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Electron Micrographs of the Agent of Feline Pneumonitis (Baker).

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The morphology of several viruses and rickettsiae has been studied with the aid of the electron microscope but no studies on any member of the psittacosis-lymphogranuloma-trachoma group have been published. Although the data presented here deal only with the agent of feline pneumonitis, similar micrographs have been obtained with agents of lymphogranuloma venereum, human pneumonitis* (S.F.) and psittacosis.*

The material for study was prepared from 10% or 20% suspensions of heavily infected yolk sacs which were shaken in Ringer's without beads for 20 minutes to liberate the agent from infected cells. From then on the procedure varied but the following gave the cleanest preparations. The supernate from shaking was twice centrifuged for 15 minutes at 2000 and at 2400 r.p.m. The agent was then sedimented twice at 15,000 r.p.m. for one hour and resuspended in Ringer's solution. After a third centrifugation at 15,000 r.p.m. for one hour the sediment was finally resuspended in the original volume of distilled water. This suspension was clarified finally at 2000 r.p.m. for 15 minutes and the supernate dried on collodion membranes in the usual manner.

As shown by the illustrations, the characteristic picture obtained is one in which a central more opaque mass is surrounded by a less opaque circular limiting membrane.

* We wish to thank Dr. Francis Gordon for supplying suspensions of these two agents which had been inactivated by ultraviolet irradiation.

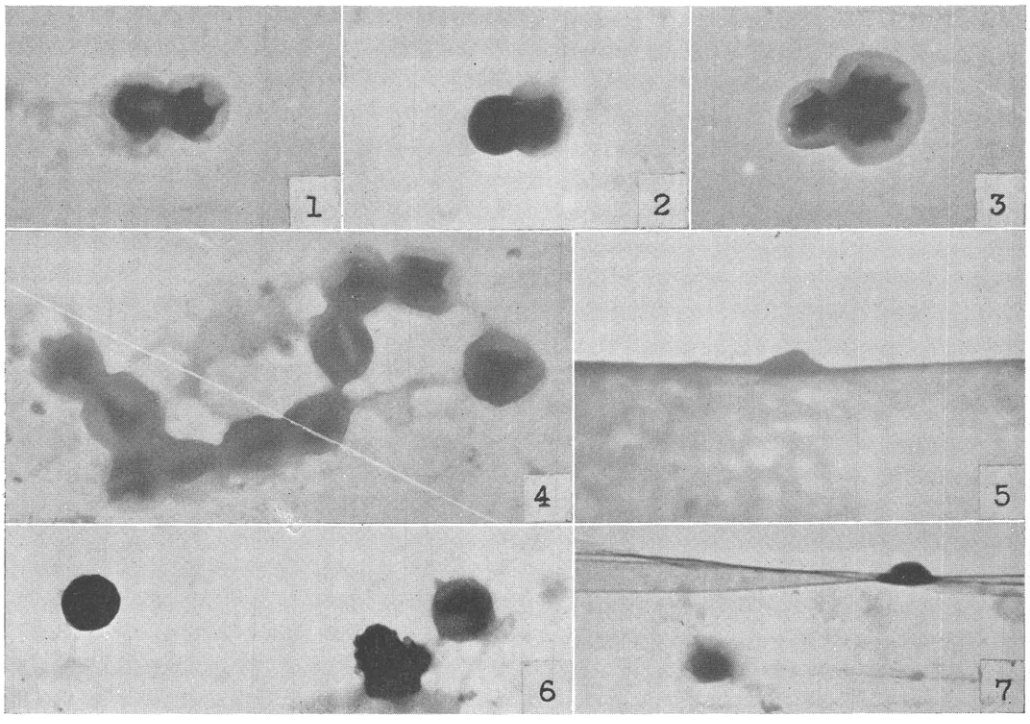

FIG. 1.
The elementary bodies of Feline Pneumonitis. Magnification 20,700 ×.

This appearance resembles closely that described for the rickettsiae[1-3] and certain bacteria,[4] less closely that shown by vaccinia[5,6] and not at all that of the smaller viruses. In the case of feline pneumonitis, the central substance is quite irregular internally as well as in outline (Fig. 1-4). The appearance suggests the irregularities of a wrinkled pea flattened on one side and in a few instances in which side views have been obtained due to rupturing of the collodion membranes, this opinion was confirmed (Fig. 5). In a very few instances dark bodies of uniform density are seen without any limiting membrane. It is not certain that these represent the bodies of the agent but since they are of the same general size and density as the central masses they may be undistorted bodies or bodies in which the substance of the limiting membrane has been lost (Fig. 2, 6). In some bodies showing the limiting membrane there was little distortion of the central mass. In side view such bodies appear like derby hats (Fig. 7).

The majority (51%) of the elementary bodies appear by direct measurement of electron micrographs to be between 440 and 490 mμ in diameter. The median diameter is 465 mμ, the average 455 mμ, and the range from 350 to 580 mμ. Such lack of uniformity is not seen with the smaller viruses. It seems probable that these diameters for the elementary bodies are larger than those existing in the viable forms. The flattened wrinkled pea form noted above suggests that the bodies resemble sacs filled with jelly which, settling down on the collodion membranes, lose their spherical shape and increase

[1] Plotz, H., Smadel, J. E., Anderson, T. F., and Chambers, L. A., *J. Exp. Med.*, 1943, **77**, 355.

[2] Weiss, L. J., *J. Immunol.*, 1943, **47**, 353.

[3] Shepard, C. C., and Wyckoff, R. W. G., *Pub. Health Rep.*, 1946, **61**, 761.

[4] Mudd, S., and Anderson, T. F., *J. Am. Med. Assn.*, 1944, **126**, 561.

[5] Green, R. H., Anderson, T. F., and Smadel, J. E., *J. Exp. Med.*, 1942, **75**, 651.

[6] Sharp, D. G., Taylor, A. R., Hook, A. E., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 259.

in diameter. Larger bodies, such as might correspond to initial bodies, are rare although at least one 770 mμ in diameter has been noted. There are two possible reasons for the paucity of initial bodies. They may be largely discarded with the low speed sediment or they may fragment, at least to the extent of losing their capsular material, in the process of repeated differential centrifugation. There is evidence that both occur. Thus examination with the light microscope of low speed supernates of preparations, originally rich in initial bodies, show complete disappearance or marked decrease in numbers—to between 1% and 2%. Such supernates are then further treated, by repeated high speed sedimentation and resuspension, and those showing any initial bodies suffer a further 90% decrease.

Pairs, chains and groups of elementary bodies are to be found (Figs. 1-4). Fairly dense intercellular bridges stretching between bodies are not infrequent and may be enclosed by limiting membranes (Fig. 4). The picture is reminiscent of that shown by certain bacteria.[4]

Summary. Electron microscope studies of the agent of feline pneumonitis suggest that the elementary bodies in nature have properties similar to those of jelly-filled sacs which in course of preparation for examination in the microscope settle to a form similar to a wrinkled pea with one flattened side. Such distorted bodies are approximately 465 mμ in diameter. They have several characteristics which resemble rickettsiae and bacteria.

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Pathogenizing Effect of Different Carbohydrates on *Eberthella typhosa.*

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It has long been known that the pathogenicity of bacteria for laboratory animals can be enhanced by addition of certain substances to the injected suspensions (cf. references in papers of McLeod,[1] Ercoli *et al.*[2]). Several investigators have used carbohydrates for this purpose. No systematic study of the relation between carbohydrate structure and pathogenizing potency has, however, been reported. The present communication reports experiments which form part of an investigation designed to clarify the chemical basis of pathogenizing activity in the carbohydrate group.

The experiments define the activity of selected sugars and polysaccharides as pathogenizing agents for *E. typhosa* in the mouse. Special attention is directed to the pathogenizing activity of several levans, including 2 natural sources and a preparation synthesized from sucrose by a cell-free system. The information obtained is inadequate to define finally in a positive sense the features of molecular pattern which determine pathogenizing activity in the carbohydrate group. It suffices, however, to show that several features of the chemical structure and physical character of a carbohydrate are unimportant to its rôle as a pathogenizer.

Test organism. E. typhosa (0901) served as the test microorganism. The bacteria were grown in plain broth at 37°C, the incubation lasting 24 hours.

Test of pathogenizing activity. The experiments were designed to show whether intraperitoneal injection of carbohydrate into

[1] McLeod, Ch., *Am. J. Hyg.*, Sec. B, 1941, **34**, 41 and 51.

[2] Ercoli, N., Lewis, M. N., and Harker, E., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 273.