

strain given intracerebrally. It may be mentioned that the histopathological lesions in the central nervous system of guinea pigs and mice that succumbed to infection with the virus isolated from the 2 patients were more severe than those generally encountered in animals infected with the virus of choriomeningitis;<sup>1</sup> not only an extensive choriomeningitis was present but also a true encephalitis.

The occurrence of disease in all guinea pigs and most mice inoculated with the human material clearly indicated that the virus of choriomeningitis had been present in the inocula and that it had not been encountered adventitiously.<sup>†</sup> In order to remove any doubt about this a portion of each tissue was taken from storage at  $-70^{\circ}\text{C}$  and after fresh suspensions had been prepared they were again inoculated into normal guinea pigs or mice. The virus was reisolated and reidentified from each specimen.

*Summary.* The virus of lymphocytic choriomeningitis was isolated from the tissues of two patients who died following an undiagnosed acute systemic disease.

### 13669 P

#### Morphological Structure of the Virus of Vaccinia

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Elementary bodies of vaccinia which represent the infective unit of the disease<sup>1</sup> are relatively large, *i. e.*, 235-250  $\text{m}\mu^2$  in diameter. They respond to osmotic influences,<sup>3</sup> are antigenically complex,<sup>4</sup> and possess certain chemical and biologically active constituents in common with bacteria and mammalian cells.<sup>5</sup> The virus of vaccinia,

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<sup>†</sup> The virus of lymphocytic choriomeningitis was also isolated by Dr. R. S. Muckenfuss, of the Department of Health, New York City, from tissue of the second patient mentioned in this report.

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<sup>1</sup> Smadel, J. E., Rivers, T. M., and Pickels, E. G., *J. Exp. Med.*, 1939, **70**, 379.

<sup>2</sup> Pickels, E. G., and Smadel, J. E., *J. Exp. Med.*, 1938, **68**, 583.

<sup>3</sup> Smadel, J. E., Pickels, E. G., and Shedlovsky, T., *J. Exp. Med.*, 1938, **68**, 607.

<sup>4</sup> Smadel, J. E., Hoagland, C. L., and Rivers, T. M., *J. Bact.*, 1941, **41**, 57.

<sup>5</sup> (a) Hoagland, C. L., Smadel, J. E., and Rivers, T. M., *J. Exp. Med.*, 1940, **71**, 737; (b) Hoagland, C. L., Ward, S. M., Smadel, J. E., and Rivers, T. M., *Proc. Soc. Exp. Biol. and Med.*, 1940, **45**, 669; *J. Exp. Med.*, 1940, **72**, 685; *J. Exp. Med.*, 1941, **74**, 69, 133.

therefore, might be regarded as a complex entity which more closely resembles bacteria than crystallizable plant viruses, differing from the former principally in respect to size and growth requirements; only living cells are capable of serving as an adequate culture medium, because it appears that the elementary bodies lack certain enzyme systems essential for independent growth and metabolism. In an attempt to detect morphological structure in the elementary bodies of vaccinia, preparations of the virus were examined by means of the RCA electron microscope.<sup>6</sup>

The present studies show that elementary bodies of vaccinia possess a definite and characteristic structure. In general their shape is roughly comparable to that of a brick; they are almost rectangular when viewed both from above and from the side. Furthermore, 5 circumscribed areas of increased density are found in the bodies. These appear as dark disks in the micrographs and the arrangement of the 5 is similar to the spots on dice. Prior to our realization of the importance of rewashing stored preparations of virus<sup>1</sup> immediately before examination in the electron microscope different results were obtained. The particles appeared somewhat oval rather than rectangular and were uniformly opaque; these pictures were similar to those obtained previously by others.<sup>7</sup> Apparently under these conditions sufficient protein was adsorbed on the surface of the particles to scatter almost all of the electrons in the electron beam. This protein in stored suspensions of virus probably appears during the course of spontaneous disintegration of a certain number of elementary bodies, since a few ghosts are found in all preparations, and this material is removed from the bodies by washing.

Alkaline treatment of elementary bodies liberates a nucleoprotein antigen from the particles without completely dissolving them.<sup>4</sup> Electron micrographs of films of elementary bodies treated with N/50 NaOH for 1/2 minute show that certain changes have occurred. Some of the bodies are swollen and show rounded outlines and indistinct edges. Their substance is less dense, resulting in an accentuation of the areas of condensation. Extraction with N/10 NaOH for 10 minutes lyses the virus particles almost completely and even the condensed areas have practically disappeared. Certain of the ghosts formed in this manner present gaps in their surfaces through which in some instances internal substances stream forth.

Control materials, consisting of supernatant fluid from which virus had been sedimented and of particles from normal kidney cells ob-

<sup>6</sup> Zworykin, V. K., Hillier, J., and Vance, A. W., *Elec. Eng.*, 1941, **60**, 157.

<sup>7</sup> von Borries, B., Ruska, E., and Ruska, H., *Klin. Woch.*, 1938, **17**, 921.

tained by the method of differential centrifugation identical with that used for the virus of vaccinia, contained no structures which resembled elementary bodies. The present observations indicate that the elementary body of vaccinia has a morphological structure similar in some respects to that of bacteria<sup>7, 8</sup> and different from that of plant viruses.<sup>9</sup>

*Summary.* The pictorial data obtained by means of the electron microscope show a remarkable regularity in the morphology of elementary bodies of vaccinia. The virus particles apparently have an internal structure and some sort of limiting membrane.

### 13670 P

#### Experiments on Transfer of Cutaneous Sensitivity to Simple Compounds.

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In the course of experiments to passively transfer skin sensitivity to simple compounds an attempt was made to induce sensitivity by injecting peritoneal exudates from sensitized animals. Guinea pigs were rendered sensitive to picryl chloride in the manner previously described,<sup>1</sup> using conjugates (guinea pig stromata treated with picryl chloride) in conjunction with intraperitoneal injections of killed tubercle bacilli. To produce exudates, killed tubercle bacilli (or tuberculin) were injected intraperitoneally about 3 weeks from the beginning of the treatment, when substantial tuberculin hypersensitivity was established.

On injecting such exudates intraperitoneally into normal animals, the recipients in most of the experiments were seen to develop sensitivity to picryl chloride; when then a drop of an oil solution of the substance was put on the skin, erythematous reactions, mostly of high color, were apparent on the next day. The phenomenon was found to be due not to the clarified fluid but to the sediment obtained upon centrifugation. Among the possible explanations, an active

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<sup>8</sup> (a) Mudd, S., and Lackman, D. B., *J. Bact.*, 1941, **41**, 415; (b) Morton, H. E., and Anderson, T. F., *Proc. Soc. Exp. Biol. and Med.*, 1941, **46**, 277.

<sup>9</sup> (a) Anderson, T. F., and Stanley, W. M., *J. Biol. Chem.*, 1941, **139**, 339; (b) Kausche, G. A., Pfankuch, E., and Ruska, H., *Naturwissensch.*, 1939, **27**, 292.

<sup>1</sup> Landsteiner, K., and Chase, M. W., *J. Exp. Med.*, 1941, **73**, 431.