THE MACROMOLECULAR PARACRYSTALLINE LATTICE OF INSECT VIRAL POLYHEDRAL BODIES DEMONSTRATED IN ULTRATHIN SECTIONS EXAMINED IN THE ELECTRON MICROSCOPE*,‡

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Plates 50 to 52

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Some insect viruses are characterized by the formation of polyhedral bodies within the nuclei of host cells. Verson (1) was the first to describe such bodies as crystals and Bolle (2) discovered that they were composed of protein. Within the polyhedra Komarek and Breindl (3) demonstrated by histochemical methods numerous small particles, which they believed to be the infectious viral agents. These were subsequently isolated (4) and shown in the electron microscope to be generally rod-shaped and to occur frequently in bundles contained within a limiting membrane (5, 6). Although previous attempts (7) to demonstrate the virus within sectioned polyhedra have been unsuccessful, because the sections were too thick to permit adequate penetration of the electron beam, a suggestion of a macromolecular lattice was observed on the cut surface of one polyhedron after shadowing (8). The purpose of the present communication is to illustrate and describe thin sections of polyhedra showing the viral particles enclosed within a macromolecular, paracrystalline lattice.

Materials and Methods

Porthetria dispar L. (gipsy moth) and *Bombyx mori* L. (silkworm) viruses were chosen for this investigation. Highly purified, air-dried polyhedra were suspended for 3 hours in 2 per cent osmium tetroxide (buffered at pH 7.4 with 0.014 M sodium acetate and sodium veronal), sedimented at 5,000 R.P.M., and resuspended for 2 hours in 5 ml. of new osmium tetroxide solution. After sedimentation they were suspended in 80 per cent, 95 per cent, and absolute ethyl alcohol, and a mixture of alcohol and methacrylate for 16, 3, 16, and 2 hours, respec-

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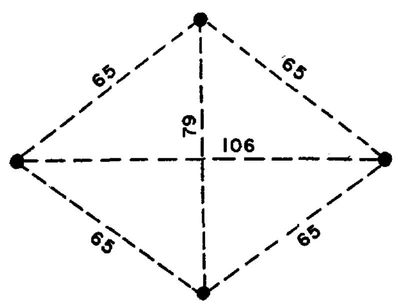
tively. The polyhedra were next suspended in liquid methacrylate for 16 hours at room temperature, sedimented, and resuspended in methacrylate, which was then polymerized at 46°C. The embedded polyhedra were sectioned on a Porter-Blum type microtome (9) equipped with a glass knife and floated onto a 30 per cent acetone–70 per cent water mixture. The sections were placed on formvar-coated copper grids and examined in an RCA type EMU 2E electron microscope. Micrographs were taken at initial magnifications of 13,400 and 18,900.

RESULTS

Fig. 1 illustrates a portion of a polyhedron of *P. dispar*. Scattered within the matrix are bundles of two to seven viral particles contained within a single membrane. Serial sections (to be published elsewhere) indicate that these particles are rods averaging 20 to 30 mμ in diameter and attaining lengths up to 330 mμ. While the majority in the field shown are cross-sectioned, several are cut obliquely. Study of many such sections revealed that the number of rods contained within one membrane varied from one to eight. Near the center of



TEXT-FIG. 1. Schematic diagram indicating spatial relationships of the macromolecules.

the field may be seen a type of bundle frequently encountered and composed of a central rod surrounded by six equally spaced rods. Irregularities in the density of the polyhedron probably represent variation in thickness of the section.

Previous investigations of polyhedra by x-ray diffraction have shown patterns which suggest a crystalline lattice (10, 11). That this lattice actually exists is demonstrated by sections of a polyhedron of *B. mori*, viewed at high magnification in Figs. 2 and 3. A similar macromolecular arrangement was also observed in sections of *P. dispar*.[1] Fig. 2 shows a nearly regular array of spherical densities, while in Fig. 3 dark bands traverse the field. The macromolecules illustrated in Fig. 2, and seen in other sections, measure from center to center 79 A on the vertical axis, 106 A on the horizontal axis, and 65 A on both oblique axes, as illustrated in Text-fig. 1. The diameter of the dense zone within the

[1] Since the paper was submitted a paracrystalline, macromolecular lattice has also been observed in capsules enclosing the rod-shaped virus of *Cacoecia murinana* (Hbn.). This virus is a typical representative of another group of insect viruses which is characterized by oblate ellipsoidal inclusion bodies of much smaller size than polyhedra.

macromolecules averages 30 A. In Fig. 3 the distance from center to center of each band measures 65 A, while the dark bands themselves average 30 A in thickness. Viral particles can be seen scattered through the field illustrated in Fig. 3. Although of similar dimensions, these viral particles differ from those seen in the polyhedra of *P. dispar* in that the bundles were less frequent, the most commonly observed form being a single rod enclosed by a limiting membrane. It should be noted that there is no disturbance of molecular array adjacent to the viral particles. Frequently the bands can be seen to enter the space separating a viral particle from its membrane. Whether superimposition within the section accounts for this phenomenon remains to be determined.

DISCUSSION

Chemical analyses together with sedimentation and diffusion measurements have revealed that the polyhedra of *P. dispar* and *B. mori* consist of 95 per cent protein with a molecular weight of 276,000 and 378,000, respectively (4). Study of the above figures, as well as of consecutive serial sections, suggests that the macromolecules are prolate ellipsoids, appearing discrete when cross-sectioned (as in Fig. 2) and, at certain orientations, forming bands by superimposition when longitudinally or obliquely sectioned (as in Fig. 3). Assuming the macromolecules to have elliptical cross-sections and to be closely packed in the orthorhombic unit cells, illustrated by Text-fig. 1, the major and minor axes can be calculated as 79 A and 52 A, respectively. On the basis of the above molecular weight, and assuming a density of 1.35, the axial length of such ellipsoidal macromolecules can be calculated to be 180 A for *B. mori*. These calculations do not take into account shrinkage which may have resulted from loss of bound water during preparation of the specimens.

Variable distortion of the macromolecular lattice has been observed in areas adjacent to zones of severe compression resulting from impact of the microtome knife. In many instances, however, the distortion appeared to be unrelated to compression and its character suggested that a disturbance of the macromolecular array had occurred before the sections were cut. It seemed likely that this disturbance caused variations in the angle at which groups of molecules were transected. Thus, in Fig. 2, the discontinuity of the pseudohexagonal lattice is believed to be an example of this phenomenon. What effects, if any, on macromolecular arrangement may be produced by fixation and dehydration, or by polymerization of the methacrylate, are at present unknown.

SUMMARY

Thin sections of polyhedra obtained from gipsy moth larvae infected with *P. dispar* virus and from silkworm larvae infected with *B. mori* virus revealed viral particles contained within a pseudohexagonal, macromolecular, paracrystalline lattice. The gipsy moth virus occurs in bundles of one to eight rods enclosed by a limiting membrane. The particles of the silkworm virus, although gen-

erally occurring singly, also possess a limiting membrane. The macromolecules appear to be dense, discrete particles when cross-sectioned and to form dense bands by superimposition when longitudinally or obliquely sectioned at certain angles. Calculations of macromolecular size have been made.

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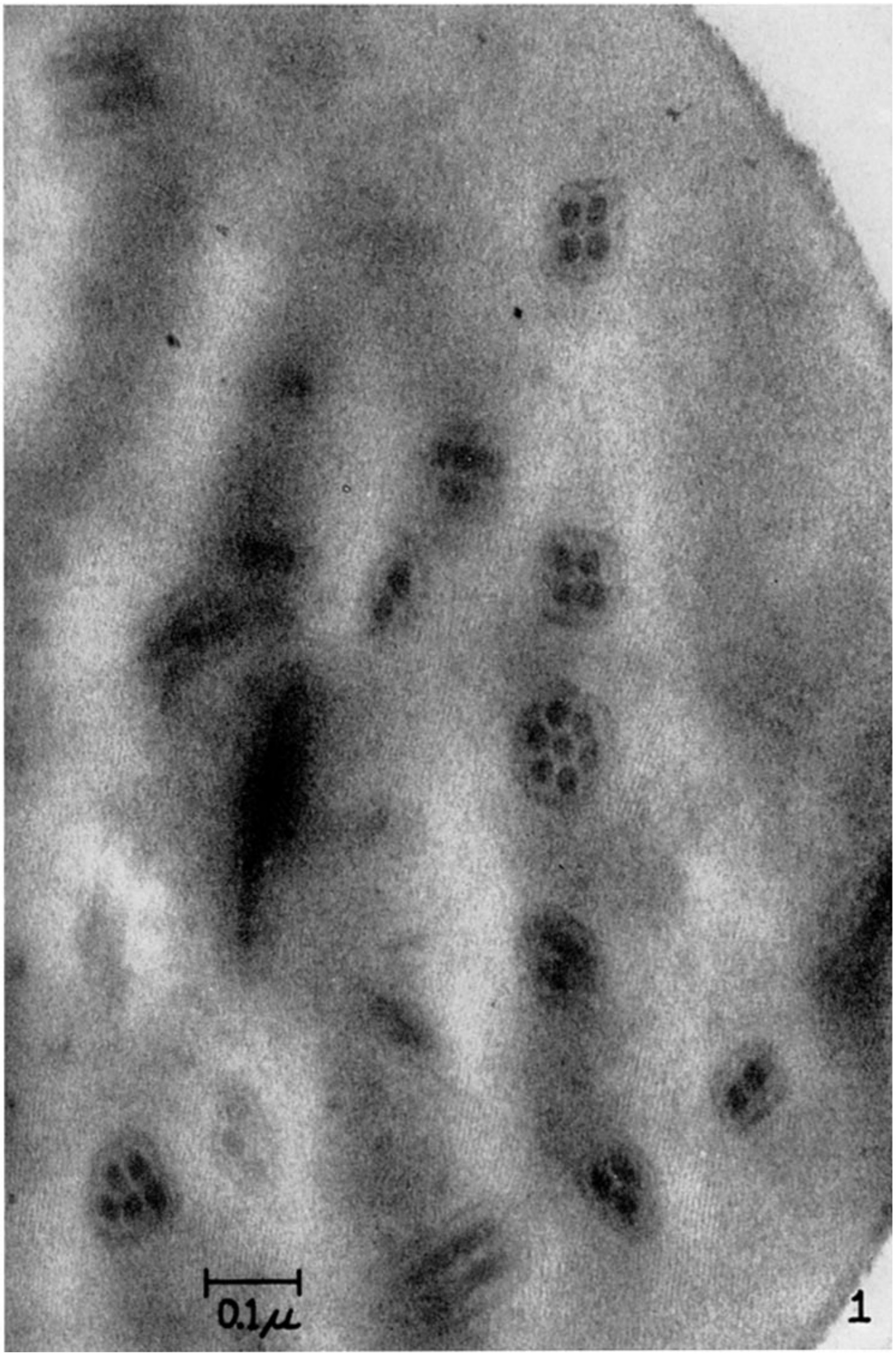
EXPLANATION OF PLATES

PLATE 50

FIG. 1. Portion of a polyhedron of *P. dispar*. Its border is visible at the upper and lower right-hand corners. Scattered within the sectioned matrix are characteristic bundles of viral particles enclosed by limiting membranes. The degree of magnification precludes adequate reproduction of the macromolecular array. × 130,000.



(Morgan *et al.*: Polyhedral bodies studied by electron microscope)

PLATE 51

FIG. 2. Portion of the matrix of a polyhedron of *B. mori*. The arrangement of the dense particles, particularly well illustrated in the upper third of the field, is consistent with pseudohexagonal close packing of macromolecules composing a paracrystalline lattice. The macromolecules are probably cross-sectioned in this plane. × 340,000.

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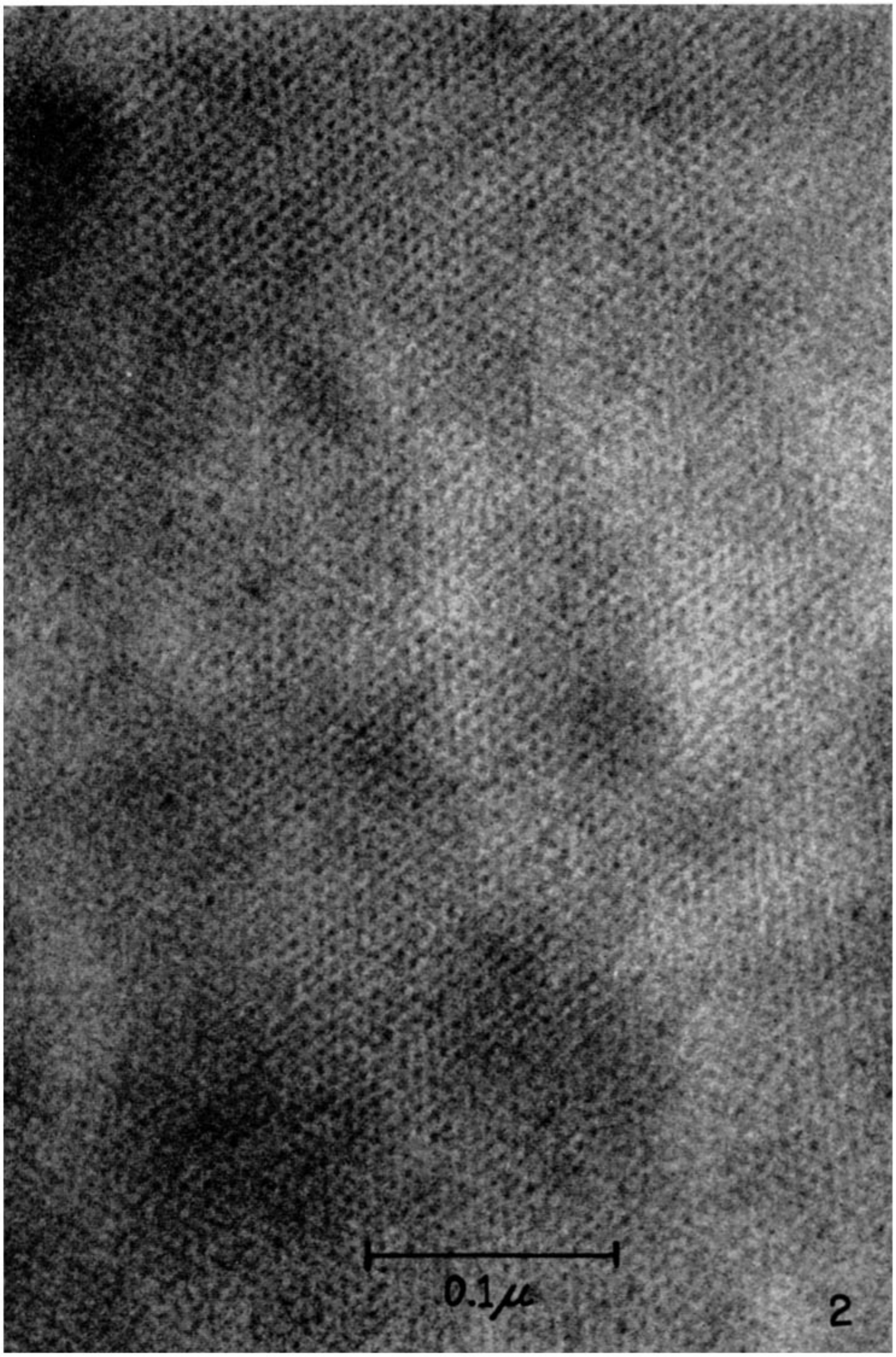
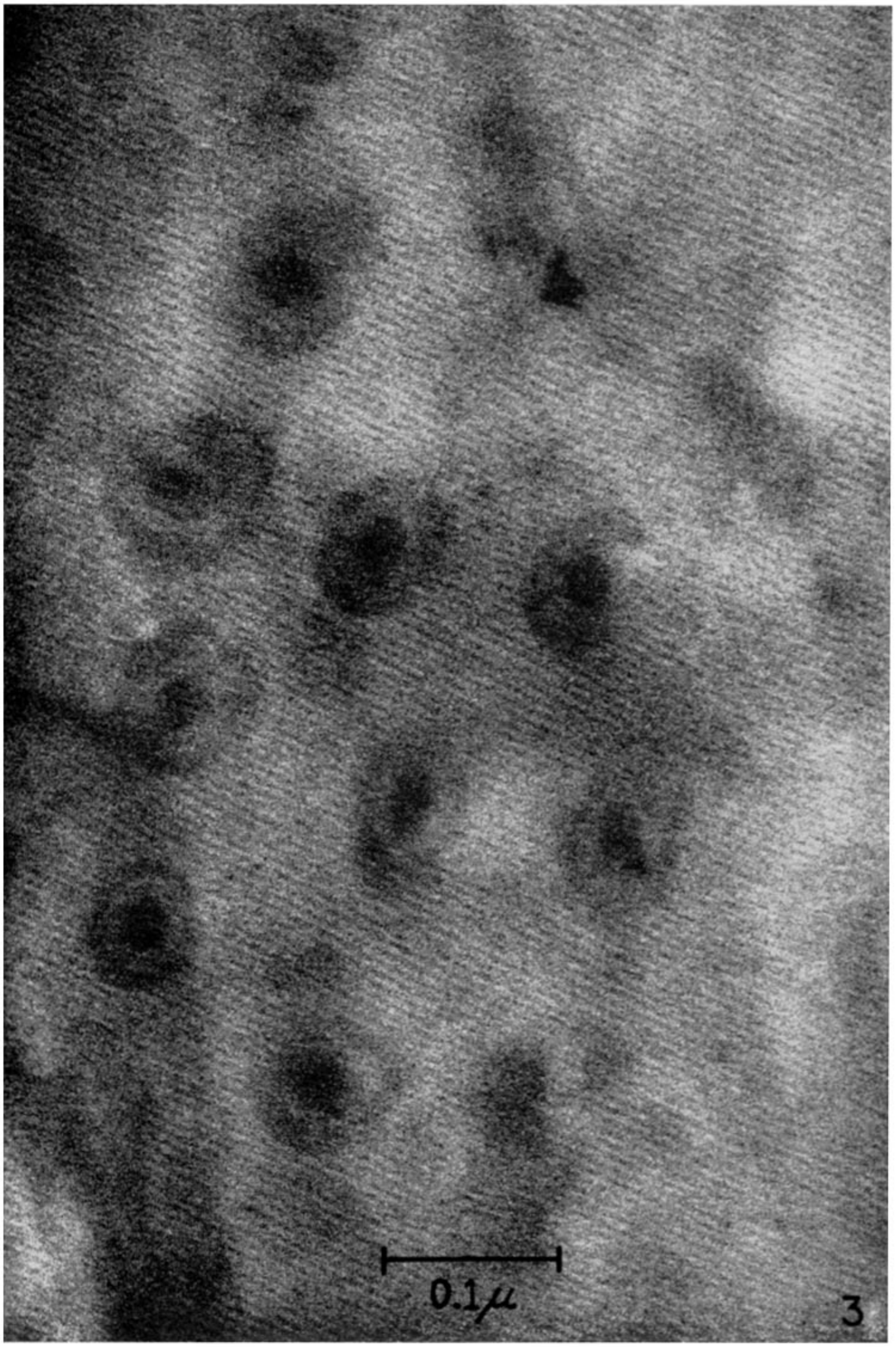


(Morgan *et al.*: Polyhedral bodies studied by electron microscope)

Plate 52

Fig. 3. Portion of a polyhedron of *B. mori*. Single viral particles possessing a limiting membrane are scattered through the field. The dense linear bands traversing the illustration diagonally are believed to represent superimposition of macromolecules sectioned obliquely or longitudinally. The spacing of these bands agrees exactly with the spaces separating the macromolecules along the short axes illustrated in Fig. 2. × 240,000.



(Morgan *et al.*: Polyhedral bodies studied by electron microscope)