Cultures for bacterial contaminants were done, aerobically only, by plating allantoic fluid on blood agar and incubating at 37°C.

Experimental. Although no attempt has been made, as yet, to identify types of tissue or to visualize and reconstruct pictorially single cells as such, a great variety of cellular detail has been observed and studied. Selected preparations illustrating, in part, the variety of minute detail which has been observed in cytoplasm, are depicted in the photographs reproduced in Fig. 1-6. Much variation has been encountered in different specimens, even in individual discs from the same egg. A number of structures previously described, such as mitochondria and Golgi bodies(1), have been recognized. In addition, many other less clearly defined structures have been seen.

Discussion. Experience derived from examination of tissues from about 50 embryos suggests that avian tissues suitable for electron microscopy may be obtained by the method described. Although most of the discs prepared in this way contain a considerable amount of tissue in the peripheral areas, many contain little or no tissue in the central areas. The fact that only the small central zone of a disc may be visualized under the electron microscope thus renders many preparations unsuitable for study. Furthermore, even when sufficient tissue is present in the central area, in some instances, it is too thick for visualization. However, when several discs are inserted into each egg usually at least one contains some tissue which may be visualized. Since the tissue supports itself, apparently by attachment to the wire strands of the discs, a supporting membrane of collodion or plastic is not required. This fact allows great contrast in portions of very thin tissue and as well perhaps allows reasonably good visualization of tissues thicker than those which could be visualized satisfactorily if a supporting membrane were present. Satisfactory specimens have been obtained in various circumstances but optimal conditions as regards age of embryo and time of removal of discs have not been established.

Mortality following insertion of the discs has varied considerably but appears to average about 34%. Bacterial contamination of the allantoic cavity has occurred in only 8% of the embryos examined. Application of the method described to the study of embryonic avian tissues infected with various agents and to the study of both normal and abnormal tissues of other species is planned.

Summary. An easy and satisfactory method of obtaining embryonic avian tissues for electron microscopy has been described. A variety of detailed cellular structure has been observed and illustrated.

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Effects of Androgen and Somatotrophin on the Os Penis of the Rat.* (17623)

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In a previous paper(1) it was reported that testosterone propionate acted as a growth stimulant to the rat's os penis in the absence of the pituitary gland. There remained the question of what influence somatotrophin (growth hormone) might have upon the penile ossicles. Would it be capable of stimulating bone growth in a penis that remains atrophic and poorly vascularized in the absence of androgens? Or would it act with an androgen to bring about a better growth than the androgen alone could accomplish? The following

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1. Thyberg, W. G., and Lyons, W. R., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 158.

TABLE I.
Showing the Effect of Testosterone Propionate and Somatotrophin on Body Weight and Os Penis of Castrated, Hypophysectomized Rats.

No. of rats	Operations*	Testosterone propionate, mg	Somatotrophin, mg	Avg wt Day 26	Avg wt Day 47	Avg wt gain, g	Avg area of lateral radiophic shadow of os penis, mm^2
5	C & H	0	0	74.8	83.4	8.6	1.28 ± .08
5	C & H	0.1	0	72.2	86.6	14.4	2.20 ± .10
5	C & H	0	1.0	75.8	145.8	70.0	1.50 ± .02
5	C & H	0.1	1.0	79.0	165.2	86.2	2.78 ± .05
4	C	0	0	68.5	156.5	88.0	1.38 ± .14
5	26 day normal	0	0	70.4	—	—	1.35 ± .07
5	47 " "	0	0	—	168.8	—	2.29 ± .19

* C—castration; H—hypophysectomy.

experiments have contributed answers to these questions.

Experimental. In this investigation the os penis was studied histologically and planimetrically in the following groups of Long-Evans rats: (1) castrated and hypophysectomized when 26 days old and injected subcutaneously on the same day and daily thereafter for 21 days with 0.1 cc sesame oil; (2) similarly operated upon and injected daily subcutaneously with 0.1 mg testosterone propionate† in 0.1 cc sesame oil for 21 days; (3) similarly operated upon and injected daily subcutaneously with 1.0 mg of somatotrophin† (pituitary growth hormone) for 21 days; (4) similarly operated upon and injected daily subcutaneously with 0.1 mg testosterone propionate and 1.0 mg of somatotrophin for 21 days; (5) castrated only when 26 days old and received no hormone; (6) 26-day-old normal rats; (7) 47-day-old normal rats. All rats were fed the customary dry diet and water *ad lib.* as well as a wet diet made up in the form of a paste especially for the benefit of the hypophysectomized animals. On the day following the last injection, or at 47 days of age, the penis and the fore-paws were fixed in 10% formalin and radiographs made of these tissues immediately thereafter. Radiographs were also made of the whole rat in some cases. Instead of comparing the penile ossicles in the different groups by merely measuring their lengths microscopically as in the previous report(1), planimetric studies were made on enlarged skiagrams (prints enlarged 3.5 X from contact films made of the lateral aspect of the distal part of the penis). Histologic studies were made on the penis and the third metacarpal; but the metacarpals will not be treated in this report except to say that they were used as control bones providing the expected evidence of an excellent growth response to somatotrophin in contrast to the os penis.

Results. In Table I, the results obtained in the various groups are summarized. The earlier findings of a slight weight increment (8.6 g in 21 days) in hypophysectomized rats of this age uninjected with hormone and the slightly greater increment (14.4 g) in those receiving 0.1 mg daily of testosterone propionate was confirmed. However such rats remained stunted in their growth when compared with normal animals or those receiving somatotrophin (see skiagrams in Plate 1). The rats injected with a daily dose of 1.0 mg of somatotrophin gained 70 g in the 21-day period as contrasted with those receiving 1.0 mg of somatotrophin and 0.1 mg of testosterone propionate which gained 86.2 g. This latter figure falls within the range of weight increments observed in normal rats during this period and happens to approximate the sum of the increments observed in the groups injected separately with somatotrophin or testosterone (70 plus 14.4).

The figures in the right hand column of Table I represent the area of the lateral aspect

† The somatotrophin was generously provided by Dr. C. H. Li, Institute of Experimental Biology, and the testosterone propionate by Ciba Pharmaceutical Products, Inc., Summit, N. J.

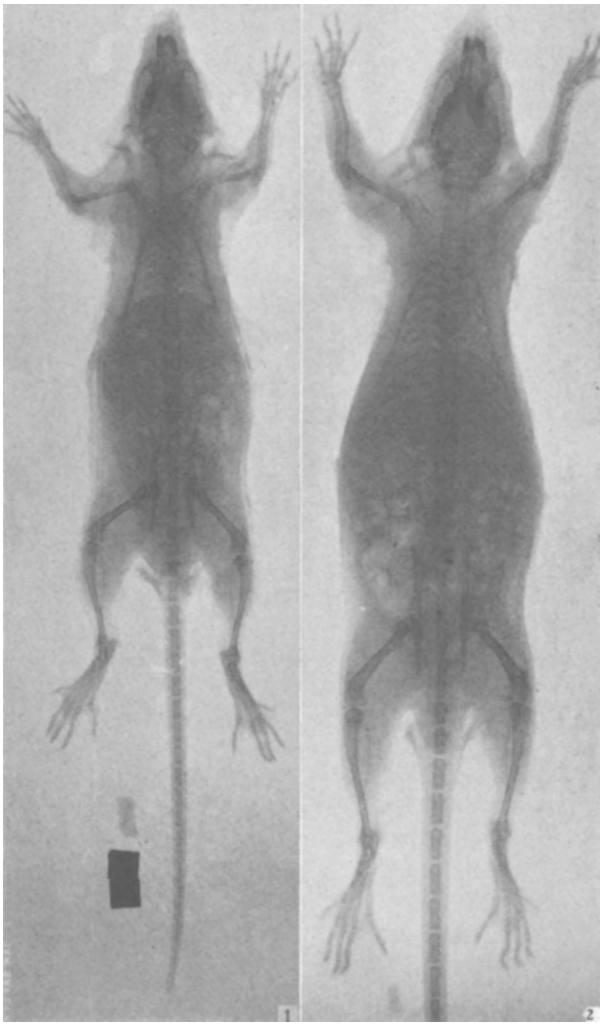

PLATE 1.

FIG. 1. Skiagram of a rat castrated and hypophysectomized at the age of 26 days and injected subcutaneously daily for 21 days with 0.1 mg testosterone propionate. × 0.5.

FIG. 2. Skiagram of a rat castrated and hypophysectomized at the age of 26 days and injected subcutaneously daily for 21 days with 1.0 mg somatotrophin. × 0.5. In each figure, the peripheral part of the penis containing its minute ossicle has been placed beside the tail. Enlarged skiagrams of os penis may be seen in the following plate. In the rat shown in Fig. 1 the skeleton remained stunted and the os penis and the penis grew, whereas in the other rat normal skeletal size was attained but the os and the penis remained small.

of the os penis as projected against a contact X-ray film. The enlarged skiagrams were measured with a planimeter and corrections then made for the enlargement. The figures of 1.28 and 1.38 mm^2 for the hypophysectomized and castrated, and the simply castrated animals respectively indicate that a growth stasis occurred in the os penis in both of these groups (Fig. 7 and 11, Plate 2). The figure obtained for the 26-day-old normal rat was

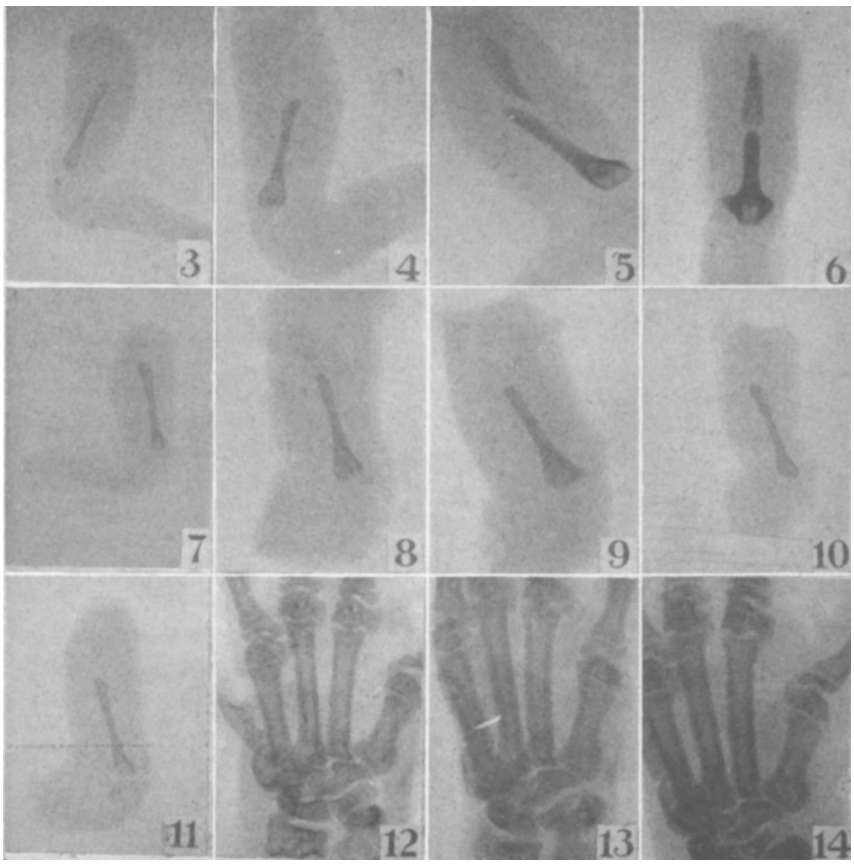

PLATE 2.

FIGS. 3-12. Skiagrams (all lateral views except Fig. 6 which is antero-posterior) of penile ossicles of rats under the following conditions or treatments. All figs. 3.5 ×.

FIG. 3. 26 days old normal.

FIG. 4. 47 days old normal.

FIG. 5. 3 months old normal showing secondary ossicle that forms in the glans penis distal to the main bone between the second and third months.

FIG. 6. Same in A-P view.

FIG. 7. Castrated and hypophysectomized on day 26 and injected daily subcutaneously with sesame oil for 21 days. Necropsy day 47.

FIG. 8. Similarly operated and injected daily subcutaneously with 0.1 mg testosterone propionate in sesame oil for 21 days. Necropsy day 47. This ossicle is almost identical in size and shape with that of the normal 47-day-old rat (Fig. 4).

FIG. 9. Similarly operated and injected as in the preceding case plus a daily subcutaneous dose of 1.0 mg somatotrophin for 21 days. Necropsy day 47. The area represented here is 20% greater than that of the preceding case receiving androgen alone. Most of the overgrowth is at the spatula-like proximal cartilaginous end.

FIG. 10. Similarly operated, but received only somatotrophin. Same regimen as in preceding case.

FIG. 11. Castrated only, day 26. Necropsy day 47.

FIGS. 12, 13, 14. Skiagrams of forepaws of rats shown in Figs. 8, 9, and 10, respectively, for comparison of the middle metacarpals (3rd from right in each case) illustrating the main finding, namely, that the rats receiving androgen showed good growth of the os penis (Figs. 8 and 9), but not of the general skeleton (Fig. 12), whereas the rats receiving somatotrophin showed good growth of the general skeleton (Figs. 13 and 14), but not of the os penis (Fig. 10). All × 3.5.

1.35 mm^2 (Fig. 3, Plate II). The doubly operated animals injected with somatotrophin alone showed only a very slight increase in size of the os penis over that of the normal 26-day-old controls (Fig. 10, Plate 2), the average area being 1.5 mm^2. Testosterone

propionate caused a growth of the os penis of doubly operated rats (Fig. 8, Plate 2) comparing favorably with that of normal controls (Fig. 4, Plate 2) at the age of 47 days (2.20 mm^2 as compared to 2.29 mm^2). The best growth of this ossicle (2.78 mm^2) was obtained in the doubly operated group injected with both somatotrophin and testosterone propionate (Fig. 9, Plate 2). Here, as in the case of the body weight increments, there seemed to be an additive effect (or synergism) of the 2 hormones. In no case did the amounts of hormones used bring about full maturation of the main ossicle or the ossification of the distal center. These processes are only accomplished by about the third month (see Fig. 5 and 6, Plate 2). In contrast to its negligible effect on the os penis, somatotrophin caused excellent growth of the general skeletal system (Fig. 1 and 2, Plate 1, and Figs. 13 and 14, Plate 2).

The bones of the fore-paws of the doubly operated rats receiving testosterone propionate alone were essentially similar to those of the doubly operated controls injected with sesame oil.

Histologic studies revealed that the sections of the os penis from castrated and hypophysectomized rats injected with somatotrophin could not readily be distinguised from the doubly-operated controls given sesame oil. In both groups an occasional small cluster of cartilage cells had persisted in some rats for the 21-day period; but in all there appeared to be a lack of the stimulus for chondrogenic mesenchyme to transform to cartilage as well as a cessation of cartilage cell growth and bone replacement. The rats castrated only, showed slightly less regression of the cartilaginous proximal cap by the 47th day; but here also, the bone remained atrophic as in the doubly operated controls. In the rats treated with testosterone propionate the main penile ossicles showed the same growth processes as were illustrated in the earlier report(1). New growth and hypertrophy of cartilage cells had kept pace with the chondrolysis that accompanied osteoblastic proliferation and ossification. Periosteal osteogenesis was comparable to that in normal animals of the same age.

This was also true of the rats treated with both hormones, but in these the ossicles were quantitatively better, as though slightly older —a finding apparently similar to that of Bok(2).

Summary. In contrast to its excellent effect in stimulating cartilaginous and osseous growth in the rat's penile ossicle, testosterone propionate, in a daily subcutaneous dose of 0.1 mg for 21 days following hypophysectomy and castration, caused no appreciable resumption of general skeletal growth. Somatotrophin (hypophyseal growth hormone) in a daily subcutaneous dose of 1.0 mg for the same period in similar animals caused excellent general skeletal growth, but did not correct appreciably the atrophic condition of the os penis. The two hormones synergized in causing better growth of the os penis than the androgen alone and a greater body weight increase than the growth hormone alone in doubly operated animals.

2. Bok, *Proc. Ned. Akad. Wesch.*, 1942, v45, 1018. Abstract in Monograph on the Progress of Research in Holland during the War, J. H. Gaarenstroom and S. E. De Jongh.

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