometer. Enough of the patellae were sectioned and stained to provide the information that this cartilage, showing only the earliest signs of ossification on day 26, changes in the 3 weeks after hypophysectomy and gonadectomy even in the untreated rats to a well ossified structure somewhat smaller than in the 47-day-old normal animals.

Results. Table I summarizes the results. It was known from previous experiments carried out in this laboratory that hypophysectomized rats of this age might be expected to gain slightly in weight. The average weight increments of 9 g in 21 days for the operated controls and 5.6 g for the rats on the 0.01 mg level of testosterone propionate are not considered unusual, but the 21.2 g gain with a range from 15-32 g in the 8 rats receiving the higher dose of androgen must be viewed as an effect of the hormone not mediated through the pituitary. Although this gain in weight (1 g per day) parallels that observed in hypophysectomized rats given low dosage of pituitary somatotrophin, it must be considered small (20%) when contrasted with the weight increment in normal control rats of this age. However the penes of the operated rats injected with the 0.1 mg level of testosterone propionate had developed at a slightly faster rate than those of the normal animals; and the average weight of their distal segments exceeded that of the normal rats by 25%. The os penis of the

androgen-treated rats, then, may be considered a bone within an organ growing even more rapidly than in the normal animals. The higher dose of androgen permitted the os penis to attain a length approximately 19% greater than the uninjected, operated controls and 92% as great as normal rats of a similar age. The lower dose was less effective as may be seen in Table I. The main histologic findings are represented in the photomicrographs and briefly summarized in the legends.

Discussion. Adequate discussion of these findings must be postponed until further experiments are carried out to determine the possible intermediary role played by organs other than the pituitary (*e.g.*, the thyroid). The growth autonomy of certain parts of the skeletal system as shown by tissue culture experiments⁴ and as re-emphasized by the Dutch investigators who witnessed bone growth in rats hypophysectomized on day 5⁵ has a place in final interpretations.

The present findings support the earlier evidence that testosterone propionate may cause skeletal growth changes¹ and body weight increase^{6,7} in rats in the absence of the pituitary. This in no way detracts from the importance of the pituitary's role in promoting growth. Rather, it suggests that along with its so-called direct growth hormone

⁴ Fell, H. B., and Robinson, R., *Biochem. J.*, 1929, **23**, 767.

⁵ Van Eek, W. F., and Freud, J., *Acta brev. Neerl.*, 1941, **11**, 43.

⁶ Leonard, S. L., *Endocrinol.*, 1943, **32**, 229.

⁷ Gordon, G. S., Evans, H. M., and Simpson, M. E., *Endocrinol.*, 1947, **40**, 375.

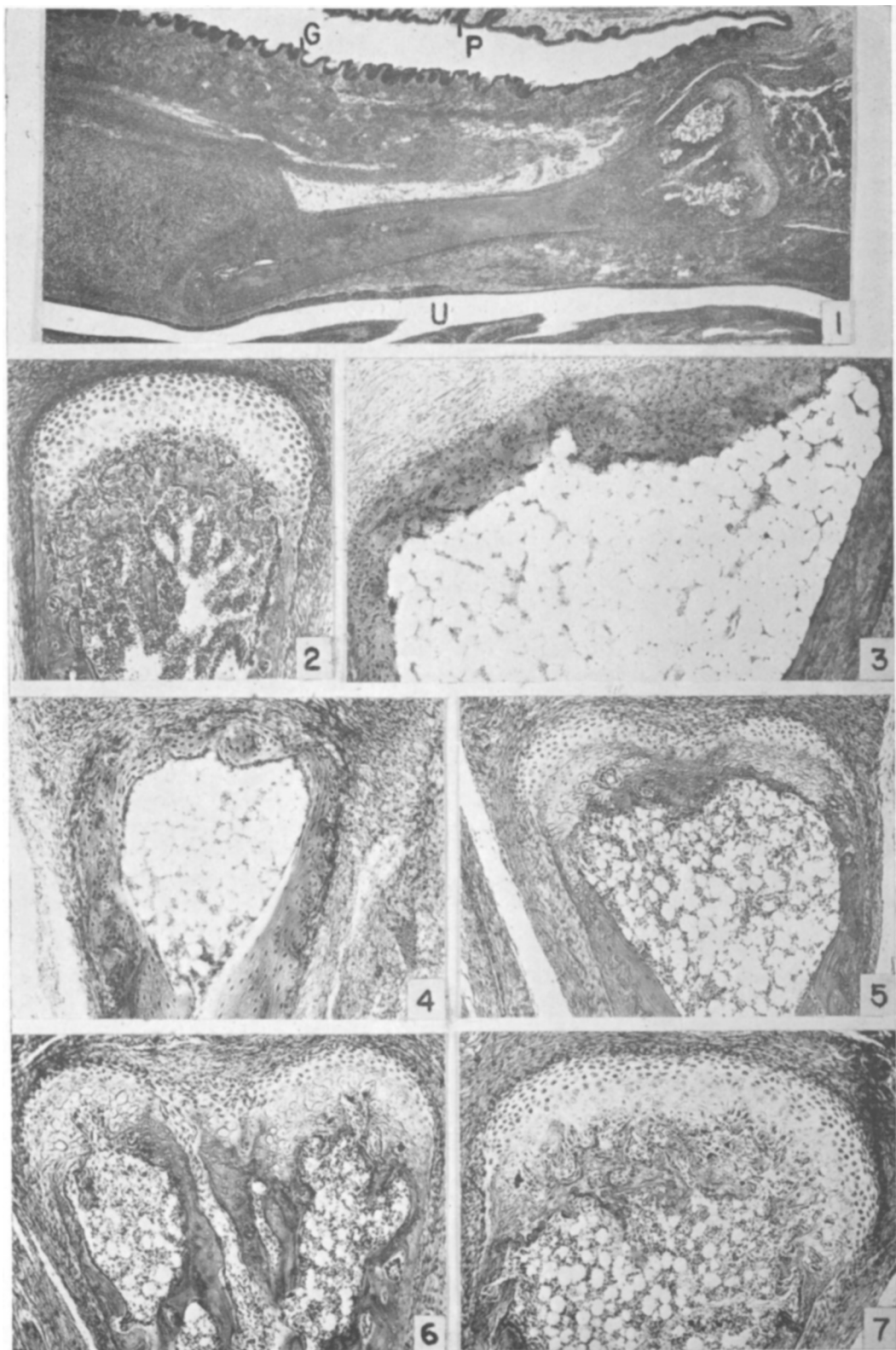


FIG. 1. The os penis of a rat castrated and hypophysectomized on day 26 and injected, subcutaneously, daily, thereafter for 21 days with 0.1 mg testosterone propionate in sesame oil. Necropsy one day after the last injection. Condyles have developed proximally. The completely ossified distal tip is in close contact with the urethra of the glans and is inserted against a cushion of dense connective tissue and fibro-cartilage which later forms a secondary ossicle. The marrow cavity is inconspicuous except in the condylar region. The balano-preputial epithelial cord has split under the influence of the androgen to form a horny, papillated covering for the glans (G) and the smooth lining of the prepuce (P). See Fig. 6 for higher magnification of the proximal end of the os. H. & E. $\times 25$.

FIGS. 2-7. All H. & E. $\times 90$. Proximal end os penis.

FIG. 2. From an onset control rat 26 days old. Chondrocytes are being formed from the chondrogenic tissue (above) and are being destroyed and replaced by trabeculae of endochondral bone. Periosteal bone is also being formed here and was observed throughout the remaining length of the os. The marrow contained but few fat cells.

FIG. 3. From a 9-month-old, unoperated, untreated, normal rat. Apparently the definitive pattern of this bone has been attained. Although no cartilage cells were recognized the possibility that some of the mesenchyme covering the bone might become chondrogenic remains. The marrow is almost entirely fatty.

FIG. 4. From a 47-day-old rat, hypophysectomized and castrated on day 26 and injected only with sesame oil as a control. Cartilage has disappeared and osteoblasts line communications between the marrow cavity and what was formerly perichondrium (above). Most of the marrow is composed of fat cells. Some of the rats in this group showed a few remaining cartilage cells in this region of the os.

FIG. 5. From a 47-day-old rat, hypophysectomized and castrated on day 26 and injected daily immediately thereafter with 0.01 mg of testosterone propionate. Cartilage has been maintained and has continued to proliferate and to be replaced by bone.

FIG. 6. Same, except for hormone dosage which was 0.1 mg. The marrow in Figs. 5 and 6 has been restored to a condition similar to that of a normal rat of this age (see Fig. 7).

FIG. 7. From a normal, untreated, 47-day-old rat.

(somatotrophin) effect and its indirect action upon skeletal tissues through thyrotrophin, one must also consider the indirect stimulus of its gonadotrophic substances responsible for the elaboration of androgen. Both male hormone and somatotrophin have been shown to perform nitrogen sparing (protein-anabolic) functions^{7,8} and Rupp and Paschkis have recently reminded us that thyroid hormone at optimal levels is also a protein anabolizer.⁹

Summary. Rats hypophysectomized and castrated on day 26 and injected immediately thereafter with 0.1 mg testosterone propionate

daily for 21 days showed an average daily weight gain of 1 g. During the same period unoperated, uninjected littermate controls gained approximately 5 g daily. The distal segment of the penis of these experimental animals weighed on an average 25% more than the normal 47-day-old controls. The ossicle in this portion of the penis of the experimental group measured 3.80 ± 0.12 mm as contrasted with the normal length of 4.1 ± 0.2 mm and the original length of 3.33 ± 0.15 mm for the 26-day-old normal and the length of 3.20 ± 0.18 mm for the doubly-operated 47-day-old controls uninjected with hormone.

⁸ Kochakian, C. D., Conference on Metabolic Aspects of Convalescence, N. Y., 1946, p. 169.

⁹ Rupp, J., and Paschkis, K. E., Abstract 58, 1948 Meeting of the Assoc. for the Study of Internal Secretions.

Addendum. Our attention has been called to similar findings reported by Sindram, I. S., *et al.* in *Acta Need. Morph.*, 1939, **2**, 236.