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Pathology and Development of the Grasshopper Inclusion Body Virus in *Melanoplus sanguinipes*

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Grasshoppers, *Melanoplus sanguinipes* (F.), infected with the grasshopper inclusion body virus (GIBV) showed a general torpor, took longer to develop, and had abnormally high rates of mortality. Infection was found only in the fat body, and developing viruses and inclusion bodies were observed in the nuclei and cytoplasm of infected cells. Although the size of the inclusion bodies in cells varied at different stages of infection, the inclusion bodies appeared to grow during the infection. Electron microscopic investigations of viral replication showed that at about 8 days after inoculation presumptive viral particles had developed as buds or protrusions from precursor granular masses; thereafter, these particles underwent internal differentiation and were incorporated into developing inclusion bodies. The GIBV was similar to insect viruses in the genus *Vagoiavirus* Weiser and to pox viruses, particularly vaccinia.

Henry and Jutila (7) reported the isolation of a polyhedrosis virus in grasshoppers, *Melanoplus sanguinipes* (F.), from Arizona and transmitted it experimentally to *M. bivittatus* (Say), *M. differentialis* (Thomas), and *Schistocerca americana* (Drury). It was then believed to be a nuclear polyhedrosis virus because of the rod-shaped particles in the inclusion body and the restriction of the infection to the fat body.

More recent studies by Jutila and Henry (*unpublished data*) on the morphological and chemical characteristics of the grasshopper inclusion body virus (GIBV) showed that it resembles nuclear polyhedrosis only in that both contain deoxyribonucleic acid. Other characteristics, the morphology of the inclusion bodies, the structure of the viral particles, and the chemical composition, differentiate it from both nuclear polyhedrosis viruses and from granulosis viruses.

MATERIALS AND METHODS

The *M. sanguinipes* used in the studies were reared by the procedure of Cowan (3). Grasshoppers that had been inoculated were maintained in a growth chamber at 30 C in continuous light and fed fresh lettuce and bran each day. In some tests, an artificial diet was offered as a supplement.

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Inocula obtained from homogenates of infected grasshoppers were introduced by injection or per os either by direct feeding or by placing inocula on pieces of lettuce which were then fed to grasshoppers. The injected inocula were prepared by passing homogenates through a series of filters to a final filter with a pore size of 0.8 or 0.45 μm. Injections were made through the ventral thoracic membranes with a 27-gauge needle attached to a microinjector apparatus.

Tissues for sectioning were fixed in Gilson's fluid, embedded in paraplast, and cut in sections 50 to 8 μm thick. The sections were then stained with either hematoxylin and eosin or by the procedures recommended by Hamm (6).

Two series were prepared for an electron microscopic study of the development of the virus. In the first, 100 fourth-instar nymphs were injected with filtered inocula, and portions of fat bodies were dissected out and pooled at periodic intervals; fat bodies dissected from untreated grasshoppers served as controls. In the second study, which was essentially a repeat of the first, 120 fourth-instar nymphs were injected and fat bodies were dissected out; one group of untreated grasshoppers and one group of starved grasshoppers were used as controls.

Tissues were fixed in a 2.5% gluteraldehyde-potassium phosphate buffer solution (*p*H 7.0) for 15 min at room temperature, then postfixed in osmium tetroxide (2%) for 2 hr in a refrigerator maintained at 4 C. Then the tissues were dehydrated through a graded series of acetone, soaked in propylene oxide, and embedded in Epon 6005. The sections were stained in 2% aqueous uranyl acetate for 2 hr and then in Reynolds'

lead citrate for 5 min and were examined with a Zeiss EM9 microscope.

RESULTS

Symptomology. Grasshoppers infected with GIBV showed a general torpor, took longer to develop, and had abnormally high rates of mortality. The bodies of heavily infected nymphs (inoculated when they were in the third instar) were frequently distended with protruding cervical membranes due to the heavy accumulation of inclusion bodies in the fat bodies. Such nymphs rarely developed beyond the last instar and persisted there in a semimoribund condition. Adults that developed from less heavily infected nymphs appeared anemic and rarely became sexually mature.

Because of difficulties in standardizing the techniques of assay, a critical analysis of the virulence of GIBV was not completed. Generally, third-instar nymphs of *M. sanguinipes* that were inoculated per os with about 10^5 inclusion bodies failed to reach the adult stage and died between 20 and 30 days after treatment; the lethal levels of infection were about 10^5 to 10^6 inclusion bodies per mg of grasshopper weight. Injections of filtered inoculants from homogenized grasshoppers that presumably contained nonoccluded infective particles were more active since most inoculated grasshoppers died 15 to 20 days after treatment. However, these injections produced fewer and smaller inclusion bodies in the terminally infected grasshoppers. Increased rates of mortality among injected nymphs were evident 7 and 8 days after inoculation, which, as will be discussed, corresponds to the appearance of the first recognizable structures involved in viral replication. As previously reported by Henry and Jutila (7), inclusion bodies were discernible under light microscopy at 12 days after per os inoculations and at about 10 days after injection.

Histopathology. The fat bodies of grasshoppers infected with GIBV became increasingly hypertrophied and eventually, in terminal infections, appeared as irregular, flaccid, grayish masses. Generally, initial infections were limited to one or several isolated parts of a fat body; later, the entire tissue became infected. As a result, different parts of this tissue in sublethally infected grasshoppers exhibited different degrees of hypertrophy and diseased condition.

Examinations of sectioned tissues with the light and electron microscopes showed that infections occurred in both the cytoplasm and nuclei of the cells of the fat body. The nuclei of the infected cells were generally larger and less granular than those that showed no signs of infection. Usually, the nuclear membranes were disrupted by the time inclusions could be discerned under a light microscope. Because adjacent cells usually differed in the degree and stage of infection, the inclusion bodies in cells in earlier stages of infection varied in size; those in terminally infected cells were uniformly large, an indication that individual inclusion bodies grow during the infection.

The hemolymph of heavily infected grasshoppers often appeared cloudy because of inclusion bodies which apparently were from disrupted cells of the fat body. Inclusion bodies were observed in hemocytes, but this was considered to be the result of the phagocytic activity of these cells.

Viral replication. Because of the asynchrony in infections and the viral development within both individual and adjacent cells, sections prepared for electron microscopy often contained cells which contained different structures involved in viral replication. The first recognizable stages were large, slightly dense masses with both granular and fibrillar characteristics (Fig. 1) observed in sections prepared 8 days after injection. In sections prepared later than 8 days after infection, other masses were observed that were smaller, more dense, and characteristically more granular and less fibrillar (Fig. 1–2). Some of the smaller and more dense masses appeared to be entirely granular and were either partially or completely enclosed in single peripheral membranes. Apparently they developed as a result of condensation of the larger, less-dense masses, and the peripheral membranes were formed from the fibrillar structures in the larger masses.

Presumptive viral particles measuring about 320 nm long by about 250 nm wide originated as protrusions or buds from the densely granular masses (Fig. 1–2). These particles always appeared on the peripheral parts of the masses and were enclosed by a membrane that apparently was derived from the membrane surrounding the granular mass. The internal structure of the particles nearest to the masses, presumably the most recently developed, was darkly granular but less dense than the internal structure of the precursor masses. Other smaller particles that were considered to be of earlier origin contained dense granules surrounded by less densely granular areas (Fig. 2). In still other particles, central cores or nucleoids which appeared dense but nongranular were surrounded by closely associated membranelike structures within densely granular outer coats. Although the cores of most particles at this stage appeared cylindrical, some dumbbell-shaped cores were observed (Fig. 3). Viral particles that appeared mature were observed

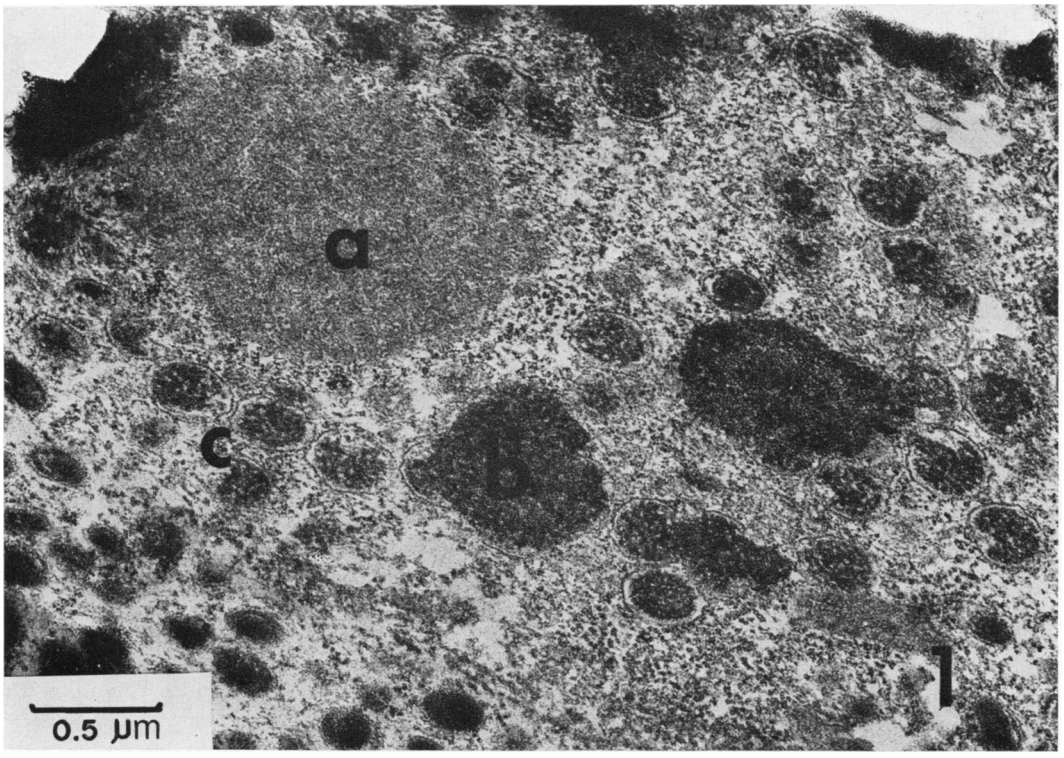

FIG. 1. *Ultrathin section of the cell of fat body of M. sanguinipes showing (a) a fibrillar-granular mass, (b) several electron-dense granular masses, and (c) presumptive viral particles observed during the replication of the GIBV 8 days after injection.*

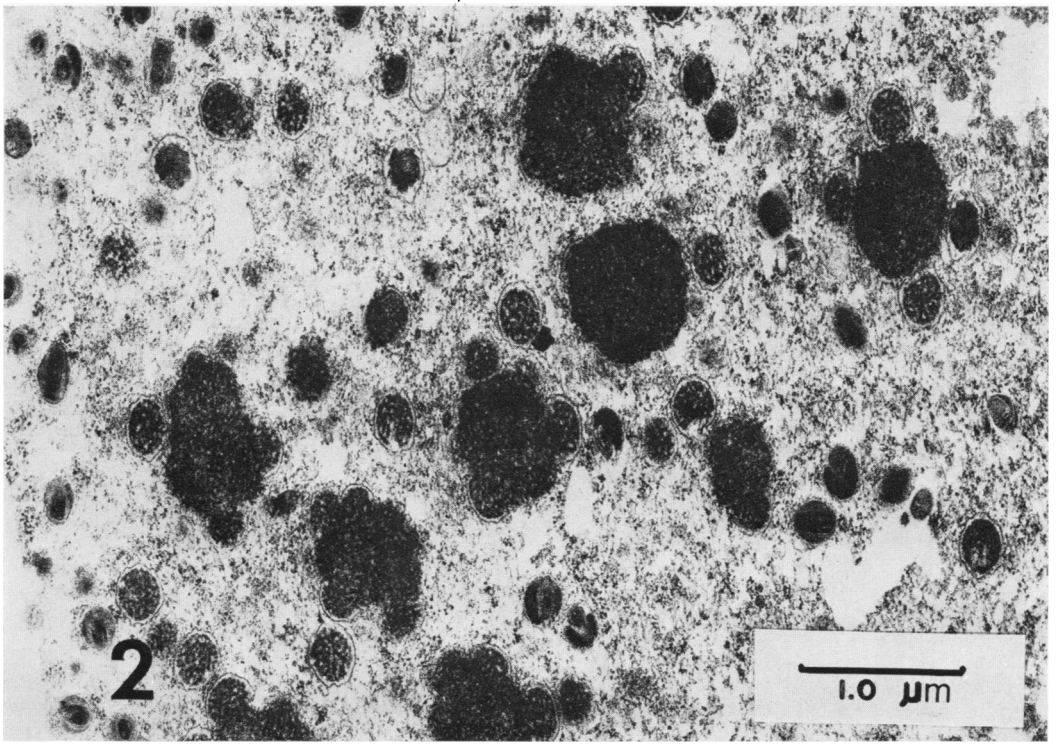

FIG. 2. *Ultrathin section of the cell of fat body of M. sanguinipes showing the formation of presumptive viral particles from an electron-dense granular mass observed during the replication of the GIBV. Note the variation in the internal structure of the presumptive particles.*

free in the cytoplasm and enclosed in developing inclusion bodies (Fig. 3–4). However, the formation of inclusion bodies did not depend on the presence of mature viral particles because immature particles were observed within the matrices of relatively small presumptive inclusion bodies (Fig. 3). Also, immature particles were observed in the peripheral parts of larger inclusion bodies although the particles in the internal portions appeared to be mature. Differentiation of particles therefore continued during occlusion.

DISCUSSION

The GIBV bears little resemblance to the better known inclusion body viruses such as nuclear and cytoplasmic polyhedrosis and granulosis. Except for the inclusion bodies, GIBV differs entirely from cytoplasmic polyhedral viruses, and is only superficially similar to nuclear polyhedral viruses in that both infect cells of fat bodies and contain DNA. However, the replication of nuclear polyhedral viruses, as reported by Xeros (13, 14) and Day et al. (5) and reviewed by Aizawa (1), differs from that observed during development of GIBV. Also, the structure and replication of GIBV particles appeared different from the development of granulosis viruses as reported by Huger and Kreig (9) and Huger (8).

Of the insect viruses reported, GIBV resembles most closely a virus reported by Vago (10) and Weiser (11) from grubs of *Melolontha melolontha* (L.) and another virus reported by Weiser and Vago (12) from the lepidopterans *Operophtera brumata* (L.) and *Acrobasis tumidella* (Zincken; *zelleri* Rag.) These viruses infected the fat bodies of their hosts and formed inclusion bodies that grew during the infection.

Recently, Bergoin et al. (2) reported that vesicular elements measuring about 250 × 450 nm were the first stages observed in the development of the virus from *M. melolontha*. Initially the vesicular objects were similar in density to the cytoplasm of the cells but contained small particles 40 to 50 nm in diameter. During growth, the internal area of vesicles increased in density and eventually differentiated into mature viruses. From this the GIBV appears to be somewhat smaller (320 × 250 nm) but this may be due to differences in the

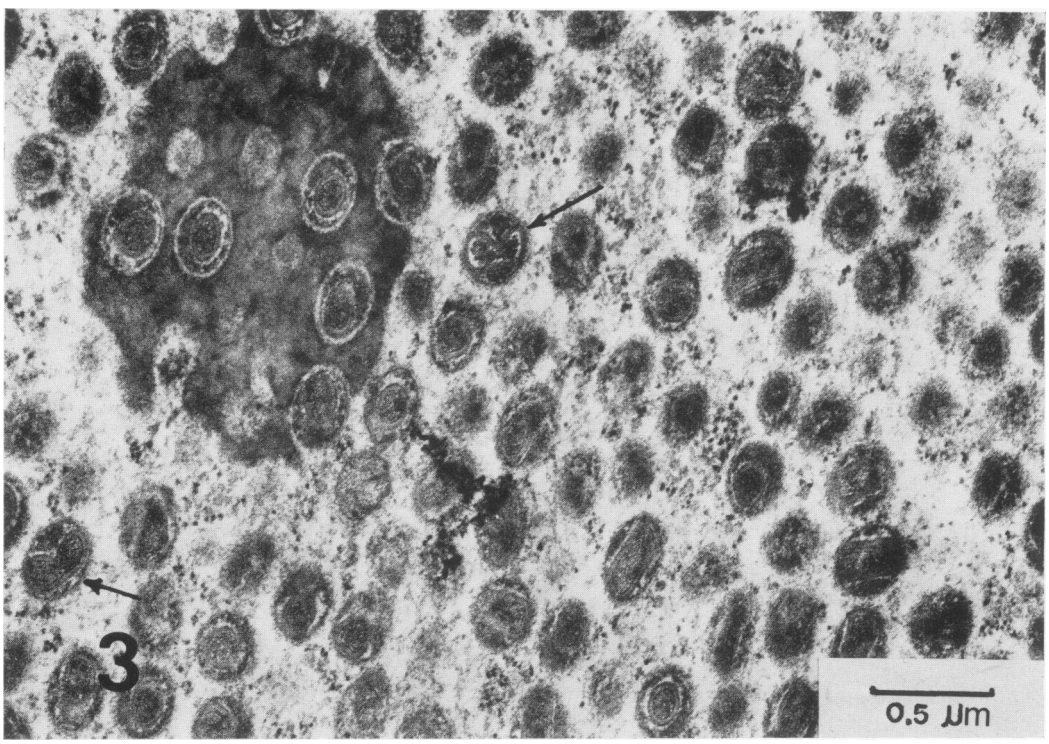

FIG. 3. *Ultrathin section of a cell of fat body of M. sanguinipes showing an inclusion body during early development, also containing a small number of presumptive viral particles of the GIBV. Several viral particles contain dumbbell-shaped cores (arrows) that appear similar to structures observed by Dales and Siminovitch (4) in vaccinia virus.*

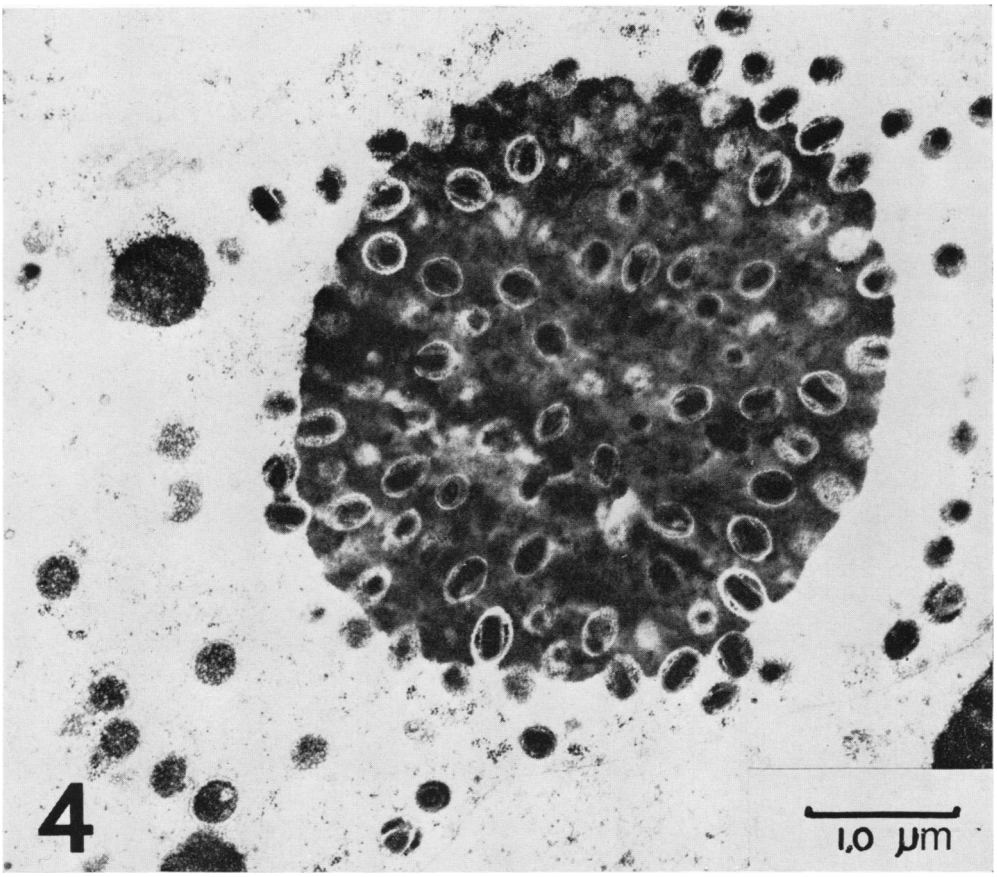

FIG. 4. *Ultrathin section of the cell of fat body of M. sanguinipes showing an advanced inclusion body containing numerous viral particles of the GIBV.*

experimental procedures. The main difference appears to be that the presumptive viral particles of GIBV develop from precursor granular masses, whereas those reported by Bergoin at al. (2) develop separately. Thus, GIBV may belong to the genus *Vagoiavirus* Weiser which Weiser (11) erected for the virus from *M. melolontha*.

In comparison with other viruses, GIBV resembles viruses of the pox group, particularly vaccinia. Dales and Siminovitch (4) reported that presumptive vaccinia particles developed from precursor masses differentiated internally and contained central cores (nucleoids) similar to those observed in GIBV. However, the development of vaccinia virus does not involve formation of inclusion bodies and may also differ in the development of the limiting membranes surrounding the viral particle.

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