THE STRUCTURE OF INSECT VIRUS PARTICLES

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Plates 61 to 63

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INTRODUCTION

By means of the new technique of cutting ultrathin sections for electron microscopy it is now possible to section the virus particles themselves and this allows, within limits, the examination of their fine structure. The viruses affecting insects are particularly suitable for a study of this kind, because of their apparently complex nature, their comparatively large size, and their peculiar relationship with the host cell.

The viruses which have been selected for this study are representative of each of the known insect virus types; these are, cytoplasmic polyhedral viruses which affect the gut cells of the larvae of *Arctia caja*, the garden tiger moth, and other species; a nuclear polyhedral virus which multiplies in the blood cells of a fly larva, *Tipula paludosa*; a recently discovered virus which multiplies in the fat body of the same larva without the formation of polyhedra, and a granulosis or capsular disease virus from the larva of *Melanchra persicariae* L., the dot moth.

Materials and Methods

For sectioning, all material was fixed in 2 per cent osmic acid, buffered at pH 7 with phosphate buffer, dehydrated with alcohol, and embedded in a mixture of 8 parts butyl methacrylate and 2 parts methyl methacrylate. To obtain sections of the virus particles themselves, freed from cellular material, these were spun down in osmic acid at a speed of 12,000 R.P.M. for 40 minutes and the pellet treated similarly to the other material. The sections were not shadowed with metal, since the methacrylate was not removed, and they were cut on a Cook and Perkins microtome, fitted with a glass knife. Figs. 6, 12, and 15 illustrate sections through pellets of virus. The unsectioned virus particles were shadowed with gold-palladium.

Some of the photographs were taken on a Siemens Elmiskop I microscope at 80 kv. and the others on a Metropolitan-Vickers EM 3 electron microscope at 75 kv. Those sections which were thin enough were photographed at a magnification of 40,000 times on the Siemens microscope and later enlarged optically. Both carbon and formvar-supporting films were used on the grids.

I. Cytoplasmic Polyhedral Viruses

Previous work (Smith and Wyckoff, 1950) has shown that this type of virus is usually spherical in shape and also suggests that the particles, as in the case of *Phlogophora meticulosa* L., are apparently composite (Smith and Xeros, 1954*a*).

The standard method of examining the nuclear polyhedral viruses on the electron microscope is to dissolve the polyhedral crystals with weak sodium carbonate and thus to liberate the rod-shaped virus particles contained within. With the cytoplasmic polyhedra, however, this method is unsatisfactory since the virus dissolves before the crystal does, and a partially dissolved framework full of round holes is left. The alternative, therefore, was to section the crystals themselves. This was not easy because they appear to be very hard and in consequence there was some compression of the crystal itself and frequent fracturing of the knife edge.

However, thin sections were eventually obtained and show very plainly, in the case of *Arctia caja*, the composite nature of the apparent virus particles. Since these cytoplasmic polyhedra are infectious and, because of this, must contain the virus, it seems reasonable to assume that these occluded particles are in fact virus particles. Each particle, although itself discrete, appears to consist of a rather loose complex of extremely minute bodies, measuring about 10 to 12 mμ. It is not easy to be sure of the exact number of subparticles but there seem to be five or six (Figs. 1 and 2).

For comparison with the virus from *A. caja*, three other cytoplasmic polyhedral viruses were examined. These are from *Phalera bucephala*, the buff-tip moth (Fig. 3), *Drepana lacertinaria*, the scalloped hook tip (Fig. 5), and *Papilio machaon*, the swallow tail butterfly (Fig. 4).

From these it would appear that there is a difference in size among the various cytoplasmic viruses. In *D. lacertinaria* and *P. machaon* the virus particles are very small and it is not possible, under the magnification available, to be sure if they are composite. In *P. bucephala* the particles seem to be larger, and careful examination of Fig. 3 will show the presence of a surrounding membrane.

The structure of the cytoplasmic polyhedra themselves is interesting and appears to differ from the close packing in a paracrystalline lattice as shown by Morgan *et al.* (1955) for the nuclear type of polyhedra. This may be partly due to lower resolution in the present case, but the substance of the crystals seems to be inhomogeneous and there may be hollows and pits inside the crystal. These appearances may, however, be the result of compression by the knife during the process of section cutting, since the crystals are very hard. When some cytoplasmic polyhedra are treated with sodium carbonate they break up into horseshoe-shaped plates and this might account for the inhomogeneity shown in sections.

II. Nuclear Polyhedral Virus from Tipula paludosa

This virus develops in the nuclei of the blood cells and the "polyhedra," which actually are crescent-shaped, possess remarkable elastic properties (Smith and Xeros, 1954 *b*). It is not possible to dissolve these polyhedra by means of alkali without also destroying the occluded virus so they were sectioned in a similar manner to the cytoplasmic polyhedra. The virus causes a

very great increase in the number of blood cells so that the larva develops a kind of leukemia.

Like all the other nuclear viruses so far investigated this virus is rod-shaped and the peculiar behaviour of the virus in the cell nucleus with its developing vesicles has already been described (Smith, 1955 *b*). It is dealt with shortly, therefore, for comparison with the cytoplasmic virus.

Sections through the virus particles (Fig. 6) show that each is enclosed in a membrane or capsule and since this is some distance away from the substance of the rod it is probable that a second intimate membrane surrounds the actual virus material itself although this is not visible (Fig. 6). The exact relationship between the membrane or capsule surrounding the virus rods shown in Fig. 6, which are sectioned in a pellet, and the vesicles present round the rods in the polyhedra (Fig. 7), is not yet clear. Sections through some polyhedra show the occluded virus rods in large numbers with some apparent orientation inside the crystal (Fig. 13).

III. A Spherical Virus from Tipula paludosa, without Polyhedral Inclusions

This virus which has been recently discovered has been briefly described by Xeros (1954) and Smith (1955 *a*).

The site of multiplication of the virus is the fat body and the amount of virus produced in the body of the diseased larva is very large.

When the virus is dried down from a suspension and metal-shadowed and studied in the electron microscope it appears as a sheet of hexagonal particles closely packed in a semicrystalline arrangement. However, sections of tissue containing the virus *in situ*, without removal of the methacrylate (Fig. 8) show the virus particles to be near spherical in shape, so it is probable that the hexagonal appearance may be an artifact. They measure about 100 mμ in diameter.

In Fig. 8 may be seen electron micrographs of sections of the virus particles, *in situ* in the tissue, at a magnification of 40,000 times. Here again a very definite membrane is visible with a darker centre or "nucleus." In some sections it is possible to make out the very fine granular nature of the central mass. It is interesting to find that some virus particles consist apparently of a thin membrane only in which no contents are visible (Fig. 12).

Although no polyhedral crystals are associated with this virus, there are formed apparent "inclusion bodies" (Figs. 9 and 11) which bear a superficial resemblance to those occurring in some virus diseases of vertebrates. These inclusion bodies vary in size from masses of many thousands of virus particles to very small groups of a dozen or so. Fig. 11 is a very thin section through one of these small virus groups, photographed at 40,000 times on the microscope. The inclusion is surrounded by a thin membrane which appears to be quadruplicate. Note the outer membrane and central mass in some of the virus particles.

Another method, besides that of sectioning, for investigating the structure

of the virus particle was used. This might be likened to a microdissection technique and consists in subjecting the purified virus to different concentrations of sodium carbonate. Virus particles at a stage of disintegration are shown in Fig. 10. This is a negative print of a dried-down preparation. Here again, the surrounding membrane is visible whilst the contents in process of dissolution can be seen.

IV. Granulosis Virus from Melanchra persicariae L.

In the granulosis diseases there are no polyhedral crystals but the rod-shaped virus particles are enclosed in a "granule" or capsule, usually one rod, but sometimes two, per capsule. When treated with weak alkali the capsules dissolve in a similar manner to the nuclear polyhedra and the virus is liberated. In Fig. 14 are shown some capsules treated in this way. It will be noticed that the partially dissolved outer capsule has collapsed, revealing an inner capsule or membrane containing the virus rod, which is visible only in sections.

Very thin sections of the capsules (Fig. 15) demonstrate clearly the triple nature of the granule. The cut edges of the outer capsule stand out widely separated from the virus rod with the enclosing inner capsule. Some rods are cut transversely and some longitudinally. There is a considerable discrepancy between the sizes of the inner capsules shown in Figs. 14 and 15, the capsule being apparently much larger in Fig. 14. This is almost certainly due to the sodium carbonate treatment which, by partially dissolving the inner capsule, causes it to spread.

DISCUSSION

An outstanding feature of the viruses affecting insects is the occlusion of the virus particle itself in a membrane or envelope of some kind, and in the case of the polyhedral viruses, of course, there is the additional envelopment of the virus in the crystal.

In the cytoplasmic polyhedral viruses, exemplified here in *Arctia caja* and other species, it is not certain whether the minute component particles of the virus are capable of inducing disease. These particles are, however, thought to occur separately in the cytoplasm.

In the nuclear polyhedrosis of *Tipula paludosa* the virus rod is enclosed in an outer membrane. Subsequently, a large thin walled vesicle apparently develops round the individual rods, and this eventually becomes an enclosing capsule within the polyhedral crystal (Smith, 1955 *b*).

In the granuloses or capsular diseases there is no enclosing crystal, the place of this being taken by the granule which gives the disease its name. Thin sections through these granules show that the same phenomenon of enveloping membranes occurs.

Even in the virus diseases without the formation of polyhedra or granules,

like the second virus disease described from *Tipula paludosa*, there is still the tendency for the development of inclusion bodies and membranes. These inclusion bodies vary greatly in size and may consist of large or small masses of virus particles. A peculiarity in this case is the development of surrounding membranes which appear to be triplicate or even quadruplicate. From the study of a large number of sections of the virus particles of this disease it has been observed that a percentage of the particles appear to be empty. These particles are much less electron-dense in the centre and seem to be genuinely empty shells and not just thin sections through the outer membrane only. In Fig. 12 some of these particles apparently cut through the centre are clearly shown. If confirmed this is one of the most interesting points arising from this study and suggests that the empty particles may possibly be similar to the "top component" particles of the turnip yellow mosaic virus, which are hollow, non-infectious, and contain no nucleic acid (Markham and Smith, 1949; Markham, 1951; Schmidt, Kaesberg, and Beeman, 1954).

SUMMARY

Thin sections have been cut of the virus particles from four types of insect virus diseases: cytoplasmic polyhedroses of lepidopterous larvae, a nuclear polyhedrosis of *Tipula paludosa* (Diptera), a granulosis from *Melanchra persicariae* (Lepidoptera), and a new virus disease without polyhedra from *T. paludosa*.

The cytoplasmic polyhedral viruses are thought to have composite particles in some cases. The shape and enveloping membranes of the different virus particles are compared. In the new virus disease of *T. paludosa* some of the virus particles appear to be empty; inclusion bodies surrounded by complicated membranes are also demonstrated.

Figures 1, 2, 6, 8, 11, and 13 are from electron micrographs taken by Mr. R. W. Horne on the Siemens Microscope in the Cavendish Laboratory. The others are from electron micrographs taken by Miss S. Vernon-Smith on the Metropolitan-Vickers microscope in the laboratory of the Virus Research Unit.

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EXPLANATION OF PLATES

PLATE 61

FIG. 1. Part of a section through a cytoplasmic polyhedral crystal from the larva of *Arctia caja*, the garden tiger moth; note the regular spacing of the virus particles which are slightly compressed by the sectioning process. × 50,000.

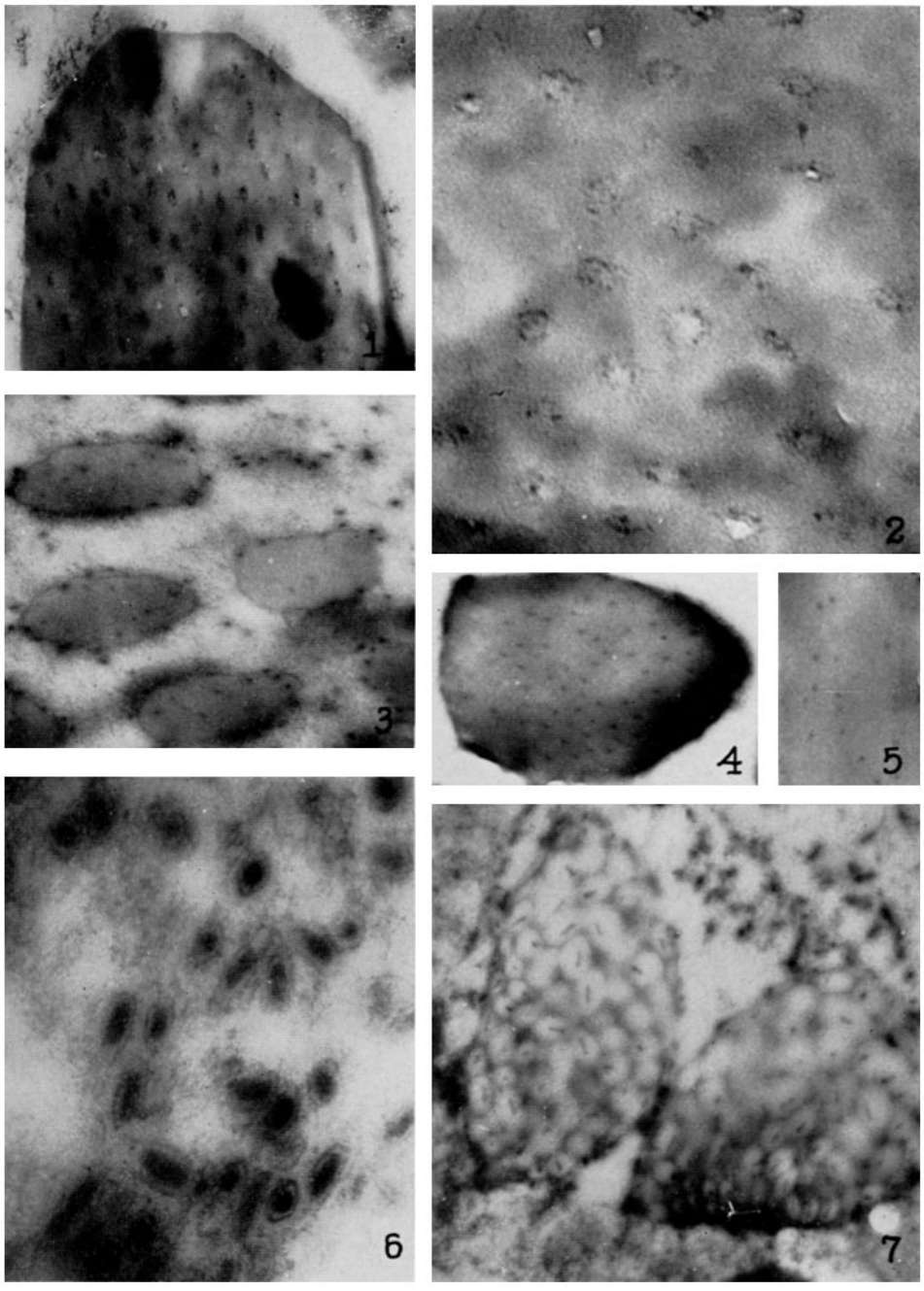
FIG. 2. Part of a similar section to that in Fig. 1, but at a much higher magnification; note the composite nature of the virus particles. × 135,000.

FIG. 3. Sections through cytoplasmic polyhedra from the larva of *Phalera bucephala*, the buff-tip moth; the virus particles, which appear to be fewer and smaller than those in the foregoing, are scattered also in the cytoplasm. There appears to be a membrane round the particles. × 32,500.

FIGS. 4 and 5. Similar sections through a cytoplasmic polyhedral crystal from the larvae of *Papilio machaon*, the swallow-tail butterfly, and *Drepana lacertinaria*, the scalloped hook tip moth, respectively. × 27,000 and × 40,000.

FIG. 6. Thin sections through a pellet of the rod-shaped virus particles from the polyhedrosis of the larva of *Tipula paludosa*; note the enclosing membrane. × 71,400.

FIG. 7. Sections through two polyhedra in a blood cell of the larva of *T. paludosa*; note the large thin-walled "vesicle" round each virus particle. × 21,400.



(Smith: Structure of insect virus particles)

PLATE 62

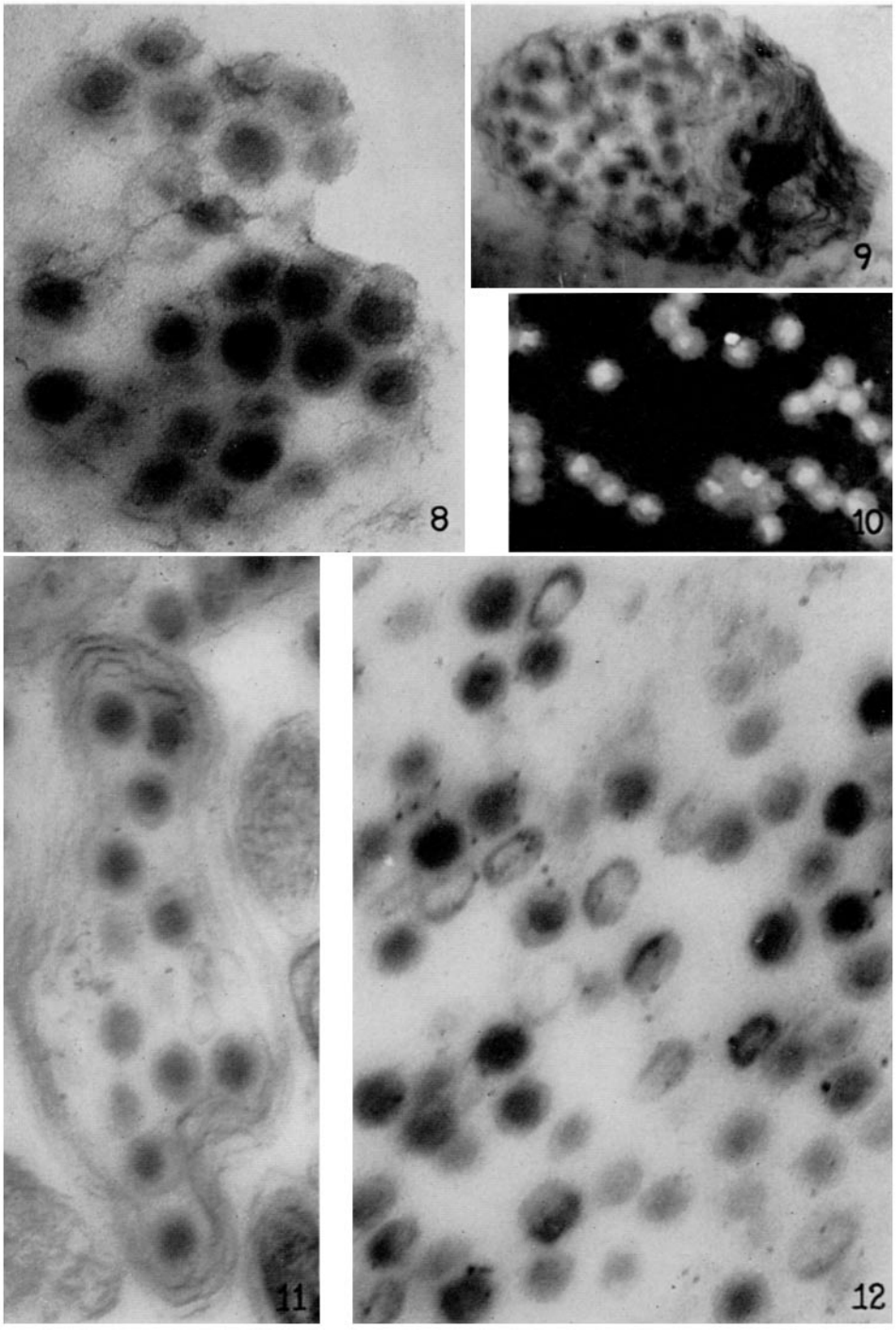
FIG. 8. Sections of virus particles of a virus without polyhedra from the larva of *Tipula paludosa*; note the outer membrane and the inner dark centre; the section is through diseased fat body. Photographed on the Siemens microscope at 40,000 and enlarged to × 78,500.

FIG. 9. Section of an "inclusion body" in the same disease as in Fig. 8; note the apparent multiple membranes enclosing the inclusion body. × 41,400.

FIG. 10. Virus particles partially dissolved in weak sodium carbonate, showing the outer membrane and the denser centre. Negative print of a dried-down preparation. Shadowed with palladium-gold. × 34,250.

FIG. 11. Thin section through a very small inclusion body showing one layer of virus particles and the multiple membranes surrounding the inclusion; photographed at 40,000 times on the Siemens microscope and enlarged to × 78,500.

FIG. 12. Section through a pellet of virus particles showing a number of apparently empty particles; photographed at 40,000 on the Siemens microscope and enlarged to × 71,400.



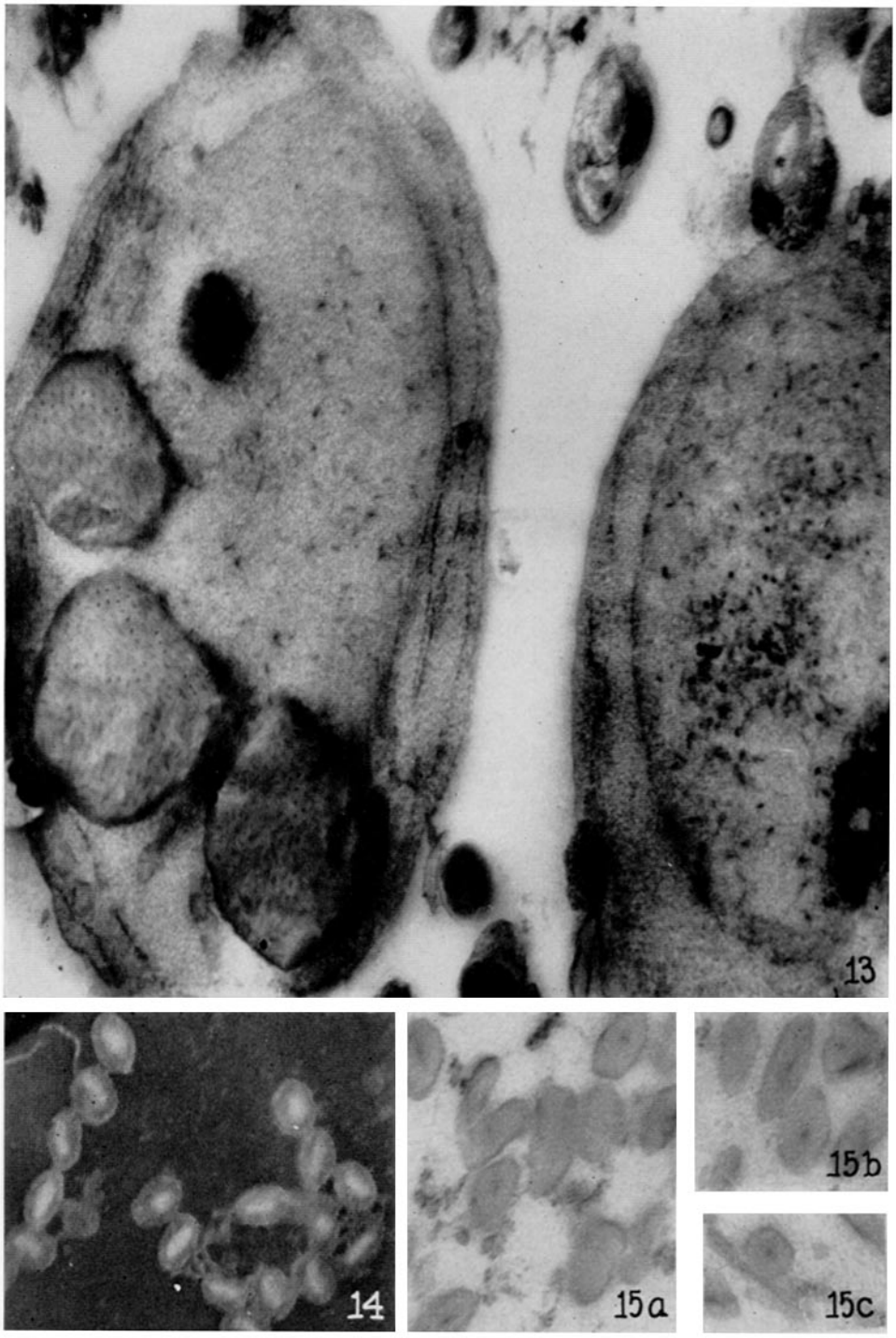
(Smith: Structure of insect virus particles)

PLATE 63

FIG. 13. Sections of two blood cells from the larva of *Tipula paludosa* infected with its polyhedral virus; the cell on the right is in an early stage of the disease before the development of the polyhedra. Note the numerous virus particles in the enlarged nucleus. The cell on the left is in a more advanced stage; note how the virus particles have disappeared, having been concentrated in the three polyhedra. Note the large numbers of particles inside the polyhedra with apparently some orientation; most of the particles are cut longitudinally, but a few are cut transversely; note also the apparent double membrane surrounding the nucleus. × 20,000.

FIG. 14. "Granules" from a granulosis disease of the caterpillar *Melanchra persicariae* L., the dot moth, treated with sodium carbonate to show the inner capsule. × 19,000.

FIG. 15 *a*, *b*, and *c*. Sections of the granules shown in Fig. 14, spun down into a pellet; note the outer capsule, the inner capsule, and the virus rod inside that. × 40,000.



(Smith: Structure of insect virus particles)