Electron Microscopy of Embryonic Avian Tissues.* (17622)

ROBERT H. GREEN. (Introduced by Francis G. Blake.)
(With the technical assistance of Ann Holloway and Ruth Peabody.)
From the Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn.

Although tissues thin enough for visualization by electron microscopy may be obtained by tissue culture(1), by sectioning(2,3) and by mounting single cells(4), the methods available are time-consuming, and require considerable skill and experience. The present report will describe a method that appears relatively easy and satisfactory for obtaining preparations of embryonic avian tissue suitable for electron microscopy. The principle of the method depends upon obtaining growth of tissue directly upon the wire mesh discs or grids on which specimens conventionally are mounted with the aid of a collodion or plastic membrane. This may be accomplished by inserting the discs into the chorio-allantoic cavity of developing chick embryos. After a day or two the discs appear to become attached to the embryonic membranes and cells grow into the meshes, attaching themselves to the edges of the wire strands.

Materials and Methods. Wire mesh discs of the type used in mounting specimens for electron microscopy were sterilized in boiling water and, without further treatment, were inserted directly into the chorio-allantoic cavity of embryonated hens' eggs which had been incubated at 37°C for 6 to 11 days. Taking care to avoid tearing the chorio-allantoic and shell membranes, a piece of shell about 0.5 cm in diameter was removed from the area overlying the chorio-allantoic membrane, in the vicinity of the embryo. A disc was then grasped with a pair of fine forceps and inserted into the chorio-allantoic cavity by directing the edge of the disc parallel to the fibers of the shell membrane and quickly forcing it through the membranes. This procedure produced a small slit in the membranes through which additional discs could be inserted. As a rule, 3 or 4 discs were placed in each egg. The exposed area was then covered with a small piece of scotch tape and the eggs replaced in the incubator. After 3 to 5 days the eggs were candled to determine viability and, in some instances, sterile phosphate buffer or normal allantoic fluid was inoculated into the chorio-allantoic sac. Not infrequently the air sac was found to have shifted to the side, and when material was injected subsequent to the insertion of discs care had to be taken to insure that the chorio-allantoic sac had been entered. Candling, however, usually revealed an area of chorio-allantoic membrane, adjacent to the newly formed air sac, through which inoculation was carried out. A small hole was drilled through the shell over this area and inoculation was done in the manner usually employed. Eggs which had been inoculated were further incubated at 37°C for several hours. Samples of allantoic fluid were then removed aseptically; the egg shells were broken open, and the contents poured into Petri dishes. The discs were removed and placed in distilled water where they were teased free of visible bits of tissue and washed for a minute or two. Discs were placed on a paper towel to remove excess water and finally allowed to dry at room temperature for 5 to 10 minutes. Preparations which appeared grossly unsuitable for electron microscopy on preliminary examination under low magnification with a light microscope were discarded. Specimens considered unsuitable

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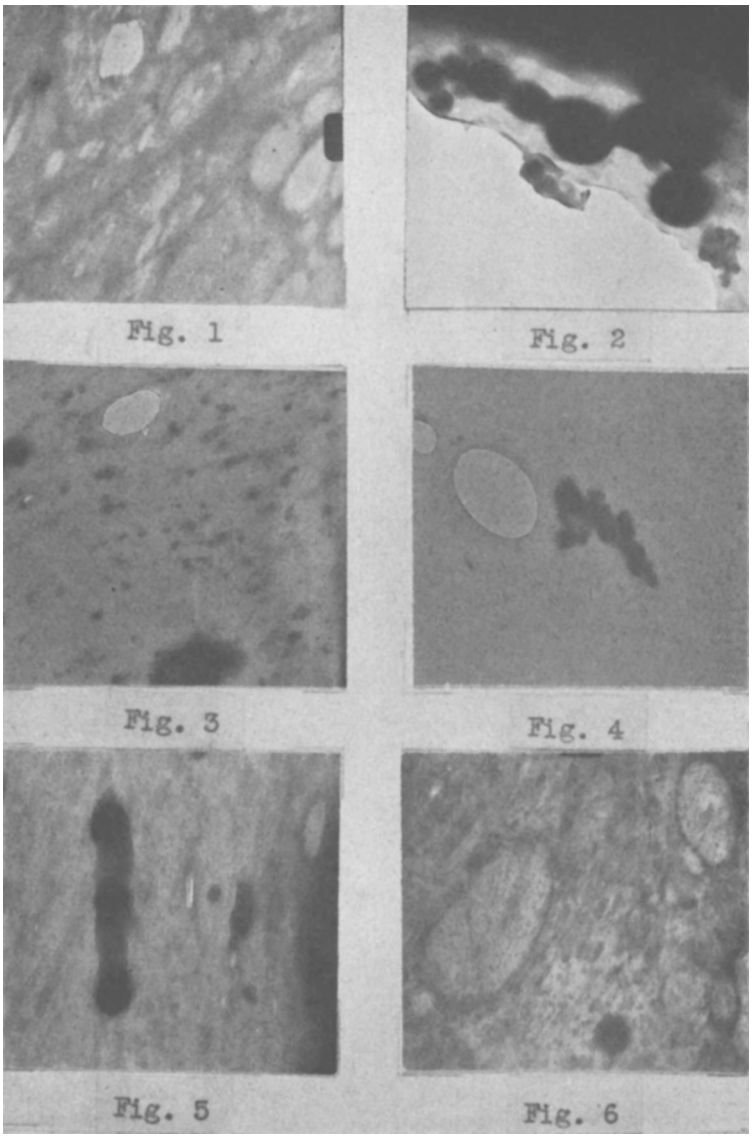

FIG. 1. Cytoplasm from 10-day embryo; grids in place 4 days.
FIG. 2. Cytoplasm from 11-day embryo; grids in place 3 days.
FIG. 3. Cytoplasm from 11-day embryo; grids in place 5 days.
FIG. 4. Cytoplasm from 11-day embryo; grids in place 5 days.
FIG. 5. Cytoplasm from 11-day embryo; grids in place 4 days.
FIG. 6. Different portion of same preparation shown in Fig. 5.
Magnification × 13,400 except Fig. 1, which is × 8400.
Preparations shown in Fig. 1-4 were not fixed; that shown in Fig. 5 and 6 was fixed by exposure to osmium tetroxide for 30 minutes.

were those in which the layer of tissue was obviously too thick as well as those in which there was no tissue within the small central zone of the disc which could be visualized under the electron microscope. Most preparations were then examined, without further treatment, in an RCA electron microscope, type EMU. All fresh specimens were examined within one to four hours after removal of the discs. A few preparations were fixed by exposing them to fumes of 2% osmium tetroxide for a period of 15 to 30 minutes.

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)

WILLIAM R. LYONS, EDWARD ABERNATHY, AND MARK GROPPER.

From the Division of Anatomy and the Institute of Experimental Biology, University of California, Berkeley.

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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