STUDIES ON THE MORPHOLOGY OF THE ELEMENTARY BODIES OF FOWL POX

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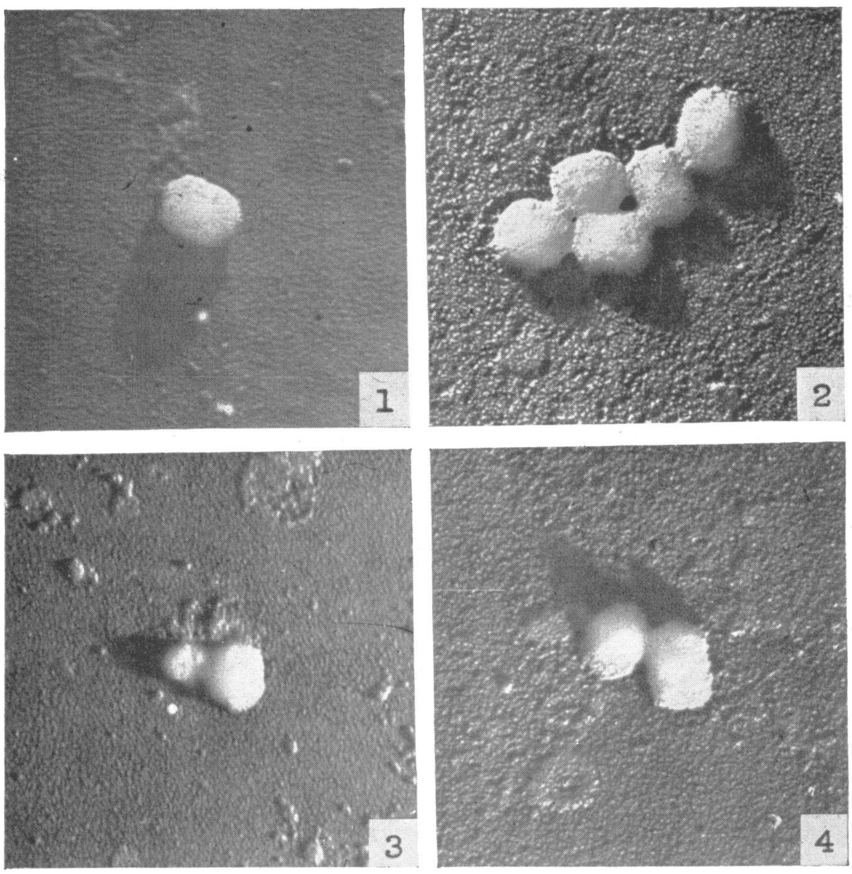
The morphological structure of the elementary bodies of vaccinia as revealed by the electron microscope was first studied by Green, Anderson, and Smadel (1942). Pictorial data presented by these workers showed that the elementary bodies are roughly rectangular in shape, possess a limiting membrane, and exhibit rounded areas of density greater than that of the remainder of the particle, suggestive of internal differentiation. These findings were confirmed by Sharp, Taylor, Hook, and Beard (1946), who, in addition, utilized the shadow-casting technique of Williams and Wyckoff (1945) and presented evidence that the elementary bodies are essentially short cylinders apparently coated with a sticky substance. Recently Groupé, Oskay, and Rake (1946) have shown that the elementary bodies of fowl pox closely resemble those of canary pox and have many morphological characteristics in common with the elementary bodies of vaccinia. In addition, it was observed that the particles of these avian poxes are most frequently attached to one another at their corners, and that the characteristically flattened corner observed on many particles probably results from the separation of particles thus joined. In the studies presented below, the shadow-casting technique of Williams and Wyckoff (1945) has been utilized to study further the morphological characteristics of the elementary bodies of fowl pox with particular reference to their internal structure and the curious budlike structures previously described (Groupé, Oskay, and Rake, 1946).

MATERIALS AND METHODS

An egg-adapted strain of fowl pox was obtained through the courtesy of Dr. F. R. Beaudette of the New Jersey Experiment Station and was maintained by serial egg passage using Burnet's technique (Burnet, 1936). Heavily infected chorioallantoic membranes were ground with sand and 10 per cent suspensions in saline prepared. Partial purification of the elementary bodies was obtained by two cycles of differential centrifugation at speeds of 1,000 rpm and 15,000 rpm for 15 minutes and 45 minutes respectively. The pellet obtained from the final high-speed sedimentation of virus was resuspended in distilled water, and a small drop of the resulting virus suspension was immediately placed on the collodion film support. After drying in air the specimens were ready for shadow casting. We are greatly indebted to Dr. H. Sidney Newcomer, who designed and built the shadow-casting device used in these studies. This device has given excellent results in the application of the technique introduced by Williams and Wyckoff (1945). Satisfactory shadowing of the elementary bodies was obtained when about 20 mg of gold were vaporized, approximately 8 to 10 cm from the specimen, at an angle ranging from 8 to 15 degrees, under a vacuum of the order of 0.00003 mm Hg. An RCA electron microscope (type EMU) was used throughout these studies.

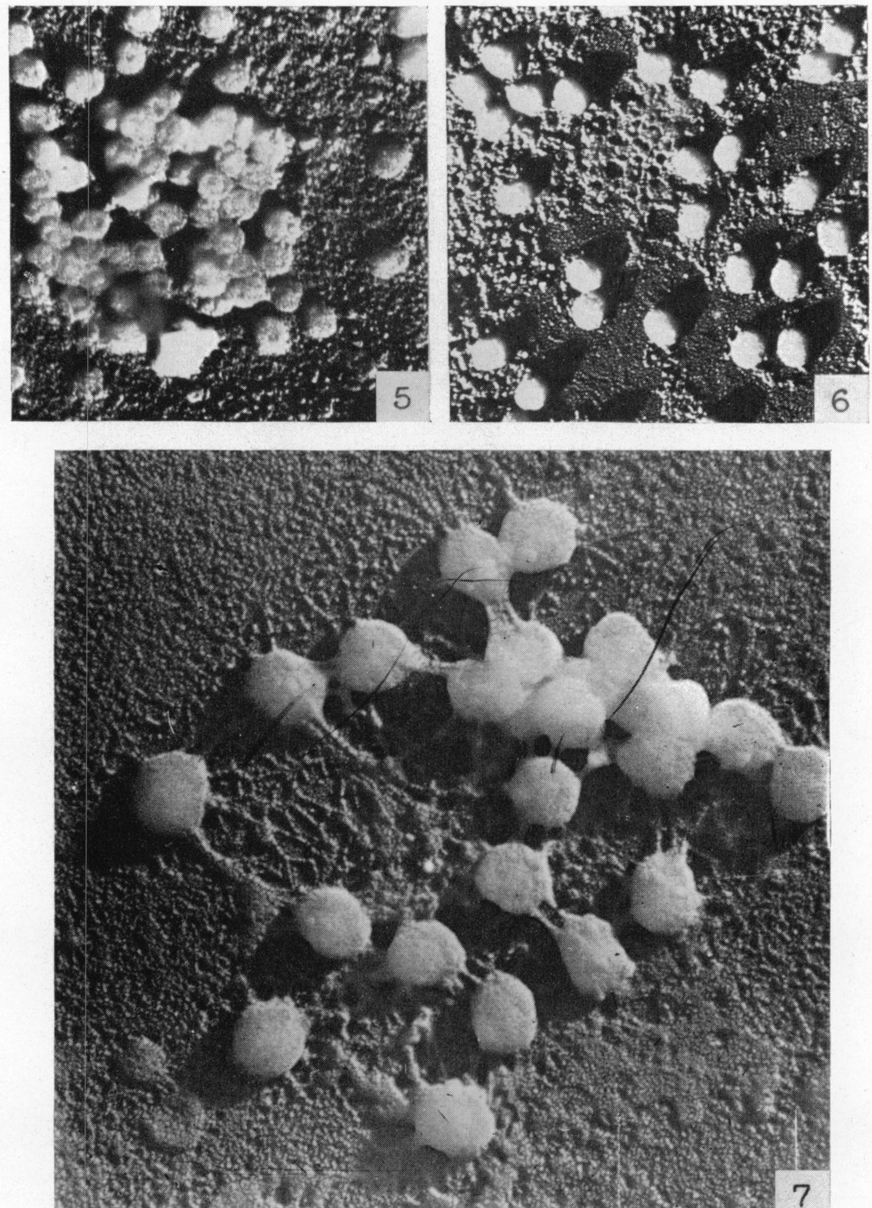


FIGS. 1–4. FOWL POX VIRUS SHADOWED WITH GOLD

1. Shadowed with 20 mg of gold at the angle tangent 1.3/9.0. 2.4 × 12,400×.
2. Shadowed with 19 mg of gold at the angle tangent 2/10. 2.4 × 12,400×.
3. Shadowed with 20 mg of gold at the angle tangent 1.3/9.0. 2.4 × 8,620×.
4. Shadowed with 19 mg of gold at the angle tangent 2/10. 2.3 × 12,400×.

EXPERIMENTAL

It will be seen (figures 1 and 2) that the elementary bodies of fowl pox appear to be flattened in varying degree and are usually rectangular in shape, although rounded forms are not uncommonly observed (figures 5, 6, and 7). It would seem that the particles settle on the collodion film with a certain amount of collapse similar to the behavior of the elementary bodies of vaccinia (Green, Anderson, and Smadel, 1942; Sharp, Taylor, Hook, and Beard, 1946) and feline pneumonitis (Rake, Rake, Hamre, and Groupé, 1946). Assuming the elementary bodies of fowl pox to be short cylinders like those of vaccinia (Sharp, Taylor,



FIGS. 5–7. FOWL POX VIRUS SHADOWED WITH GOLD

5. Shadowed with 20 mg of gold at the angle tangent 1.3/9.0. 2.4 × 5,900×.
6. Shadowed with 20 mg of gold at the angle tangent 1.3/9.0. 2.4 × 5,900×.
7. Shadowed with 19 mg of gold at the angle tangent 2/10. 2.3×12,400×.

Hook, and Beard, 1946), the presence of rounded forms could be explained by the manner in which the particles settle on the collodion film.

It has been noted by Groupé, Oskay, and Rake (1946) that the particles of fowl pox and canary pox are usually attached to one another at their corners and that the characteristically flattened corner frequently observed on many particles (figure 1) probably results from the separation of two such particles. A typical group of elementary bodies characteristically joined at their corners by an interparticular bridge is presented in figure 2. These characteristics are also evident in the micrographs of vaccinia virus of other investigators (Green, Anderson, and Smadel, 1942; Sharp, Taylor, Hook, and Beard, 1946). The curious budlike structures previously described, which usually also appear at the

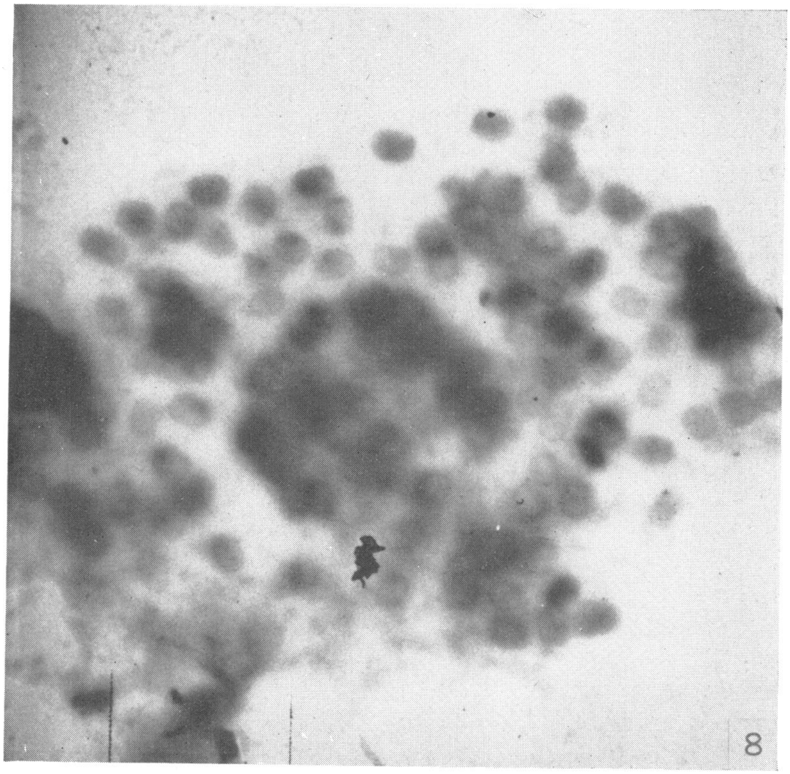


FIG. 8. FOWL POX VIRUS. 2.4 × 5,900×

corners of the particles, were not infrequently encountered in shadowed preparations. Two typical examples are shown in figures 3 and 4. It will be seen that such structures vary considerably in size and are definitely connected with their respective elementary bodies. It would seem reasonable to suppose that these structures result from unequal division of the elementary bodies. Similar unequal division has been observed with the bacteria (Mudd and Anderson, 1944).

Inasmuch as the large central area of condensation in the particles of vaccinia described by Green, Anderson, and Smadel (1942) appeared as a central rounded area of elevation when similar preparations were shadow-casted with gold by Sharp, Taylor, Hook, and Beard (1946), it was of particular interest to determine whether such central areas would be evident when the elementary bodies of fowl pox were similarly shadowed. Although not observed in all preparations studied, it will be seen in the micrograph presented in figure 5 that such central areas of elevation are readily discernible and probably represent intraparticular material (figure 8) which becomes apparent as a result of partial collapse of the particle. In addition to the central moundlike protrusions described above it will be seen from the pictorial data presented in figure 7 that numerous strands of taffylike material, apparently under tension, connect many particles with one

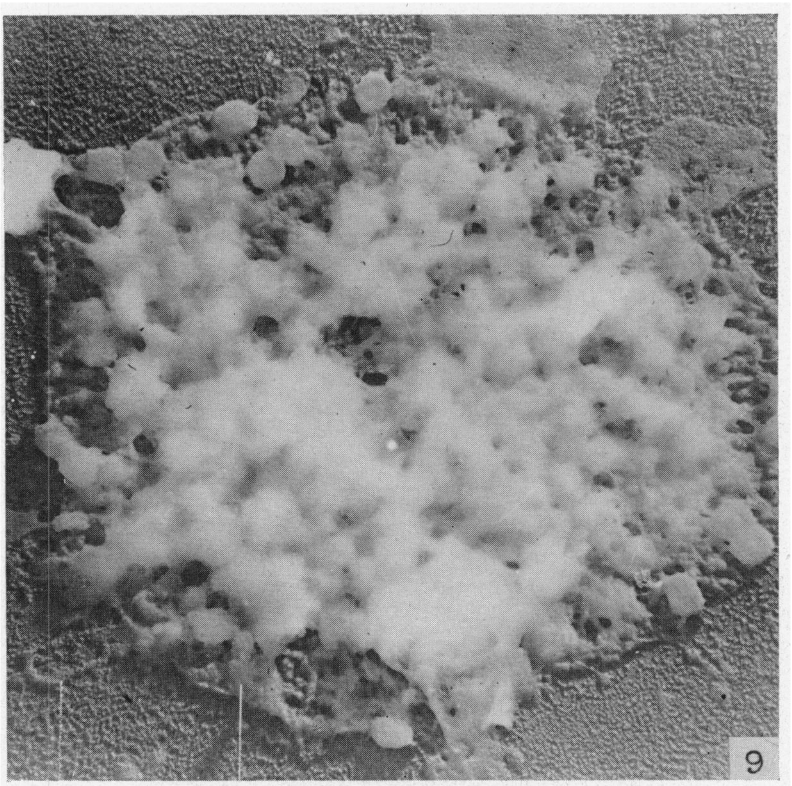


FIG. 9. FOWL POX VIRUS SHADOWED WITH 19 MG GOLD AT THE ANGLE TANGENT 2/10. 2.4 × 5,900×

another, and that these strands are easily distinguished from the interparticular bridge shown in figure 2. These findings are of interest in view of the observations of Sharp, Taylor, Hook, and Beard (1946) that the particles of vaccinia also appear to be coated with a sticky material which causes coherence of some of the particles.

The sharply circumscribed, piled-up mass of elementry bodies presented in figure 9 is interpreted as representing a Bollinger or inclusion body. It will be seen that the particles are imbedded in a matrix, and that many cross-hatching strands of sticky material are present. The elementary bodies are associated with a matrix or ground substance (figures 5, 6, and 9) only when localized masses of particles are encountered during the examination of a specimen. Whether this material represents only material from the host cell or is linked with a soluble antigen such as has been described by Shepard and Wyckoff (1946) for the rickettsiae remains to be determined.

DISCUSSION

Although the elementary bodies of fowl pox are somewhat larger than those of vaccinia, the similarities between the two are striking. The particles of both appear to be approximately rectangular in shape and possess large central mound-like elevations. In addition, both seem to be coated with a sticky substance and are frequently joined to one another at their corners. That classic examples of both avian and mammalian strains should so closely resemble one another morphologically adds still another link to the chain of evidence that has bound the viruses of the pox group together. The presence of forms suggesting unequal division of elementary bodies, together with the characteristics mentioned above, supports the suggestion of Green, Anderson, and Smadel (1942) that the pox viruses have morphological characteristics that approach those of the bacteria rather than those of the plant viruses.

ACKNOWLEDGMENT

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SUMMARY

The elementary bodies of fowl pox are approximately rectangular in shape, possess a central rounded area of elevation, and appear to be coated with a sticky material easily differentiated from interparticular bridges. The particles are most frequently joined to one another at their corners, and not infrequently forms are seen that suggest unequal division of the elementary bodies.

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