during the fourth week of virus growth, may be the effect of different mediums rather than different culture technics.

Size of a culture unit did not appear to be a limiting factor in striving for large yields of virus. Cultures were successfully grown in six-liter rectangular culture bottles. These extremely large flasks were abandoned in favor of the Roux flasks, which were more manageable and not as prone to contamination with molds.

Summary. Poliomyelitis viruses were propagated in large stationary tissue cultures of plasma imbedded monkey testes. Large volumes of supernatant fluids with high virus titers were achieved. The virus yield, as measured by titer and duration of maximum growth, was almost identical with that obtained in roller tubes, and greater than that reported for suspended cell flask cultures. The method had another advantage in that equipment for rotating bottles was not required. Since the Roux flasks could be stacked one on top of another, a minimum of incubator space was required.

1. Enders, J. F., Weller, T. H., and Robbins, F. C., *Science*, 1949, v109, 85.

2. Robbins, F. C., Enders, J. F., and Weller, T. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 370.

3. Youngner, J. L., Ward, E. N., and Salk, J. E., *Am. J. Hyg.*, 1952, v55, 291.

4. Scherer, W. F., and Syverton, J. T., *J. Exp. Med.*, 1952, v96, 369.

5. Melnick, J. L., Riordan, J. T., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 208.

6. Svedmyr, A., Enders, J. F., and Halloway, A., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 296.

7. Ledinko, N., Riordan, J. T., and Melnick, J. L., *Am. J. Hyg.*, 1952, v55, 323.

8. Unpublished data.

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Electron Microscopic Examination of Inclusion Bodies of Herpes Simplex Virus. (20145)

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Despite extensive investigation(1) there is still doubt concerning the relation of herpes simplex virus to the intranuclear inclusion bodies which are characteristically seen in infected tissues. Recent studies with the light microscope of such inclusions stained by the Feulgen method suggest that they contain virus and do not represent solely an abnormal product of cellular metabolism(2,3). This paper reports preliminary observations made with the electron microscope of thin sections of cells infected with herpes simplex virus.

Materials and methods. Chick embryo chorioallantoic membranes heavily infected with the HRE strain of herpes simplex virus (4) were ground with sterile sand in a mortar and suspended in nutrient broth containing penicillin and streptomycin. After centrifugation to remove large tissue particles, the supernatant fluid was diluted to contain approximately 300 ID_{50}/ml. Two-tenths milliliter of this suspension was inoculated onto the chorioallantoic membranes of 11-day-old chick embryos. Following 48 to 72 hours incubation at 35°C the membranes were removed and immediately fixed in 1% buffered osmium tetroxide, according to the method of Palade (5). Small pieces, showing pocks grossly, were excised from the fixed membrane, dehydrated in ethyl alcohol, embedded in methacrylate, and sectioned by the thermal expansion method. The sections were mounted on formvar screens and the methacrylate was removed by immersion in amyl acetate. After light shadowing with palladium the preparations were examined in an RCA type EMU electron microscope. Adjacent portions of membrane prepared by routine methods and

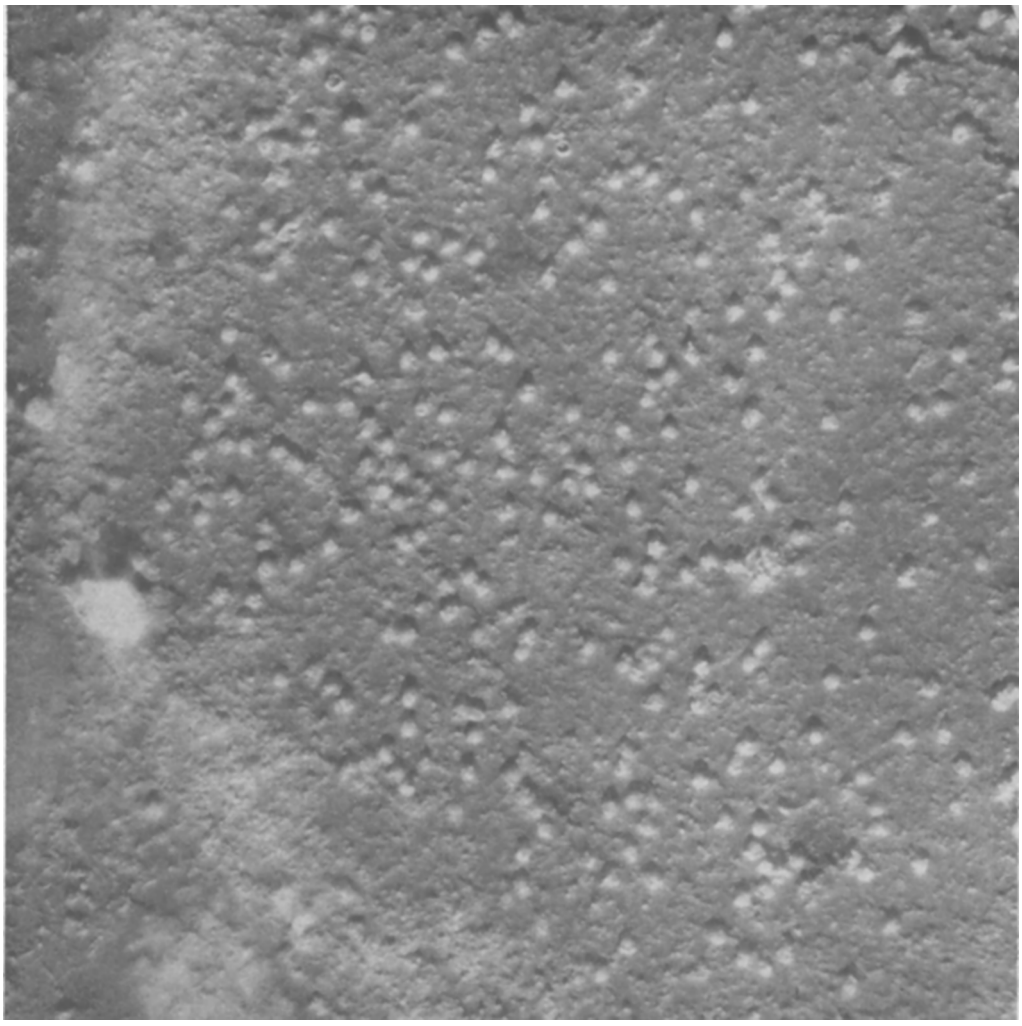

FIG. 1. Part of an inclusion body containing numerous spherical particles. Cytoplasm of host cell lies at the left. This and the following are negative prints. × 19500.

stained with hematoxylin and eosin showed numerous typical inclusions when viewed with the light microscope.

Results. The nuclei of many ectodermal cells of the chorioallantoic membrane were found to be replaced by large oval or spherical bodies readily visible in the electron microscope. Although the size of these bodies varied according to the plane of transection, they resembled both in shape and in position within the cell the inclusion bodies seen in the light microscope.

Fig. 1 shows part of an inclusion. Its border runs from the upper left hand corner downward and across the bottom. The cytoplasm of the cell is at the left margin of the picture. Distributed at random through the finely granular structure of the inclusion body are small, dense, sharply demarcated, spherical particles of nearly uniform size. Although the particles are usually dispersed through the inclusion in this fashion, occasional closely packed clusters are seen. Fig. 2 shows the margin of such an inclusion at somewhat higher magnification. The cytoplasm of the cell occupies the lower left hand corner. Clusters of particles replace the wall of the inclusion at the upper left and are partly embedded within the wall at the lower right. Some ring or shell forms are present. A few

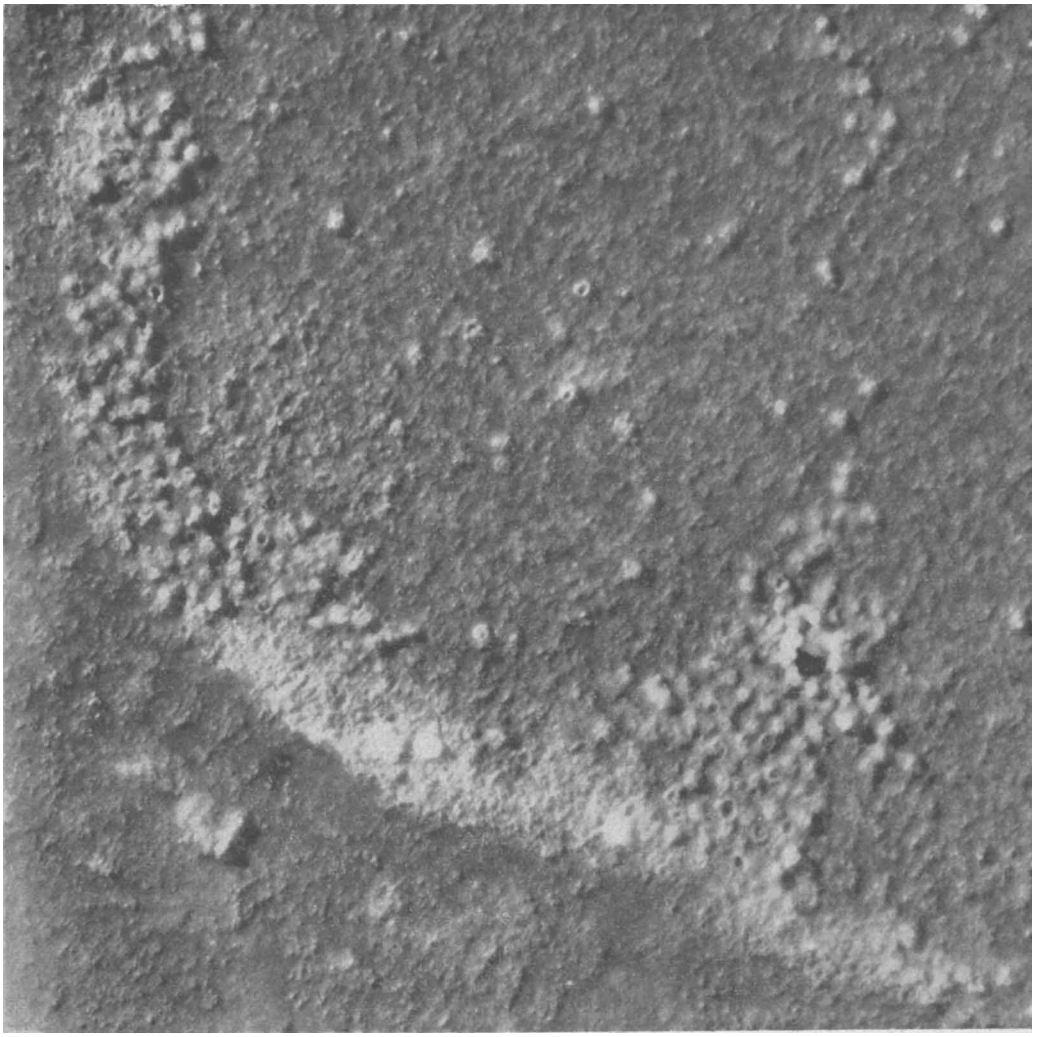

FIG. 2. The wall of an inclusion body containing clusters of particles. Cytoplasm is at the lower left corner. × 22800.

particles lie singly within the substance of the inclusion body. Sections examined before removal of the methacrylate showed inclusions containing similar particles. Most of the particles measured between 120-125 mμ, although a few ranged from 100 to 130 mμ.

Within the inclusions so far examined the number of particles has varied considerably. This is probably due in part to differences in thickness of the sections, but may also represent stages in the development of the inclusion body. Larger particles similar in size to those described by Coriell *et al.* (133-233 mμ)(6), and Evans and Melnick (200-220 mμ)(7) have been seen within the cytoplasm and at the surface of infected cells. The manner in which the inclusion body becomes differentiated and the relation of the particles within the inclusion to the larger forms seen outside are being investigated.

Summary. Intranuclear inclusion bodies were readily identified by the electron microscope in thin sections of chorioallantoic membranes infected with herpes simplex virus. Such inclusions contained numerous particles of uniform density, shape and size presumed to be elementary bodies of the virus. The particles observed within the inclusions were

smaller than other particles, also considered to be virus, seen in the cytoplasm and at the surface of cells.

The technical assistance of Miss Annette Dietsch is gratefully acknowledged.

1. van Rooyen, C. E., and Rhodes, A. J., Virus Diseases of Man, 2nd Ed., New York, 1948, Thomas Nelson and Sons, p. 184.

2. Crouse, H. B., Coriell, L. L., Blank, H., and McNair Scott, T. F., *J. Immunol.*, 1950, v65, 119.

3. Wollman, M., and Behar, A., *J. Inf. Dis.*, 1952, v91, 63.

4. Rose, H. M., and Molloy, E., *J. Immunol.*, 1947, v56, 287.

5. Palade, G. E., *J. Exp. Med.*, 1952, v95, 285.

6. Coriell, L. L., Rake, G., Blank, H., and McNair Scott, T. F., *J. Bact.*, 1950, v59, 61.

7. Evans, A. S., and Melnick, J. L., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 283.

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Pulmonary Tumors in Strain A Mice Exposed to Mustard Gas. (20146)

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Sulfur mustard and nitrogen mustards have been shown to be carcinogenic when injected into mice and rats. In tests in this laboratory(1,2) both the nitrogen mustard, methyl bis (2-chloroethyl) amine hydrochloride and sulfur mustard, bis (2-chloroethyl) sulfide, when injected intravenously into strain A mice increased the incidence and average number of pulmonary tumors to an extent comparable with that Shimkin and McClelland(3) had observed from approximately the same amount of methylcholanthrene. Boyland and Horning (4) injected stock mice subcutaneously with the nitrogen mustards, methyl bis and methyl tris, and of 14 that survived 250 days, 10 had tumors of a variety of types, one of which was a spindle-cell sarcoma at the site of injection. In this laboratory(5) tumors including sarcomas that were probably fibrosarcomas, sarcomas that were neurogenic in origin, a rhabdomyosarcoma, papillomas, and a squamous-cell carcinoma were induced in mice of strains A, C3H, and $C3H_f$ at the site of subcutaneous injection of sulfur mustard and of the nitrogen mustard, methyl bis. Griffin *et al.*(6) have reported a variety of tumors in both mice and rats injected with methyl bis and with methyl tris.

From these results, particularly those in respect to the induction of pulmonary tumors, it seemed desirable to ascertain whether or not the fumes from any of these mustards would be carcinogenic. An experiment was, therefore, set up to test the fumes of sulfur mustard, bis (2-chloroethyl) sulfide.†

Methods. Mice of strain A were used. This is a highly inbred strain that because of its high genetic susceptibility to pulmonary tumors has been used extensively for testing the carcinogenic action of chemicals particularly when injected intravenously. The incidence of spontaneous pulmonary tumors in the strain is approximately 90% in animals 18 months of age and 50% in animals 12 months of age(7). One hundred sixty individually identified strain A mice (80 males and 80 females) from 2 to 3 months of age were divided equally into control and experimental groups with littermates distributed among the 2 groups. Mice of the experimental group were placed in small individual cages of $\frac{5}{8}''$ mesh wire and in lots of 8 were placed in an 8 liter desiccator. One hundredth cc sulfur mustard absorbed on a strip of filter paper to permit maximum evaporation was suspended in the desiccator which was immediately sealed. A small electric fan in the bottom of the desiccator circu-

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