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Electron Micrography of the Western Strain Equine Encephalomyelitis Virus.*

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A material behaving biologically as the Western strain equine encephalomyelitis virus has been isolated as previously noted[1] from extracts of chick embryo tissue diseased with this agent. Recent work in this laboratory has resulted in the isolation of the virus in preparations of high homogeneity as indicated by the character of sedimentation diagrams in the analytical ultracentrifuge. Among the studies made on the purified material was the examination of it by means of the electron microscope. The results obtained with this instrument are reported briefly in the present paper.

The Western strain of equine encephalomyelitis virus was cultured in 11 or 12-day-old chick embryos in a manner similar to that employed for the Eastern strain of this virus.[2] The diseased tissue was extracted in Ringer solution of pH 8.6 to 8.8 for 4 days between 5° and 8°C. The extracts, cleared by low-speed centrifugation and filtered with celite, were subjected to 1 or 2 ultracentrifugal cycles in alternate high (30,000 g) and low (17,000 g) centrifugal fields. The purified product in Ringer solution was cleared of aggregates by angle centrifugation (7000 g) for 5 to 10 min. Electron microscope preparations were made by applying to the collodion film thin layers of virus in undiluted Ringer solution or Ringer solution diluted with water to 0.03 M salt concentration. Some of the screens were allowed to dry and examined without further treatment. Others were dried and then washed with Ringer solution or with distilled water. The purified virus was examined the day of isolation and at intervals thereafter for 40 days. Parallel studies were made on changes in infectivity and sedimentation characters.

Electron micrographs of fresh virus preparations giving a sharply sedimenting boundary in the analytical ultracentrifuge showed the presence of circular images with little evidence of extraneous material. An example of the findings is given in Fig. 1 obtained 3 days after isolation of the material. The association of virus properties with such preparations and the absence of images of this sort in micrographs of healthy embryo tissue indicated the probability that the images represented electron micrographs of the virus particles.

The virus particles were consistently difficult to photograph immediately and within a few days after isolation. The images were of low contrast and their edges indefinite. This is illustrated in Fig. 1 where the micrograph of 15,700 × magnification was increased to 52,500 × by enlargement from the negative. In older preparations the contrast was greater. The images in micrographs of fresh specimens were for the most part rounded. Some were oval in appearance while others appeared definitely elongated. Of especial interest was a clearly discernible differentiation of structure within the images. This was seen in the presence of a dense inner region surrounded by an enveloping region of considerably less density. In general the denser area was rounded, centrally placed, occupied much of the image and appeared to be approximately the same density throughout. In many cases, however, the inner area appeared to be of approximately half the total diameter of the particle and was oval or irregular in outline.

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1 Sharp, D. G., Taylor, A. R., Beard, D., Finkelstein, H., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 459.

2 Taylor, A. R., Sharp, D. G., Beard, D., and Beard, J. W., *J. Infect. Dis.*, in press.

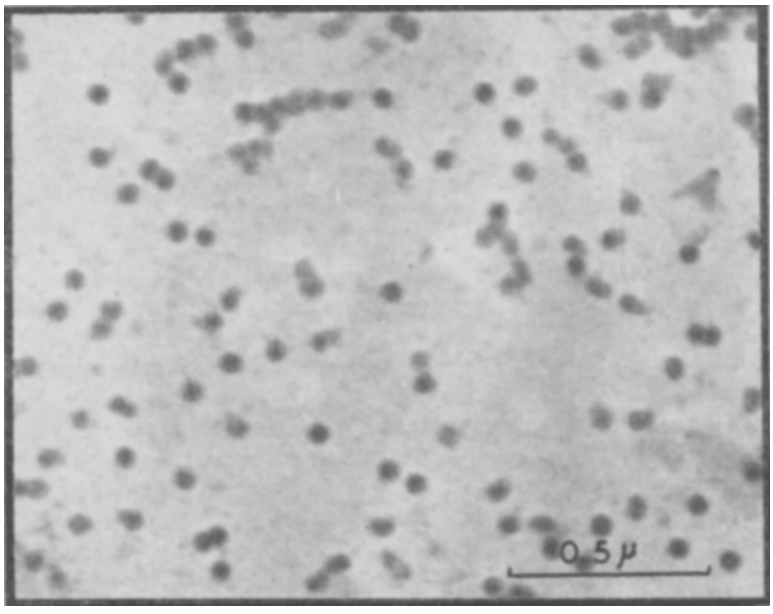

FIG. 1.

Electron micrograph of Western strain equine encephalomyelitis virus (52,500 ×) obtained 3 days after ultracentrifugal purification. The micrograph was made at 55 kilovolts. The magnification in the final pictures was calculated on the basis of measurements on tobacco mosaic virus rods which have been reported to be 15mμ in diameter.

Occasionally the inner structure appeared double. These findings were consistent whether the specimen was examined without washing on the screen or was washed with Ringer solution. Washing with distilled water markedly decreased contrast and definition of the less dense enveloping region.

The general features of the fresh preparations were seen also in the older specimens. With aging, however, the images appeared definitely to gain in contrast and to change in shape. The predominant image in preparations 3 weeks after isolation was comma-shaped, with the denser area forming the eccentrically placed head and the less dense material constituting the tail piece. The infectivity of such preparations had decreased 3 decimal dilutions but the sedimentation pattern and constant had not changed.

Measurements of diameter on 20 images in each of 4 plates of 3 different preparations gave values of 39.6, 39.3, 38.0, and 42.1 mμ, with an average value of 39.8 mμ for all. These values were calculated on the basis of comparison with the tobacco mosaic virus which has been reported[3] to be 15 mμ in diameter.

The present findings indicate that the Western strain equine encephalomyelitis virus is a spherical or disc-shaped particle of approximately 40 mμ in diameter. Electron-micrographic images reveal an internal structure characterized by a round or oval region of relatively high density surrounded by an enveloping material of less density.

3 Stanley, W. M., and Anderson, T. F., *J. Biol. Chem.*, 1941, **139**, 325.