A POLYHEDRAL VIRUS DISEASE OF A PASTURE CATERPILLAR, *PTEROLOCERA AMPLICORNIS* WALKER (ANTHELIDAE)

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Summary

A new virus of the genus *Borrelina*, causing a polyhedral disease in larvae of the anthelid moth *Pterolocera amplicornis*, is described. The polyhedral bodies are small and unusually resistant to alkaline solution. Electron micrographs reveal the presence of rods, spheres, and rods projecting from spheres in alkali-digested polyhedra. The relations between these bodies are not clear.

I. Introduction

Recent reviews (Steinhaus 1949*a*, 1949*b*; Smith and Wyckoff 1951) have shown that viruses of the genus *Borrelina* Paillot, causing polyhedroses in at least 17 families of larval Lepidoptera, are widespread in the northern hemisphere. Examination of a number of larval Lepidoptera from laboratory cultures and field collections in Australia during the last 3 years has failed until now to reveal a polyhedral disease. A polyhedrosis in field-collected larvae of the anthelid *Pterolocera amplicornis* Walker has now been discovered in the vicinity of Canberra. This paper presents some details of the host, the disease, and the pathogen.

II. The Insect Host

The family Anthelidae is composed of medium to large moths, and is confined to the Australian region, including New Guinea and the Celebes. The genus *Pterolocera* Walker contains only two described species. The type species, *P. amplicornis*, which has a wingless female, occurs from southern Queensland to Tasmania and in South and south-western Australia, and is a pest of native grass pastures. The species as at present constituted is extremely variable. *P. isogama* Turner has a winged female and occurs in south-western Australia. Its larvae feed on *Acacia acuminata* Benth. The desirability of including both species in the same genus is open to doubt.

Although of some economic importance (see Evans 1943), nothing of the life history of *P. amplicornis* has been published. At Canberra it completes a single generation each year. Adults, which do not generally live more than 24 hr, have been take in mercury vapour light traps from the end of February until early April, with the peak in the latter half of March (Fig. 1). Eggs laid in autumn hatch in about 7 wk at laboratory temperatures, and the larvae

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develop slowly during the winter. Larval growth is accelerated in early spring and mature larvae (Plate 1, Fig. 1) occur during October and November. They are hairy caterpillars, just over 2 in. long, and pupate in the soil in shallow tunnels in which they spin a silken cocoon. The prepupal and pupal periods occupy about 16 wk.

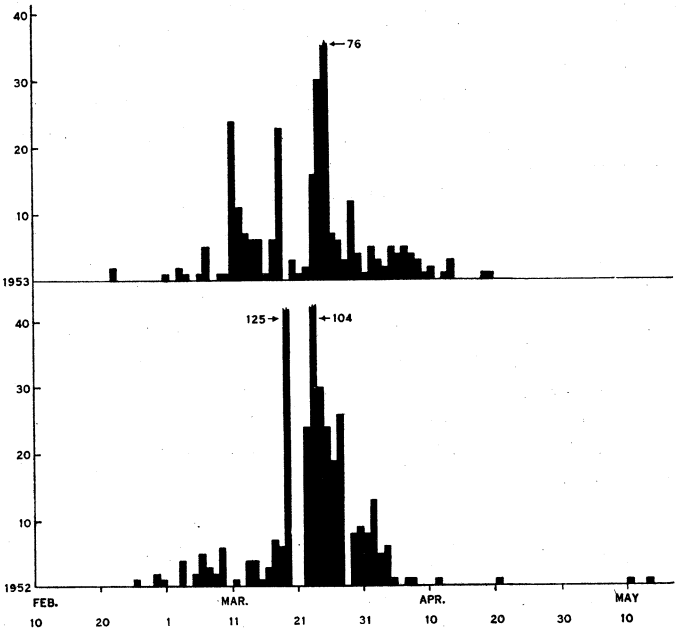

Fig. 1.—*Pterolocera amplicornis* moths collected in mercury vapour light trap, Canberra, 1952-53.

III. The Disease

Larvae infected with polyhedrosis were first noticed early in October and occasional infected specimens were found until the majority of larvae had pupated. Less than 1 per cent. of larvae collected in the field were infected.

When larvae were fed on grasses sprayed with a suspension of polyhedra, symptoms appeared in some, but not all, larvae in about 4 days at laboratory temperatures. The first symptom is a loss, starting from the posterior end of the body, of the typical silvery appearance, owing to the deposition of a dark pigment in the cortex of the long unpigmented hairs on the dorsal surface. This darkening is continued anteriorly until the entire body is darker than normal (Plate 1, Fig. 1). A similar loss of the silvery appearance of the hairs occurs when these are immersed in alcohol. At the same time the infected insects become sluggish, flaccid, and do not curl up when handled as do the uninfected specimens. The cuticle of the moribund larva is fragile and when punctured permits the escape of a thick, creamy fluid containing very large numbers of polyhedra and bacteria. Insects in this condition were found in the field on the low grass upon which they feed or on the soil surface.

The histopathology is similar to many polyhedroses previously described. The polyhedra are observed first in the nuclei of fat body, hypodermis, tracheal epithelium, and haemocytes. Eventually, all tissues are attacked, with the exception of gut epithelia, malpighian tubules, silk glands, nervous tissue, and muscular tissue. As the number of polyhedra increases in the nucleus, the residual chromatin is clumped in the centre, and the nucleus increases greatly in size (Plate 1, Figs. 2 and 3) until it eventually ruptures, liberating the polyhedra into the haemocoele. Smears of polyhedra from *Pterolocera* are uncoloured by Feulgen's stain and by methyl green. After treatment with alkali the polyhedra stain weakly green with the Unna-Pappenheim stain. After treatment with Schweitzer's reagent they stain with either methyl green or pyronin when these are used separately.

It has not been possible to test the infectivity of the disease to a variety of other Lepidoptera. However, mature larvae of a noctuid (*Persectania ewingii* (Westw.)) were in culture at the same time as infected larvae of *P. amplicornis* occurred in the field. Attempts to infect *P. ewingii* by feeding them grass sprayed with the polyhedra were unsuccessful. In view of the observation of Smith and Xeros (1952), that cross infection may occur more readily between viruses from insects that are not closely related than between those from closely related hosts, an attempt was made to infect the larvae of *Cacoecia australana* (Lewin) (Tortricidae) by feeding foliage sprayed with a heavy suspension of polyhedra. No infections resulted.

IV. The Polyhedral Body and the Virus

Infection of larvae is readily diagnosed under the dark-field microscope (Plate 1, Fig. 5). The polyhedral bodies are easily distinguished (Plate 1, Fig. 4) from the less highly refractive bacteria which are present in large numbers during the later stages of the disease. The polyhedral bodies are smaller than any hitherto described, so that accurate measurements of their dimensions and determination of their shape is possible only by means of electron micrographs (Plate 2, Figs. 6, 7, and 8). The polyhedra are irregular in shape and their diameters range from 0.7 to 1.3 μ with a mean of about 1.0 μ. They scatter electrons so effectively that no internal details can be observed with a 50 kV electron microscope.

Early attempts to free the virus from the polyhedra proved the latter to be capable of resisting attack by 0.01M NaOH solution (pH 12) for 2 hr at room temperature (Plate 2, Figs. 7 and 8). Plate 3, Figure 9, shows polyhedra treated for 60 min at 56°C with 4 per cent. Na_2CO_3 ($\cong$ 0.38M, pH $\cong$ 11.0) while standing on a collodion supporting membrane. It is apparent that the dissolution of the nucleoprotein component is only partially complete. Plate 3, Figure 11, and Plate 4 show polyhedra from which identical treatment removed almost all the nucleoprotein. The polyhedra of Plate 3, Figures 10 and 12, were treated by suspension in 4 per cent. Na_2CO_3 for 180 min at 50°C. The length of the shadow cast by a completely empty membrane (Plate 4, Fig. 14) suggests that the membrane is only 30 Å thick. This must be regarded as a lower limit as it is possible that the membrane is partially buried in a layer of breakdown

products deposited on the collodion supporting film. Some membranes exhibit ridges (Plate 4, Fig. 16), not previously recorded, that probably indicate the edges of the crystals.

The dissolution of the nucleoprotein of the polyhedra permits the escape of both thin rods (3500 Å long by 1500 Å wide) and thick rods (3200 Å long by 1700 Å wide) and of spheres (1400 Å in diameter) (Plate 3, Figs. 10-12; Plate 4, Figs. 13-16). Both the rods and the spheres appear to be flattened, which may be due to collapse on drying and to their being partially embedded in a contaminating layer of breakdown products deposited on the supporting membrane. Little light is shed by the micrographs on the relationships between these three types of bodies. Examples are found of thin rods projecting from spheres (Plate 4, Fig. 14) but none of the micrographs suggests that the thick rods consist of bundles of thin rods. The number of rods and spheres contained in each polyhedron is also obscure. Plate 3, Figure 10, indicates that some six or more thick rods appear in each polyhedron, and Plate 4, Figures 13 and 15, suggests that the polyhedra each contain numbers of thin rods. These two micrographs, however, are atypical and most fields contained few thin rods. A few thin rods and flattened spheres are visible in untreated specimens (Plate 2, Fig. 6) and also in preparations subjected to 0.01M NaOH for 120 min at room temperature, which is apparently insufficient to dissolve the polyhedra (Plate 2, Figs. 7 and 8).

On the basis of the above description it is evident that the virus has not been previously described and the name *Borrelina anthelus* sp. nov. is therefore proposed for it.

V. Discussion

The virus causing the polyhedrosis described in this paper is the first of the genus to be recognized from Australia. It is also the first recorded in the family Anthelidae, although many polyhedroses of other noctuids have been reported.

The relationships of species of the genus *Borrelina* have not been thoroughly examined and the criteria for interspecific comparisons are few. *B. anthelus* sp. nov. has small polyhedra like those from the hymenopteran *Gilpinia* (Bird 1952) and the virus elementary bodies occur in both spherical and rod-shaped forms. However, the thick rods are not obviously composed of the thin rods as they are in all previously described polyhedral diseases. Hughes (1950) considers that there is a correlation between the size of the polyhedra and the number of virus bundles they contain. The *Pterolocera* polyhedra appear to confirm this generalization.

Smith and Wyckoff (1951) mention that polyhedra from different species differ greatly in their resistance to alkaline treatment, but the most resistant that they studied were dissolved by 5 min treatment with 4 per cent. Na_2CO_3. The polyhedra of *Pterolocera* withstand this concentration for 30 min and a treatment of at least 60 min at 56°C was necessary to dissolve them completely. *B. anthelus* is thus far more resistant to alkali than any previously described species.

Rods and spheres have been found by Smith and Wyckoff (1951) in polyhedra causing different diseases. Bergold (1950), however, found both to occur in a single disease and considered they represented stages in the life cycle of the viral organism. A similar conclusion was reached by Bird (1952) who studied the polyhedral disease of the hymenopteran *Gilpinia*; in this virus the spheres were found in early stages of infection and rods in later stages. In the *Pterolocera* polyhedra the spheres and rods have been found together in almost every preparation. The conclusion of Hughes (1952) in respect to the granulosis disease of the looper caterpillar *Sabulodes caberata*, that the relationship between the spheres and rods has not yet been clarified, is further exemplified by the observations on the virus of *Pterolocera*.

It is not known whether the polyhedral disease of *Pterolocera* caused greater destruction in 1952 than in previous years. The season was wetter than usual, and 5.65 in. of rain were recorded in October, whereas the monthly average over 27 years is 2.40 in. (maximum 6.59 in. in 1934, minimum 0.34 in. in 1940). The percentage of infected larvae in the field was somewhat higher in parts of the pastures near surface water. From general observations the incidence of the disease appeared to be higher than in previous years. It is probable also that the larval population was higher in 1952 than in the previous year. Light trap records show that populations of *Pterolocera* moths in March 1952 were very much higher than in the previous year. The resultant larval population may have therefore been larger and this could have contributed to a higher incidence of the disease. It is evident, however, that the epizootic did not reduce the population in 1953 to a level below that of the previous season (Fig. 1).

VI. Acknowledgments

Thanks are due to Miss P. Sinclair, Division of Entomology, C.S.I.R.O., who brought the first infected larvae to our attention, to Mrs. M. J. Bennetts for the histopathological preparations, and to Mr. D. Wilson for the photomicrography. Meteorological data were kindly furnished by the Australian Forestry School, Canberra.

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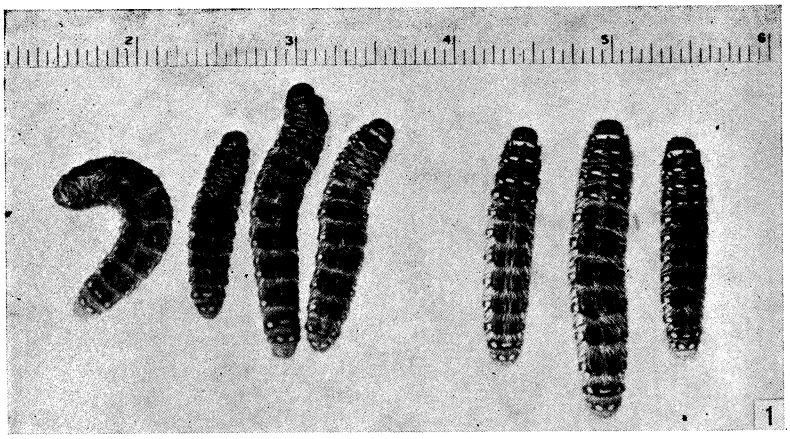
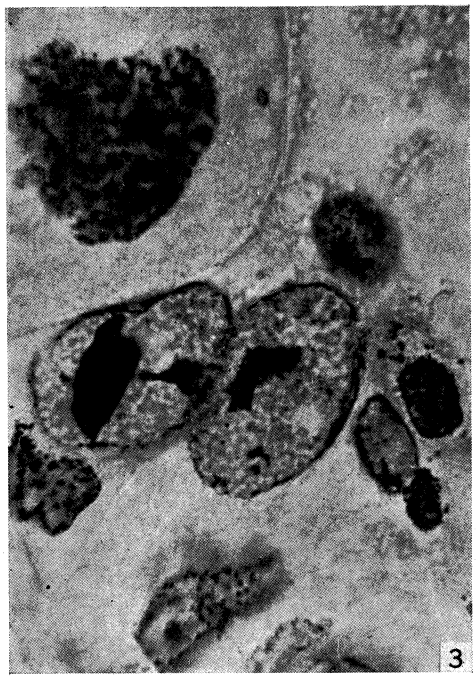
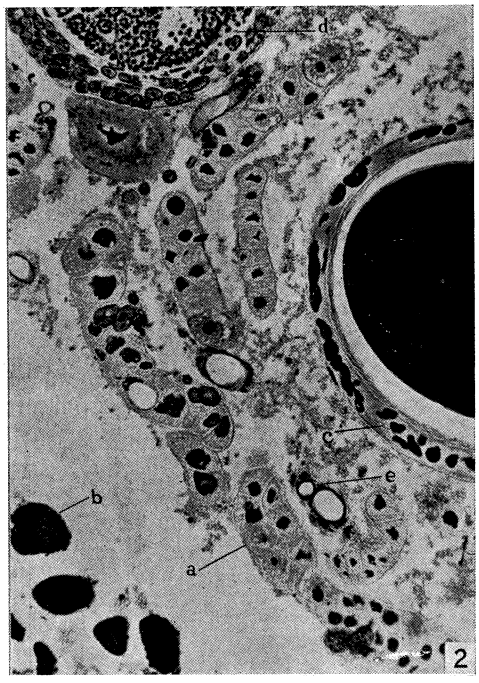
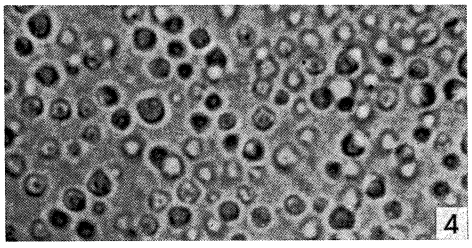
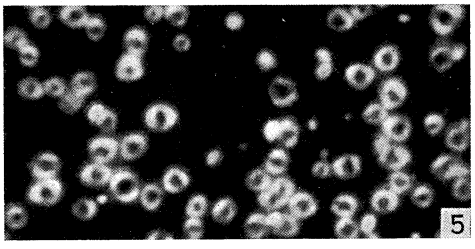
POLYHEDRAL VIRUS DISEASE OF A CATERPILLAR











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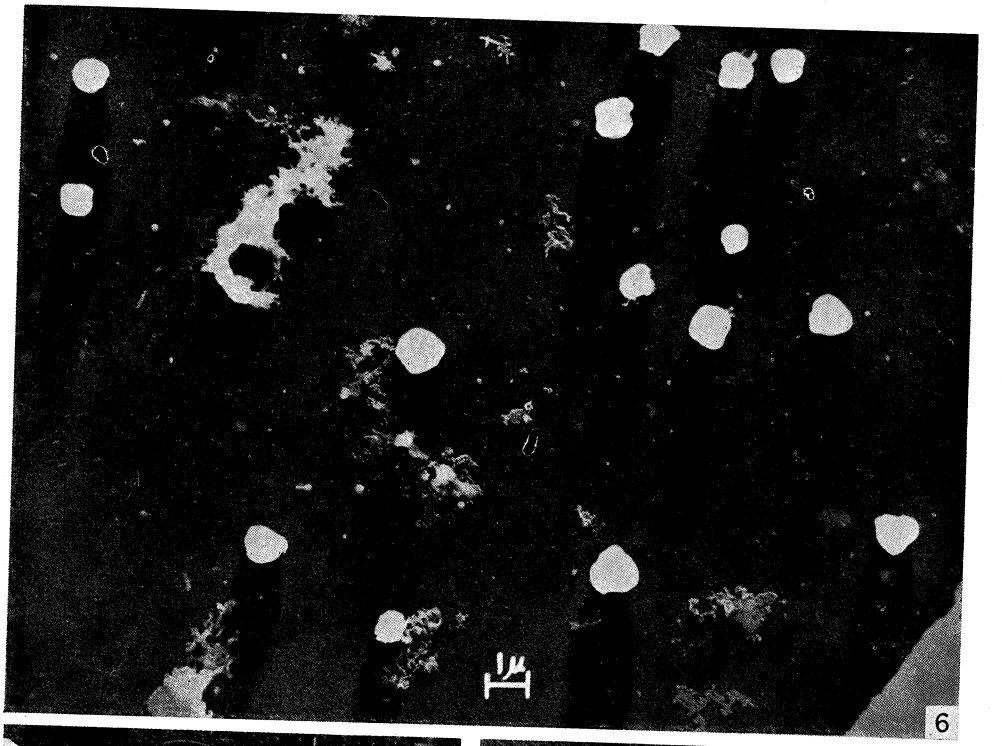

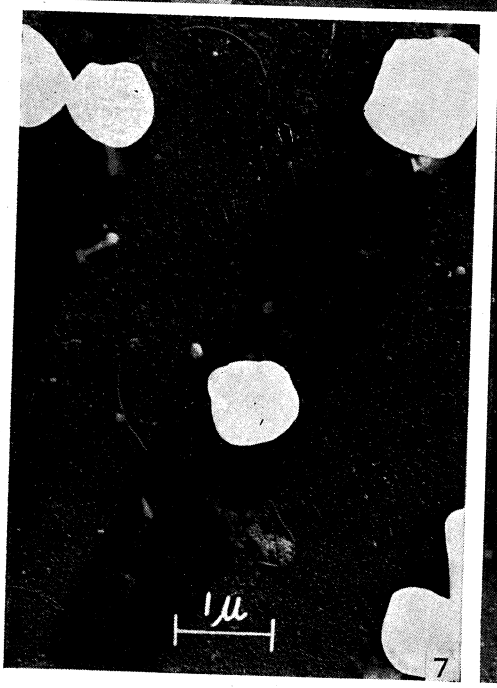

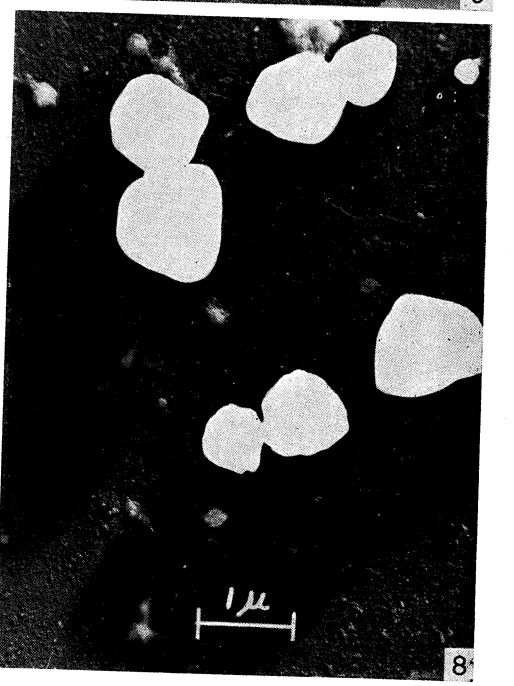

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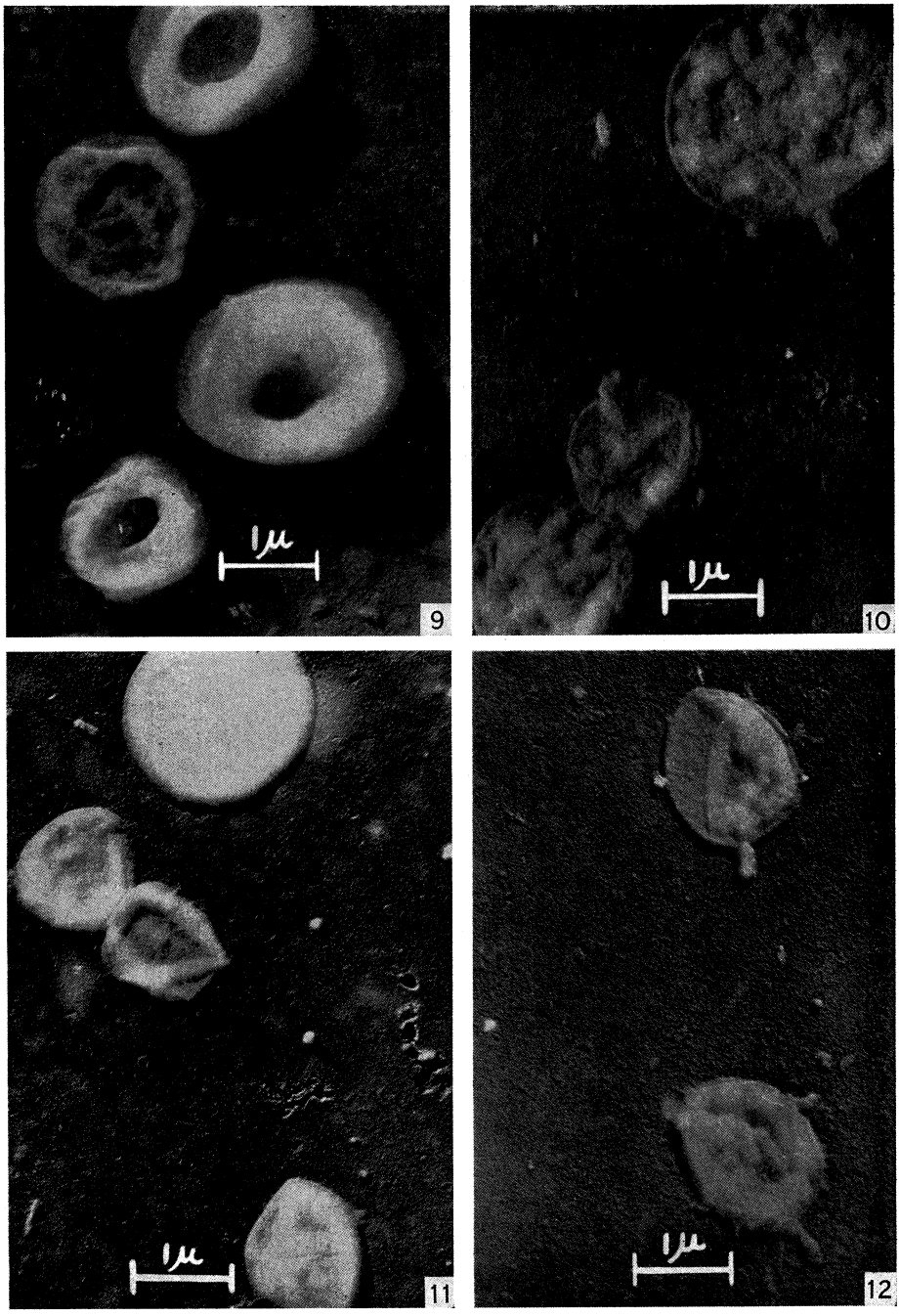

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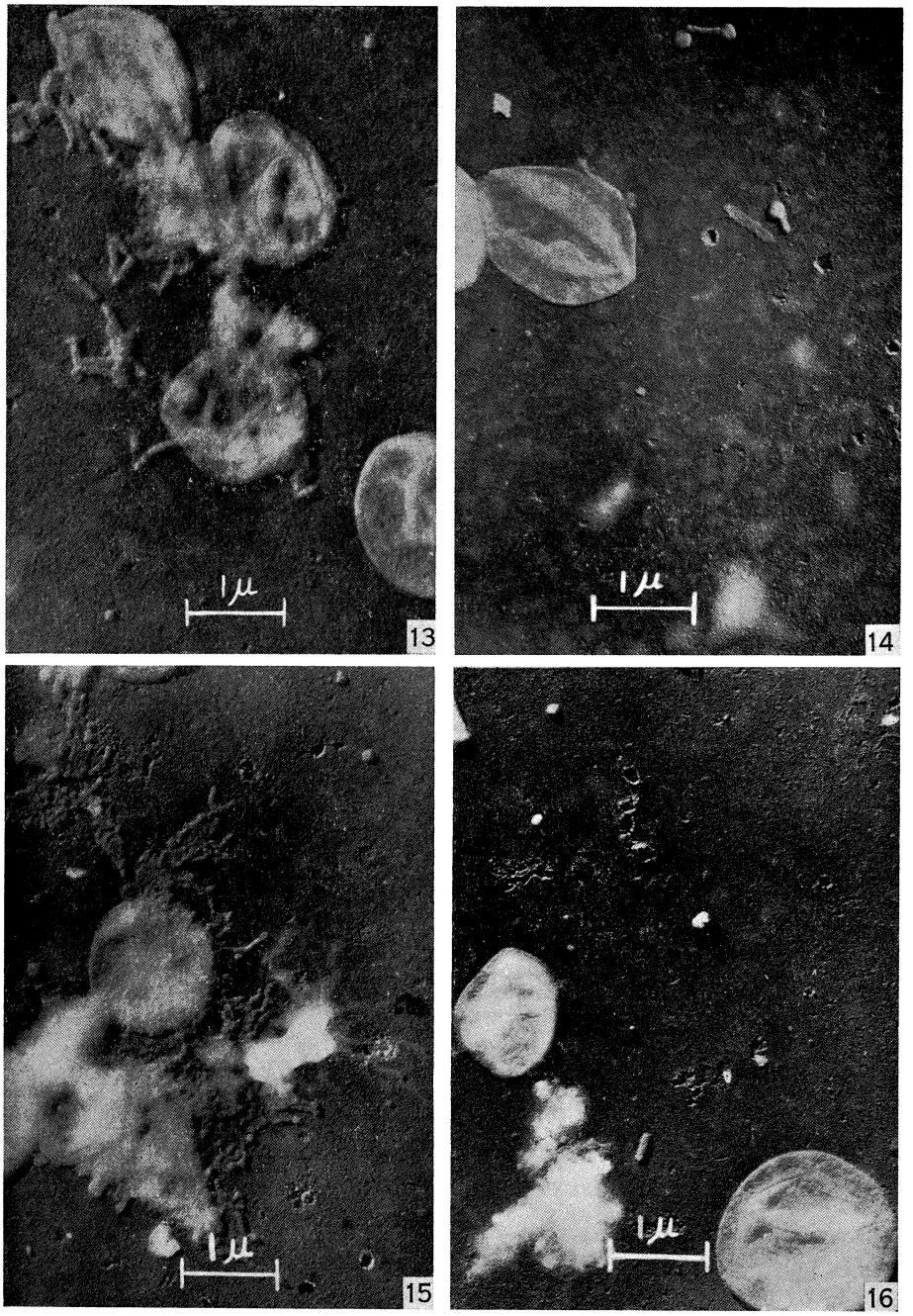

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EXPLANATION OF PLATES 1-4

PLATE 1

Fig. 1.—Mature larvae of *Pterolocera amplicornis*. Three normal larvae (right) and three larvae infected with polyhedrosis. Note dullness of body hairs of the infected larvae.

Fig. 2.—Histopathology of *P. amplicornis* infected by polyhedral disease. Note hypertrophied nuclei in fat body and in tissue surrounding the testes. (*a*) Fat body, (*b*) muscle, (*c*) silk gland, (*d*) testes, (*e*) trachea.

Fig. 3.—Same as Figure 2 under oil immersion objective. Polyhedra can be distinguished in the hypertrophied nuclei.

Fig. 4.—Polyhedra in haemolymph of infected larva. Mean diameter of polyhedra approximates 1.0 μ.

Fig. 5.—Same as Figure 4 with dark field condenser.

PLATE 2

Fig. 6.—Untreated polyhedra from infected larvae of *Pterolocera amplicornis*. Note presence of thin rods and flattened spheres. Shadowed with uranium. Ratio of shadow length to particle height is 6.

Figs. 7 and 8.—Polyhedra apparently unaffected by treatment with 0.01M NaOH solution for 120 min at room temperature. Thin rods and flattened spheres present. Shadow ratio is 4.

PLATES 3 AND 4

Fig. 9.—Incompletely digested polyhedra after 60 min treatment with 4 per cent. Na_2CO_3 solution at 56°C while standing on the collodion supporting film. Shadow ratio is 4.

Figs. 10 and 12.—Polyhedra after suspension in 4 per cent. Na_2CO_3 solution for 180 min at 50°C. Shadow ratio is 4.

Figs. 11, 13-16.—Polyhedra after 60 min treatment with 4 per cent. Na_2CO_3 solution at 56°C. Polyhedra treated while standing on the collodion supporting film. Note thin rods in Figures 11 and 14 and flattened spheres in Figures 11, 13, 14, 15, and 16. Shadow ratio is 4.