membrane. The findings showed a close correlation with the dilution factor and with the number of particles calculated from chemical and physical data obtained with the virus.

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Electronmicroscopy of Cells from Tissue Cultures Infected with Vaccine Virus.

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One of the obvious needs in electronmicroscopy is a satisfactory method for good visualization of intact cells to provide information on their internal structure and the presence of virus particles in infected preparations. Progress in this field has been made in the use of specially prepared histological sections[1] and in the studies of individual avian cells cultured on plastic membranes.[2,3] The present report describes recent observations on the intracellular growth of vaccine virus observed by electronmicroscopy with a modification of the plastic membrane technic.

Materials and methods. The technic for cultivation of mammalian epithelial cells on plastic membranes and their preparation for visualization by the electron microscope has been described in detail elsewhere.[3-5] In brief, this is as follows: .02 cc of a 0.6% solution of "Formvar" (Shawinigan Falls Products Co., Shawinigan Falls, Quebec, Canada) in ethylene chloride is dropped into a petri dish full to overflowing with chilled Ringer's solution. A cover slip held by forceps is inserted under the membrane covering the fluid in the petri dish and the cover slip is raised through the membrane; this provides a smooth non-adherent plastic coating with a distinct growth-differentiating effect; epithelial growth is enhanced and fibroblasts are inhibited.[2,3] Six such cover slips with the plastic membrane facing upward are placed on a specially prepared glass slide. Small tissue explants are placed on each of the 6 plastic membranes. Special nutrient fluid (plasma-extract supernate, embryonic extract and Tyrode) is added to each culture and maintained in place by a small glass ring sealed with vaseline. The 6 cover slips with their membranes and cultures are covered with a petri dish and infected at the start or after growth has taken place. The nutrient medium is changed at 3-4 day intervals if indicated. Strict asepsis is observed throughout the tissue culture procedure. At an appropriate time, usually 3-5 days for vaccinia, the cover slips with the plastic membrane to which are now attached the growing tissue cells are lifted from the culture chamber. A cover slip with the plastic surface facing up is placed in a petri dish containing Ringer's solution; under these conditions the membrane detaches itself completely from the cover slip and floats on the surface. The floating membrane in the petri dish is examined under low power with an ordinary microscope and the desired fields are selected. These are fixed in the center of the metallic mount by a modification[4] of the usual technic employed in electronmicroscopy.

In the present work, minute explants (weighing about 0.1 mg) prepared from the renal cortex of adult rabbits were used. Cultures were inoculated with a washed suspension of neurovaccinia elementary bodies. Elec-

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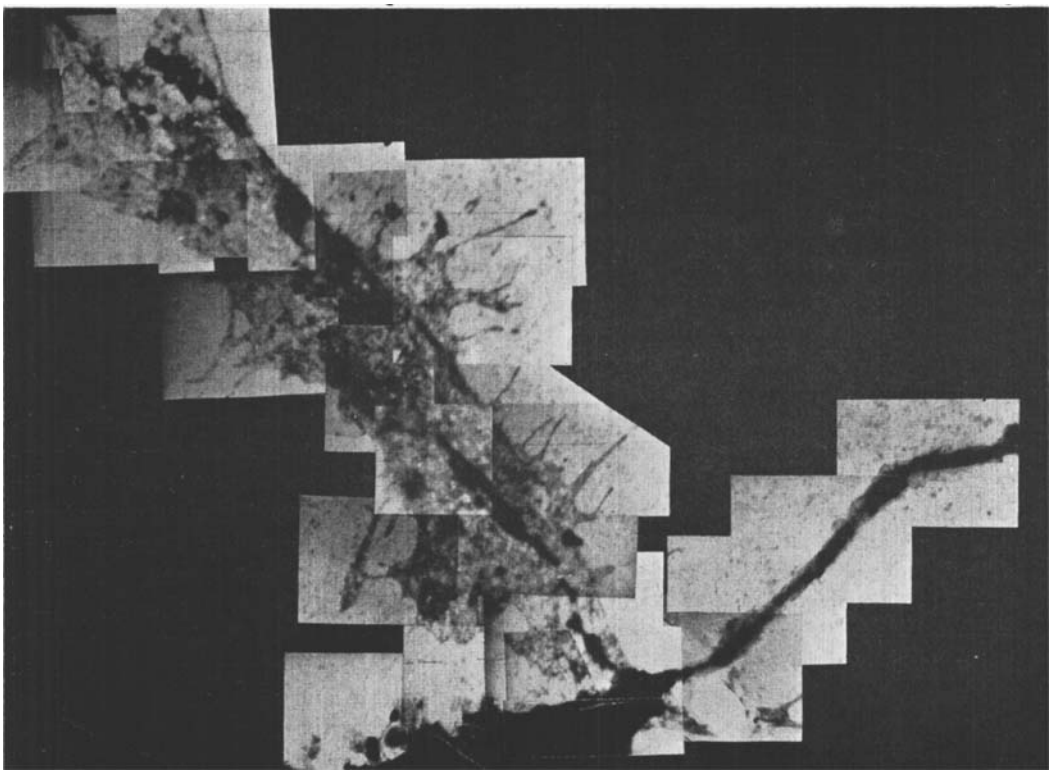

FIG. 1.
Composite electron micrograph of a cell infected with vaccinia. Elementary body-like structures are seen in upper left portion of picture.

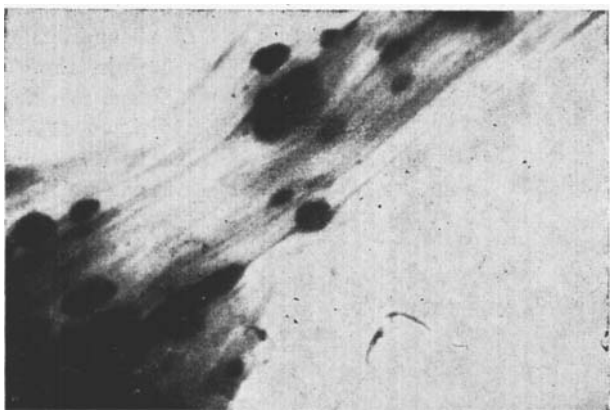

FIG. 2.
Electron micrograph representing a portion of the cell containing numerous elementary body-like structures.

tron micrographs were taken with the C.S.F. electrostatic electron microscope.

Results. Cultures of explants from kidneys of adult rabbits grown by the method described provide essentially pure growth of epithelial cells in a single layer. The technic presents several advantages over the technics previously employed: (1) It provides a rela-

tively simple means for maintaining true cultures a number of days or weeks[6] without transfer; (2) the method does not require the use of a plasma coagulum (fibrin being eliminated); and (3) a monolayer of living cells, almost entirely epithelial, can be obtained for special studies.

A topographical survey of a single cell infected with vaccinia is illustrated in Fig. 1. The individual electron micrographs which make up the composite picture were taken at a magnification of 21,000; after assembly the composite picture was reduced somewhat. The opaque spherical structures occurring in the cytoplasm at the upper left corner are interpreted as elementary bodies of vaccinia seen individually and in clusters. Fig. 2 reproduces one of the micrographs containing the elementary body-like structures which make up Fig. 1. This electron micrograph was also taken at a magnification of 21,000 but has not been reduced. An idea of the reduction in size of the composite picture is obtained by comparing Fig. 2 with the individual components of Fig. 1. Spherical opaque structures such as those observed in Fig. 1 and 2 are regarded as elementary bodies since they have not been observed in similar preparations of uninfected rabbit kidney tissue culture cells. The present method adds another approach to the study of the intracellular growth of vaccine virus which has in the past been fruitfully investigated by microscopic technics employing visible light.[7,8]

Summary. A technic has been devised which provides true cultures of mammalian cells for electronmicroscopy. The usefulness of this procedure in the study of viruses is indicated.

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Adrenalectomy and Pituitary Adrenocorticotrophic Hormone Content.*†

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Alterations in the weight and chemical composition of the adrenal gland have been used as quantitative indices for measurement of the rate of discharge of adrenocorticotrophic hormone (ACTH) from the adenohypophysis following application of stress.[1] These indices have been called "target gland indices". It is now possible to complement our knowledge of the pituitary-adrenal system gained from such indices with data on adenohypophyseal content of ACTH. The present report is one of several studies concerned with factors regulating the synthesis and discharge of pituitary ACTH, and deals especially with the changes following adrenalectomy in the rat.

Methods. Male rats from the Sprague-Dawley farm were used. Twenty rats, 230-275 g in body weight, were employed in two experiments conducted at different times. In Experiment I, 6 rats were bilaterally adrenalectomized and 4 rats served as controls; in Experiment II, 4 rats were bilaterally adrenalectomized, 3 rats were subjected to sham adrenalectomy and 3 rats served as controls.

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† With the statistical assistance of Marion A. Sayers.

‡ Fellow of the American Bureau for Medical Aid to China.

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