USE OF SERIAL SECTIONS TO DELINEATE THE STRUCTURE OF PORTHETRIA DISPAR VIRUS IN THE ELECTRON MICROSCOPE*‡

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Plates 4 to 6

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In a previous communication (1), viral particles of *Porthetria dispar* L. and *Bombyx mori* L. were shown to be contained within a pseudohexagonal, macromolecular, paracrystalline lattice composing the matrix of the polyhedral body. In order to clarify further the structure of *P. dispar* viral particles, 29 consecutive serial sections of 6 polyhedra have been examined. The purpose of the present communication is to describe representative serial sections of this insect virus and to indicate certain problems encountered in the interpretation of such sections.

Materials and Methods

Purified polyhedra of *P. dispar* (gipsy moth) virus were air-dried, fixed in buffered osmium tetroxide, dehydrated in ethyl alcohol, embedded in methacrylate, and sectioned, according to the methods previously described (1). The sections were examined in an RCA type EMU 2E electron microscope.

RESULTS

Figs. 1 to 8 illustrate consecutive serial sections of part of one polyhedron. Figs. 9 to 12 show consecutive sections of the same polyhedron at a different level. Bundles of 2 to 7 viral rods[1] enclosed by a dense, sharply defined limiting membrane are distributed at random within the polyhedral body. When sectioned approximately perpendicularly, *e.g. A* to *G*, the rods in the bundles can be followed through 6 consecutive sections. Note that bundle *E*, which is

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[1] In other sections isolated rods as well as occasional bundles of 8 rods have been encountered.

slightly oblique to the section, changes position with respect to *B* and *F*. Bundle *H* appears in the section preceding Fig. 1 and was thus cut 6 times. Bundles *I*, *J*, and *K* extend through 4 additional sections not reproduced and have been cut 7, 7, and 6 times, respectively. The rods measure 18 to 22 mμ in diameter, which is considerably less than that of viral rods liberated from polyhedra (2). This difference will be discussed in a forthcoming paper. The rods are separated from the limiting membrane by distances varying between 15 and 20 mμ. In cross-sections of the bundle the membrane tends to reflect the arrangement of contained rods, thus appearing triangular when enclosing 3 rods (bundle *H*, Fig. 1), square when enclosing 4 rods (bundle *A*, Fig. 3), or hexagonal when enclosing 7 rods (bundle *F*, Fig. 6). Occasionally a bundle is transected near one end at such a level that the rods do not appear within the plane of section, whereas the membrane is included, as shown by bundles *F* and *B*, Figs. 2 and 7, respectively. The random distribution of viral bundles suggests that they are not oriented with respect to the paracrystalline, molecular array composing the substance of the polyhedron. Although the material separating the rods has approximately the same density to the electron beam as the polyhedral substance, examination of consecutive sections at high magnification rarely reveals a molecular lattice within this material. When the molecular lattice does appear to penetrate the bundle, it can be explained by superimposition within the section.

Although many rods appear to be sectioned longitudinally, it can only be inferred that their whole length is contained within the plane of section when the entire limiting membrane has been cross-sectioned and appears sharply defined at all points on the periphery, as shown by bundle *L*, Fig. 12. Superimposition probably accounts for the apparent lack of separation of the rods within this bundle. They measure 300 mμ in length. Rods measured in other sections varied between 215 and 310 mμ in length, with an average of 280 mμ. The length of the rods bore no relation to the number contained within the bundle. Since the majority of rods, appearing to have been cut at right angles to their long axes, could be identified in 6 sections, the average thickness of the sections was calculated to be 47 mμ. This average was greater than had been calculated for serial sections of chorioallantoic membranes (3), but it should be emphasized that considerable variation occurs. For example, the rods are relatively dense compared to the polyhedral protein in Fig. 3, whereas in Fig. 4 less contrast is apparent, indicating that the latter section is thinner. Since 47 mμ at the magnification employed measures 3 mm., it can readily be seen that the larger bundles parallel to the plane of section would be cut more than once. Indeed, bundle *L* appears to be present in Fig. 11, the membrane being indistinct because it was sectioned eccentrically. Bundles *M*, *N*, and *O*, Figs. 9 to 12, can also be identified in 2 sections.

When a bundle lies oblique to the plane of section its membrane may appear to be incomplete, suggesting a stage of development as proposed by Xeros (4). However, because the electron density of the membrane is only slightly greater than that of the polyhedral protein it will not be visible if transected at an angle. One might assume, for example, on the basis of its appearance in Fig. 3, that bundle *P* was in the process of developing a membrane, whereas its appearance in Figs. 1 and 2 indicates that its long axis lies oblique to the plane of section and only part of its entire length appears at any one level. Bundles *Q* and *R*, Figs. 9 to 11, illustrate a similar effect.

Two major types of distortion have been repeatedly observed in thin sections of many tissues and are clearly illustrated in the accompanying figures; namely, variable compression and zones of differing thickness. Compression caused by impact of the microtome knife was commonly found in the thinnest sections, as illustrated by Fig. 4. In it bundle *F* has been compressed along an axis perpendicular to the knife edge,[2] with corresponding elongation in the opposite direction. That such compression may be variable even within one section is indicated by the distances separating different bundles. Thus bundles *C* and *D* are considerably closer in Fig. 4 than in Fig. 3, but no significant change in the distance separating bundles *B* and *E* is apparent. It is of particular interest that elongation of bundle *F* in the direction parallel to the knife edge is not reflected by a general elongation of the section in this dimension. The distances separating bundles *A* and *H*, or *F* and *G* remain unchanged.

Thick zones alternating with thin ones, best illustrated in Figs. 1, 3, and 5, traverse the section in a direction roughly parallel to the knife edge. The zones are of variable width and length, rarely persisting as a continuous band even within the small fields illustrated. Such differences in section thickness help to explain the appearance of certain viral rods. A thin zone, for example, crosses bundle *S*, Fig. 1, resulting in interruption of the continuity of the lower rod. The upper rod, presumably lying at a slightly different level within the section, remains intact. Bundle *T*, Figs. 2 to 6, changes position considerably with respect to bundles *A*, *B*, and *F*, indicating that its long axis is somewhat oblique to the plane of section. In Figs. 2, 3, and 5, where the rods lie within relatively thick zones, superimposition of structure causes them to appear indistinct. In thinner areas, Figs. 4 and 6, the rods traverse less distance before passing out of the plane of section, appearing therefore both discrete and more sharply defined. Bundle *U* reflects a similar situation in thick zones in Figs. 3 and 5, a thinner zone in Fig. 2, and in thin areas in Figs. 4 and 6. The distortion of the rods in bundles *D* and *H*, Fig. 4, is difficult to explain. Although some rods, as bundle *V*, Fig. 2, are curved, this cannot cause superimposition in one

[2] The direction of cutting can be determined by the orientation of knife scratches. None are shown in these figures.

section with clear demarcation of structure in other sections, both superficial and deep.

DISCUSSION

It is evident from the foregoing that consecutive serial sections provide two types of information which single sections cannot supply. First, they permit observation of structures at consecutive levels with the consequent opportunity to visualize their forms and spatial relationships in three dimensions. Second, by comparison with adjacent levels, they allow more accurate interpretation of the distortion inherent within any given section. Although average section thickness may be estimated, the wide variation from section to section and differences in thickness within individual sections make concise, quantitative reconstruction in three dimensions hazardous. The thick and thin zones traversing the sections appear to reflect periodic distortion of the embedding plastic and its contained tissue caused by impact of the microtome knife. Such distortion is not uncommon, but in many sections, particularly those of tissues less compact than the polyhedral bodies illustrated, differential sublimation and slight flow of the methacrylate occurring in the electron beam at the time of examination may efface the alternating zones of thickness (5).[3] The degree of compression produced by the microtome knife also varies, not only from section to section but within different areas of the same section. Although the compression is generally more marked in the thinner sections, there is no consistent correlation between the extent of such compression and the severity of periodic distortion. It may be noted that the viral bundles are compressed in such a manner that their diameters perpendicular to the knife edge vary in inverse proportion to their diameters parallel to the knife edge and that this distortion may not be reflected by consistent compression of the section as a whole.

SUMMARY

Consecutive serial sections of polyhedra obtained from gipsy moth larvae infected with *P. dispar* virus revealed bundles of viral rods scattered and oriented at random within the polyhedral body. Each bundle was entirely surrounded by a dense, sharply defined membrane. The rods measured 18 to 22 mμ in diameter and averaged 280 mμ in length. No spherical viral particles were encountered. The effects of variable compression and periodic distortion of the sections on the appearance of the virus are discussed.

REFERENCES

1. Morgan, C., Bergold, G. H., Moore, D. H., and Rose, H. M., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 187.

[3] These observations will be presented in a separate communication.

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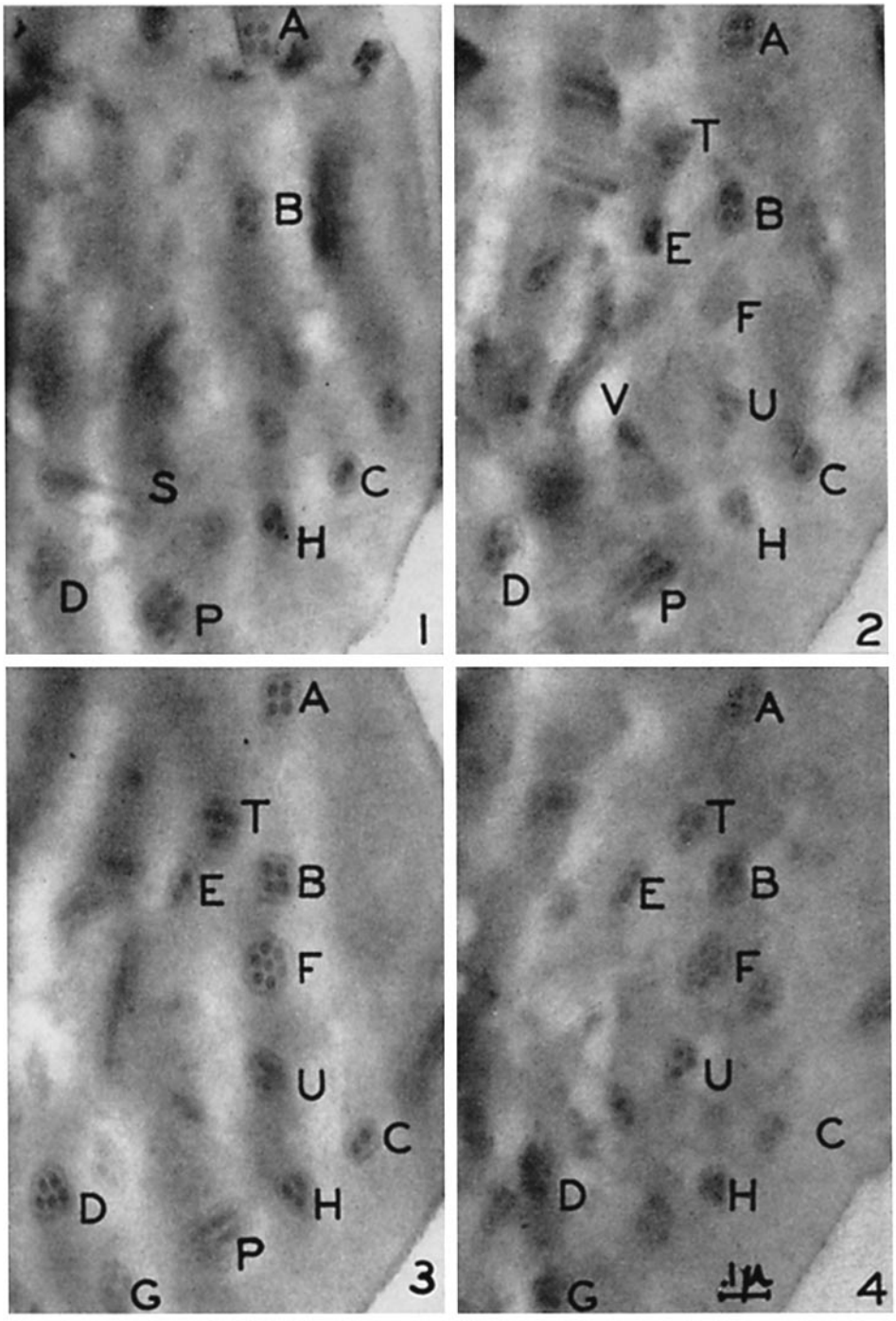
EXPLANATION OF PLATES

PLATE 4

The figures on this and the following plate illustrate consecutive serial sections of a polyhedron of *P. dispar* (gipsy moth) virus. Bundles of viral rods enclosed by a membrane are transected at different angles. Dust on the surface of the section partially obscures bundle *E* in Fig. 2.

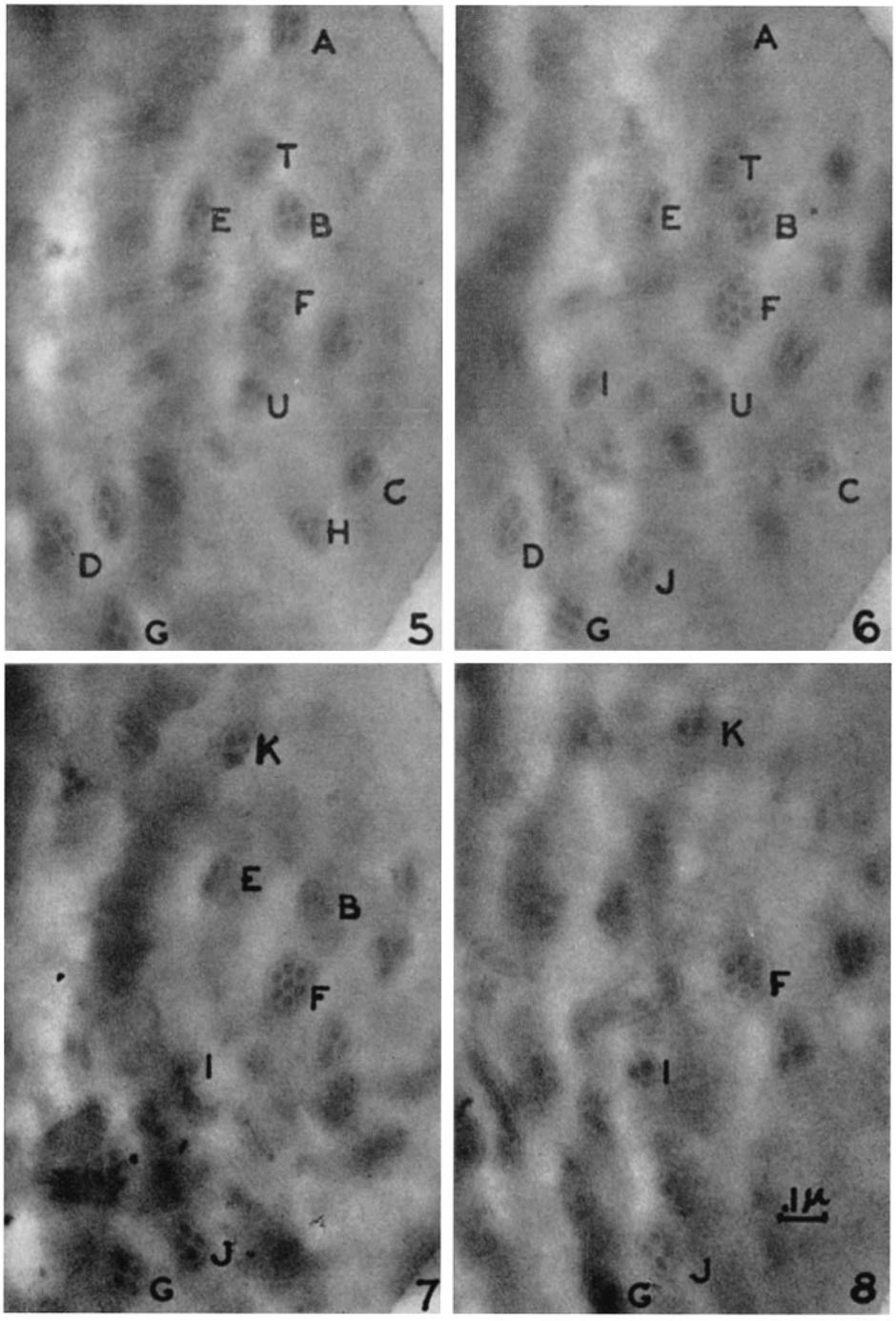
Magnification of all figures, 65,000.



(Morgan *et al.*: Serial sections of *Porthetria dispar* virus)

PLATE 5

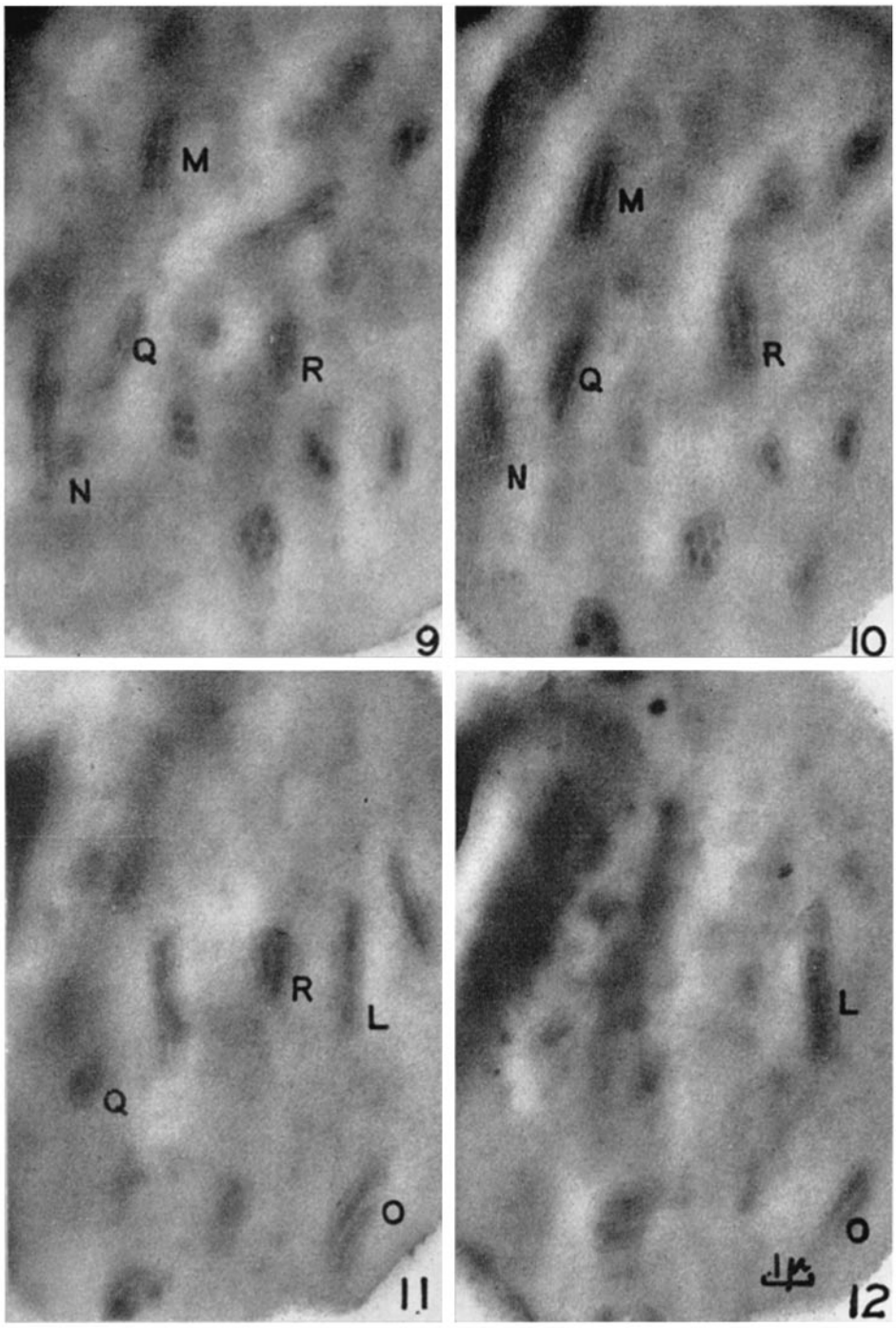
FIGS. 5 to 8. The border of the polyhedron is visible near the upper and lower right corners. It does not appear to possess a clearly defined limiting membrane. A few particles of dust are present in the lower left third of Fig. 7.



(Morgan *et al.*: Serial sections of *Porthetria dispar* virus)

PLATE 6

FIGS. 9 to 12. Consecutive serial sections of the same polyhedron at a different level. Bundle L is sectioned longitudinally. The incompleteness of the membrane surrounding bundle R appears to reflect the oblique angle at which it has been cut.



(Morgan *et al.*: Serial sections of *Porthetria dispar* virus)