

Reovirus-like Particles and Their Vertical Transmission in the Mexican Bean Beetle, *Epilachna varivestis* (Coleoptera: Coccinellidae)^{1,2}

E. W. KITAJIMA

Departamento de Biologia Celular, Universidade de Brasilia, 70910 Brasilia DF, Brazil

AND

K. S. KIM,³ H. A. SCOTT, AND R. C. GERGERICH

Virology and Biocontrol Laboratory, Department of Plant Pathology, University of Arkansas, Fayetteville, Arkansas 72701

Received May 23, 1984; accepted February 4, 1985

Examination of thin sections of organs and tissues from the Mexican bean beetle, *Epilachna varivestis*, adults and larvae revealed the consistent presence of isometric, reovirus-like particles in the cytoplasm and rarely in the nuclei. Cytoplasmic inclusions having a dense and finely granular matrix with virus-like particles at their periphery were noticed occasionally. These inclusions were, however, very frequent in the nurse cells in the germarium of the ovariole. Virus-like particles were found in the cytoplasm of oocytes and eggs as well as embedded in the nuclei of sperm, thus suggesting vertical transmission of these particles, and explaining the 100% infection of the Mexican bean beetle colony maintained at the Virology and Biocontrol Laboratory of the University of Arkansas. Partially purified extracts from ovarioles or entire beetles contained particles which, in negatively stained preparations, resembled reoviruses without the external protein coat. It is suggested that these particles represent a latent virus of the Mexican bean beetle. © 1985 Academic Press, Inc.

INTRODUCTION

The genus *Epilachna* contains the only leaf-feeding beetles in the family Coccinellidae. The Mexican bean beetle, *Epilachna varivestis*, occurs throughout the continental United States except for the West Coast, and is considered a serious pest (Ossig, 1926; Douglas and Portman, 1965). Its role as a plant virus vector was first described by Jansen and Staples (1970) when

they succeeded in transmitting cowpea mosaic virus with this beetle. Later, other viruses were shown to be transmitted by the Mexican bean beetle: southern bean mosaic and bean pod mottle (Fulton and Scott, 1974), bean mild mosaic (Waterworth et al., 1977), and bean curly dwarf mosaic (Meiners et al., 1977).

Recently, using *E. varivestis* as a model system, Gergerich et al. (1983) demonstrated that specificity of virus transmission by beetles is associated with an inhibitor(s) present in the regurgitant of these beetles. The origin of this inhibitor(s) is not known. Beetles do not have salivary glands but possess gnathal or head glands. Preliminary observation of these gnathal glands with the electron microscope demonstrated the presence of virus-like particles (VLPs) ca.

¹ This work was done at the Virology and Biocontrol Laboratory, Department of Plant Pathology, University of Arkansas, where the senior author spent a sabbatical leave as a Fellow of the Fulbright International Exchange of Scholars Program.

² The research was partially supported by the Arkansas Agricultural Experimental Station and published with the approval of its Director.

³ To whom correspondence and reprint requests should be addressed.

70 nm in diameter (Valverde, unpubl.) which were essentially similar to those found in the hemocytes of the bean leaf beetle, *Cerotoma trifurcata*, and the spotted cucumber beetle, *Diabrotica undecimpunctata*, both of the family Chrysomellidae (Kim and Scott, 1978; Kim, 1980).

A more detailed investigation was carried out to determine the distribution of these VLPs within *E. varivestis*. It was found that these VLPs were present throughout the organs and tissues, including sperm and egg cells of all individuals of the colony of *E. varivestis* maintained at the University of Arkansas, suggesting that the particles are vertically transmitted through male and female gametes. This paper describes these findings.

MATERIALS AND METHODS

The Mexican bean beetles were raised in screened cages in a greenhouse on bean plants (*Phaseolus vulgaris* cv. "Pinto"). Adults were dissected in phosphate-buffered saline, and several organs (mid- and hindgut, Malpighian tubules, flight muscle, fat body, testes and accessory gland, common oviduct, vagina, ovariole, epidermis, brain and subesophageal ganglion, heart, and maxillary gland) were removed and immediately transferred into modified Karnovsky's fixative (2% paraformaldehyde, 2% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.2). Larvae in different stages of development were also dissected. Some of these larvae were separated from the main colony soon after hatching and were raised in complete isolation from the

main colony. Ovulated oocytes and mature, laid eggs were also fixed after puncturing one of the extremities to facilitate the penetration of the fixative. After postosmication for 2 hr in 1% OsO₄ in 0.05 M cacodylate buffer, pH 7.2, and dehydration in ethanol series, the tissues were embedded in Spurr's low-viscosity embedding medium, sectioned, double-stained with uranyl acetate and Reynold's lead citrate, and examined in a JEOL 100 CX electron microscope. Some ovarioles were fixed with Karnovsky's aldehyde fixative alone and the sections were treated according to Bernhard's (1969) EDTA regressive stain technique to demonstrate whether the VLPs and associated inclusions contain DNA.

Semithin sections mounted in immersion oil were observed in the phase-contrast microscope to monitor the tissues and organs.

RESULTS

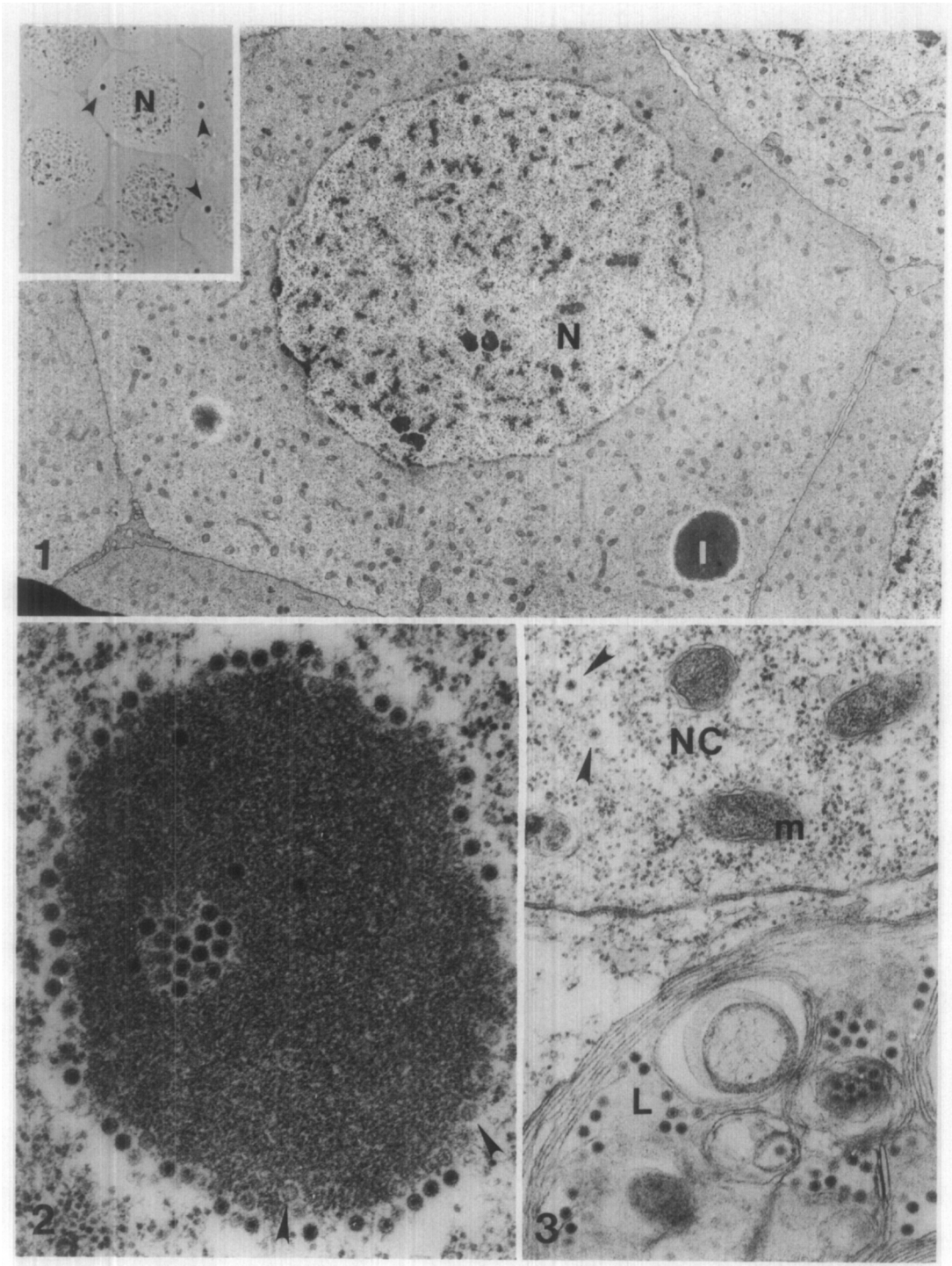
This report represents the examination of 15 adults (8 females, 7 males), six larvae of different sizes, three ovulated oocytes, and three mature and laid eggs. All of these were picked at random from the Mexican bean beetle colony. Observations of three larvae raised in complete isolation from the main colony are also included in the description.

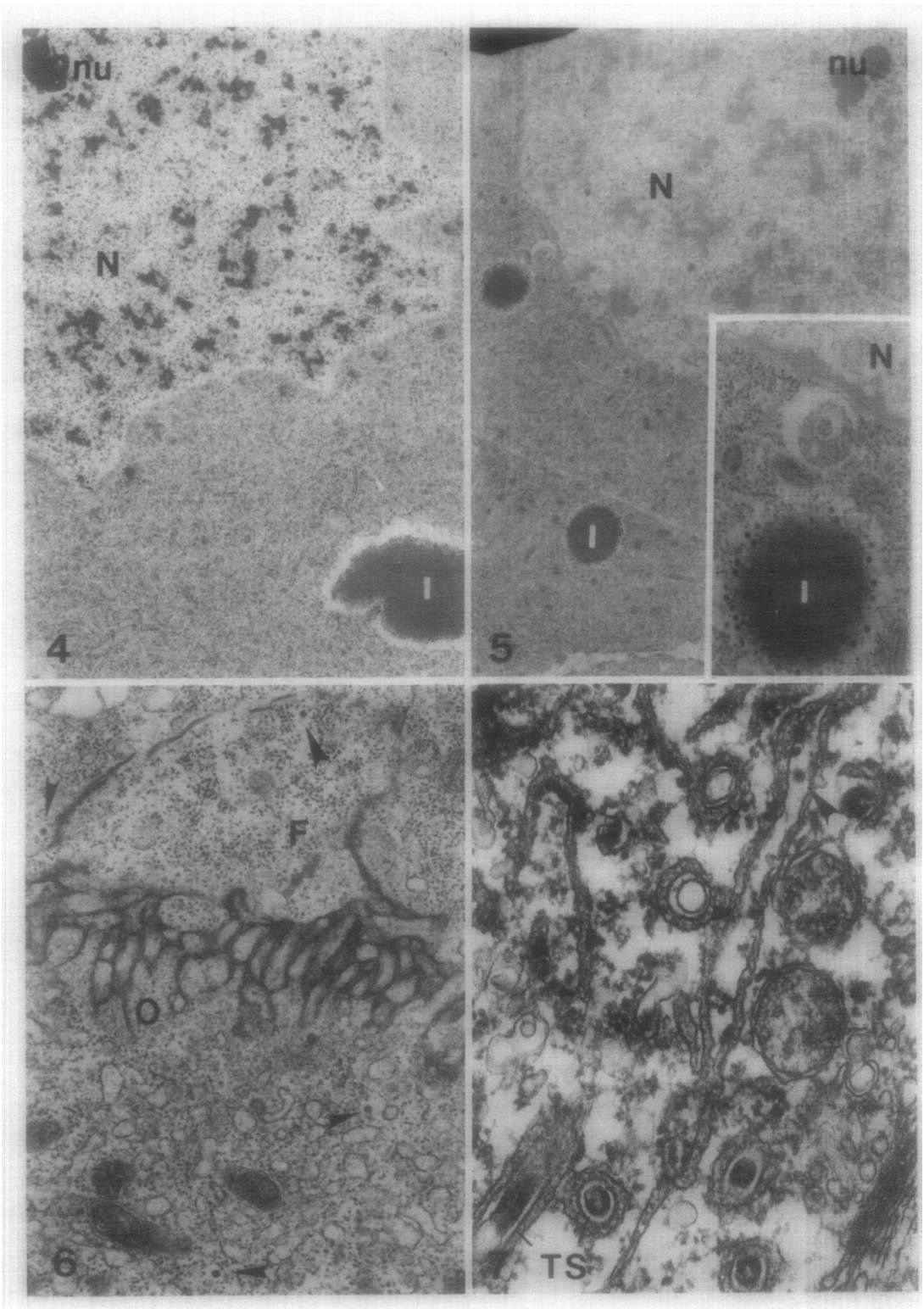
General observations. Examination of tissues and organs from adults, larvae, ovulated oocytes, and mature eggs showed the consistent presence of spheroidal, virus-like particles (VLPs), ca. 70 nm in diameter (Figs. 2–26). They were discretely scattered in the cytoplasm, usually with a clear halo around them, or in cytoplasmic,

FIG. 1. Low-magnification electron micrograph of a nurse cell in the germarium of the Mexican bean beetle ovariole (telotrophic type). Note the prominent nucleus (N) and the cytoplasm populated mostly by ribosomes and slender mitochondria. Two dense inclusions (I) appear in the cytoplasm. $\times 4000$. Insert shows a phase-contrast light micrograph of an equivalent cell, depicting the nucleus (N) and the inclusions (arrowheads). $\times 500$.

FIG. 2. Detail of the viroplasm-like inclusion. It is made up of a dense and finely granular material and numerous virus-like particles that are present either at the periphery or embedded in the dense matrix. Apparently empty particles are indicated by arrowheads. $\times 50,000$.

FIG. 3. Part of the distal region of the germarium. A large lysosome (L) of a prefollicular cell contains VLPs. In a nurse cell (NC) next to it VLPs appear scattered in the cytoplasm (arrowheads). Note the electron-lucent halo around the particle. M, mitochondria. $\times 33,000$.





single-membrane-bound cavities interpreted as lysosomes (e.g., Fig. 3), but no crystalline arrays of the VLPs were observed. Less frequently, in some cell types (muscle fibers, neurons, and gametocytes) the VLPs appeared intranuclearly, commonly associated with the chromatin material (Fig. 26). Particles were also found in association with cytoplasmic inclusions composed of a dense and finely granular material, generally having a circular to elliptical profile of the type described by Shikata (1977) for the "leafhopper-borne subgroup of plant reoviruses" (Figs. 1, 2). Particles were located at the periphery of these inclusions and were occasionally embedded in the inclusions (Figs. 2, 17). Such inclusions were relatively rare in somatic cells and testes, but they occurred consistently in most of the cells in the germarium of the ovariole.

The VLPs consist of a dense core 30–40 nm in diameter surrounded by a thin shell-like structure 5- to 7-nm thick which is separated from the core by an electron-lucent zone 10- to 15-nm wide (Figs. 2, 21). In no instance were VLPs found associated with cytomembranes exhibiting a "budding" configuration. When particles were present in the lysosomes, many of them were uniformly dense, lacking the electron-lucent area between the core and the shell (Figs. 3, 14, 15).

A more detailed description of the VLPs in each organ as well as a brief comment on the ultrastructure of the cells containing these particles is as follows.

Ovariole. The ovary is composed of an average of 54 ovarioles (two pairs of 27) of the telotropic type (Telfer, 1975). The gen-

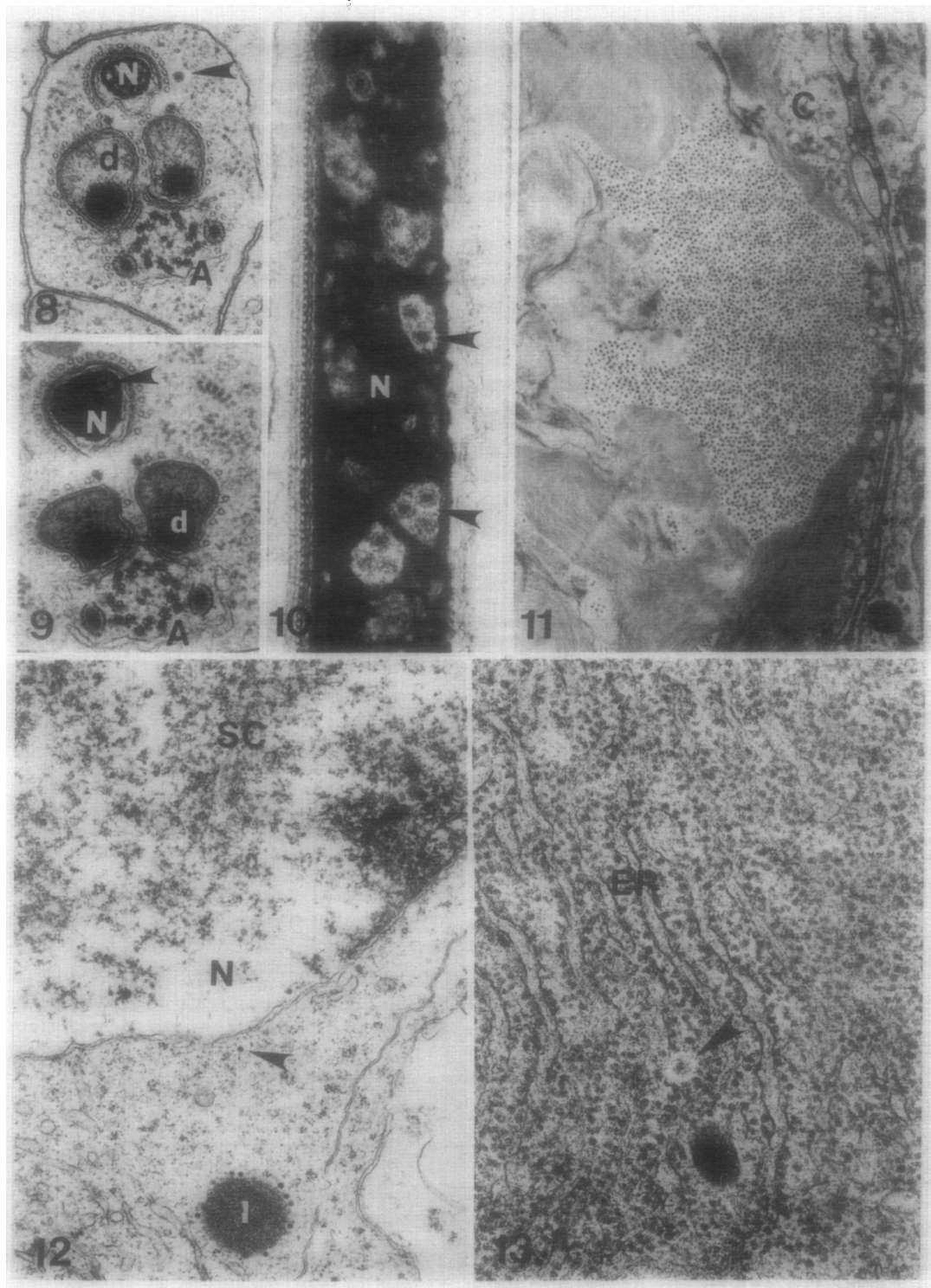
eral organization of the ovariole is similar to the descriptions given for some other beetles (Büning, 1978). The germarium is surrounded by a thick basal lamina (tunica propria) without external epithelium and contains relatively large and uniform nurse cells. These cells commonly have intercellular bridges, a prominent nucleus with well-spread chromatin, and cytoplasm which contains mostly ribosomes and slender mitochondria (Fig. 1). Most of these cells contained one or more dense cytoplasmic inclusions with VLPs at their periphery (Figs. 1, 2, 4, 5). These inclusions, which we refer to as viroplasms, were up to 5 μ m in diameter and were large enough to be seen easily by phase-contrast light microscopy in semithin sections (Fig. 1, insert) or in fresh squashes of ovariole. Nurse cells usually had one viroplasm per cell, but sometimes up to four small ones could be seen in a single cell. Virus-like particles could also be seen scattered in the cytoplasm of these nurse cells (Fig. 3). At the lower part of the germarium, where nurse cells appear intermingled with prefollicular cells and young oocytes, VLPs appeared also within lysosomes in the prefollicular cells (Fig. 3). Viroplasms were not seen in oocytes or follicular cells, where few VLPs would be detected scattered in the cytoplasm (Fig. 6). A regressive staining method using EDTA as a bleaching agent indicated that the particles and viroplasms probably do not contain DNA since the viroplasms remain densely stained following EDTA treatment (compare Figs. 4 and 5).

Ovulated oocyte and egg. In both ovulated oocytes and eggs the cytoplasm is full of granules of different sizes and consisten-

FIGS. 4, 5. Nurse cells of an ovariole fixed with aldehyde alone. Figure 4 is the control section, while Figure 5 represents a section treated with EDTA between uranyl acetate and lead citrate staining. Note that the chromatin material in the nucleus (N) in Figure 5 is bleached, while the nucleolus (NU), ribosomes and inclusions (I) remain dense. Insert in Figure 5 shows a detail of an inclusion and the periphery of the nucleus. $\times 6500$ and $\times 7500$.

FIG. 6. Contact area between follicular cells (F) and the previtellogenic oocyte (O) in the egg chamber. There is an extensive interdigitation of plasmalemma from both cells. A few virus-like particles (arrowheads) are noticeable in the cytoplasm of oocyte and follicular cells. $\times 25,000$.

FIG. 7. Periplasmic region of a mature, laid egg. A single virus-like particle (arrowhead) is seen among membranes and tubular structures (TS). $\times 52,000$.



cies except at the periplasma where a large number of tubular structures surrounded by a membrane-bound body occurs (Feldman, 1979). Virus-like particles were rarely interspersed among these structures (Fig. 7).

Testes and accessory gland. The male reproductive system consists of two masses of tubular testes which converge to form the vas deferens and two pairs of accessory glands. Spermatocytes in different stages of differentiation appear in different cysts, and the whole testis is surrounded by an epithelial layer. Viroplasm was observed in a few primary spermatocytes (Fig. 12), while VLPs could be seen scattered in the cytoplasm and less frequently in the nucleus. In some elongating spermatids, VLPs were seen either in the cytoplasm (Fig. 8) or in the condensing nucleus (Fig. 9), and they persisted in mature sperm present in the vas deferens (Fig. 10) and even within the spermatheca of the female. Intranuclear VLPs could be clearly seen in longitudinal sections of the sperm nucleus (which follows most of the sperm length from the acrosomal tip to almost the tail end). Most VLPs in the testis epithelial cells occurred in the lysosomes. An interesting feature of cysts containing spermatids in the final stages of differentiation was the presence of huge lysosomes with large masses of myelinic figures and densely packed masses of VLPs (Fig. 11) some-

times interspersed with bundles of microtubules.

The tubular accessory gland has an epithelium formed by a single layer of cells extremely rich in rough endoplasmic reticulum (Fig. 13). Dense granules appear at the apical part of these cells whose contents are released into the lumen, which is filled by a fine, dense substance. Few VLPs could be seen scattered in the cytoplasm (Fig. 13).

Maxillary gland. A single layer of epithelial cells forms the wall of the tubular, maxillary gland and surrounds a collecting duct formed by the cuticle as described for other Coleoptera (Srivastava, 1959). The apical part of the epithelial cells is covered by relatively thick microvilli and the cytoplasm contains tubular elements of the endoplasmic reticulum. The VLPs appeared within lysosomes or scattered in the cytoplasm among the tubular elements of the smooth endoplasmic reticulum (Fig. 14). Particles were found very rarely between the collecting duct and the apical part of the epithelium.

Malpighian tubule. Slender Malpighian tubules arise from the first third of the midgut and end at the hindgut. The epithelial cells have a large nucleus and the cytoplasm is rich in mitochondria, endoplasmic reticulum, lysosomes, and granules of several types. Microvilli are long and

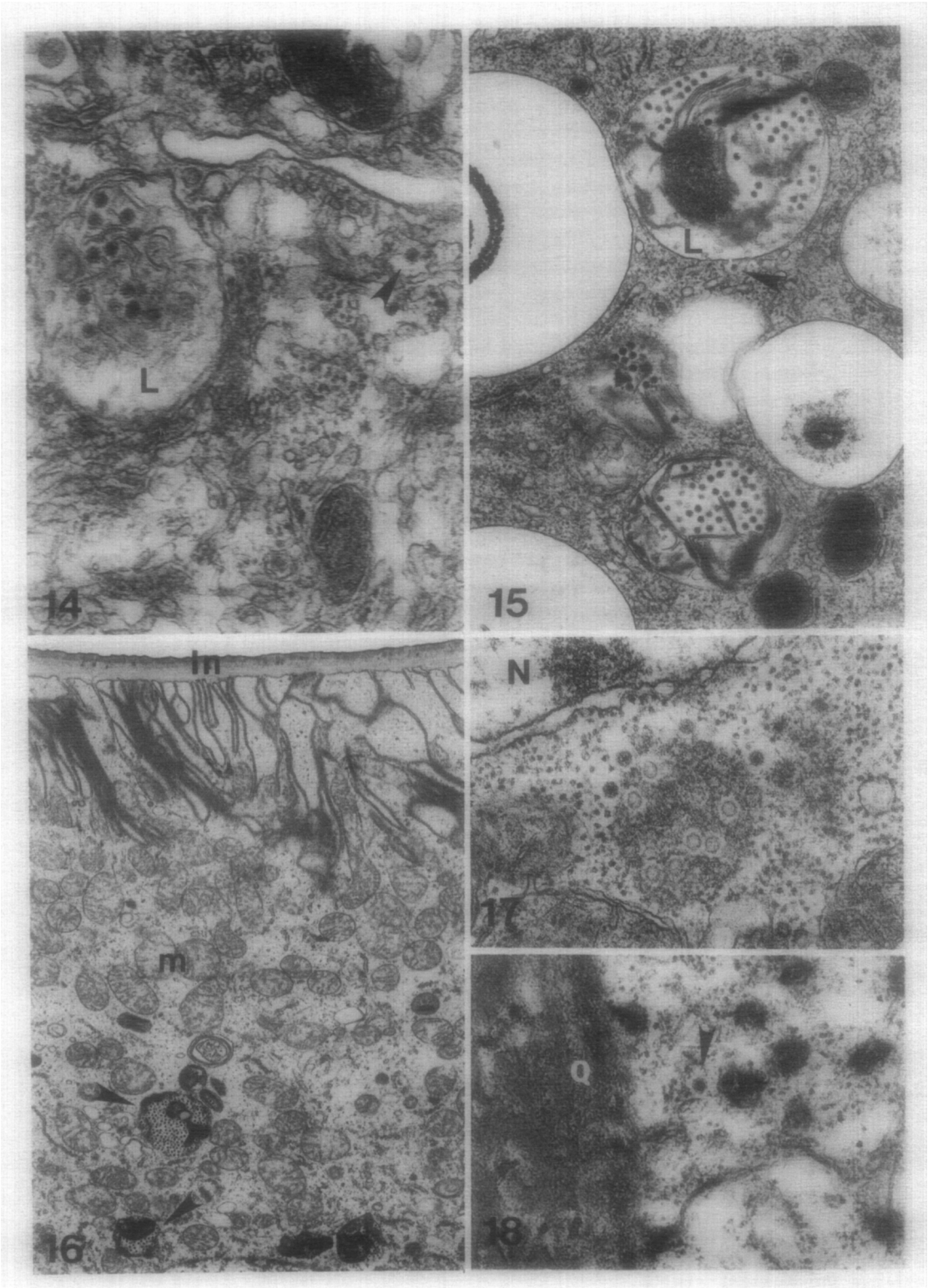
FIGS. 8, 9. Cross sections from developing spermatozoa in the testis. In Figure 8 a single virus-like particle is close to the condensing nucleus (n) (arrowhead), while in Figure 9 it is within the nucleus. d, mitochondrial derivative; A, axoneme. $\times 40,000$.

FIG. 10. Longitudinal section of the nucleus (N) from a mature sperm in the vas deferens. Several virus-like particles (arrowheads) appear embedded in the condensed chromatin. $\times 60,000$.

FIG. 11. Cyst cell from a cyst in which spermiogenesis is almost complete. Cytoplasm contains large lysosomes (L), probably digesting the cytoplasmic remnants from the developing sperm. Note a large mass of virus-like particles and myelinlike membranes. $\times 10,000$.

FIG. 12. Primary spermatocyte in early prophase of meiosis. Note the synaptonemal complex (SC) in the nucleus (N). A viroplasm (I) as well as an isolated virus-like particle (arrowhead) can be seen in the cytoplasm. $\times 16,500$.

FIG. 13. Part of the cytoplasm, extremely rich in rough endoplasmic reticulum (ER) of the epithelium from the accessory gland of the male reproductive system. A single virus-like particle is indicated by the arrowhead. $\times 60,000$.



profuse, most of them containing slender mitochondria. The VLPs were mostly found in the lysosomes, but a few of them appeared in the ground cytoplasm (Fig. 15).

Mid- and hindgut. The epithelial cells in the more distal portions of the hindgut form deep plasmalemmal invaginations where they contact the hindgut intima. The VLPs were found mostly in the lysosomes of these epithelial cells (Fig. 16).

In the midgut, the regenerative cells did not form crypts as in most Coleoptera but were buried at the base of the epithelium in the so-called nidus, essentially as described by Burgess (1932). The VLPs appeared either scattered in the cytoplasm or within lysosomes and, in a few cells, viroplasms were present, usually near the nucleus (Fig. 17).

Epidermis. Just beneath the chitinous exoskeleton (cuticle) there is a monolayer of epidermal cells, some of which contain large masses of microtubules. Cells from the epidermis have well-developed endoplasmic reticulum, Golgi complex, and dense granules at the apical region. A few VLPs were found scattered in the cytoplasm (Fig. 18).

Nervous system. The ganglial structures of the nervous system have the basic organization described for insects with layers of neurons at the periphery and a complex mass of axons and dendrites in the central region. The VLPs were found commonly in the neurons within lysosomes or scattered in the cytoplasm, and rarely, within the nucleoplasm (Fig. 19). Virus-like particles

were rare in the axons, but they were seen occasionally interspersed among the microtubules and vesicles (Fig. 20).

Fat body and oenocyte. Fat body is ubiquitous in several parts of the beetle body in both the larval and adult stages. It is made up of a cluster of several cells surrounded by a common basal lamina. Each cell usually has a prominent nucleus and a cytoplasm filled with large lipid droplets and glycogen granules. The VLPs were noticed scattered in the cytoplasm (Fig. 21) or within lysosomes. Cells identified as oenocytes are very large, with a prominent nucleus and cytoplasm rich in small vesicles, endoplasmic reticulum, and mitochondria. As in most of the cells, the VLPs were found in the cytoplasm or within lysosomes (Fig. 22).

Hemocytes. Attempts to concentrate hemocytes from hemolymph produced by reflexive bleeding from the tibio-femoral joint (Happ and Eisner, 1961) yielded very few cells, because the hemolymph coagulated quickly in the buffer or fixative solution. On the other hand, a few hemocytes were seen in situ, either in larvae or adults, trapped near internal organs. Only a few VLPs were found scattered in the cytoplasm or within lysosomes (Fig. 23). Large concentrations of VLPs and the presence of viroplasm as described in the bean leaf and spotted cucumber beetles (Kim and Scott, 1978; Kim, 1980) were not observed.

Tracheocytes. The tracheal system is scattered throughout the body of *E. varivestis* and appeared in most of the sections.

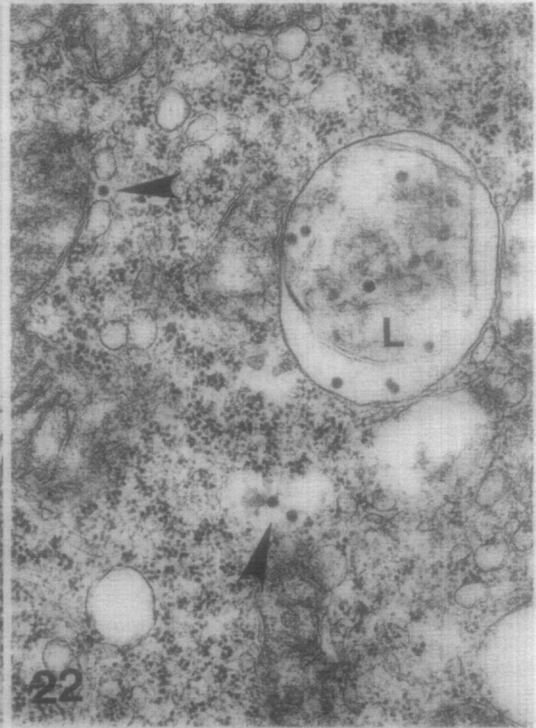
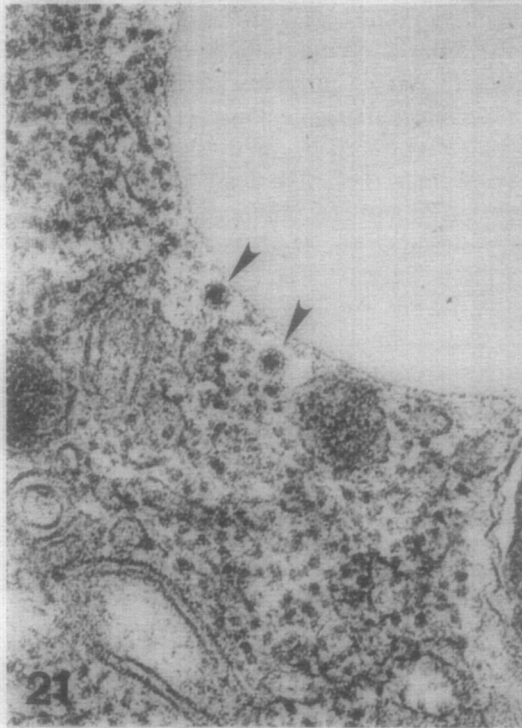
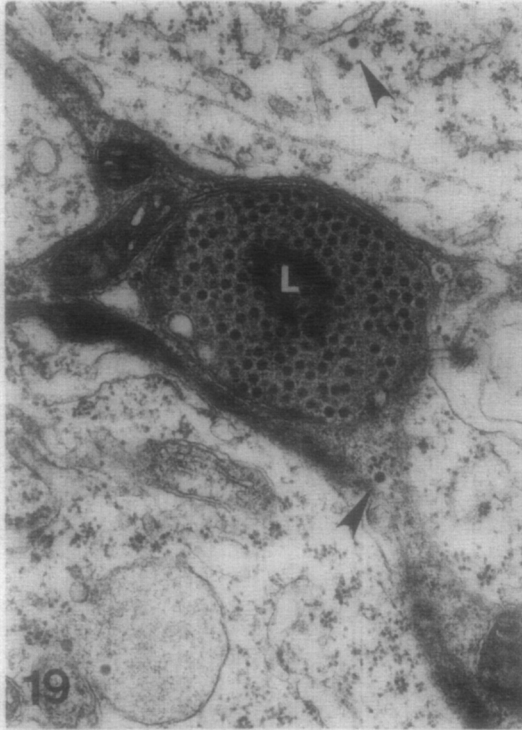
FIG. 14. Part of the epithelium of the maxillary gland. Virus-like particles can be seen either in the cytoplasm (arrowhead) or within a lysosome (L). The cell is rich in smooth and tubular endoplasmic reticulum. $\times 50,000$.

FIG. 15. Lysosomes (L) containing virus-like particles in the epithelium of a Malpighian tubule.

FIG. 16. Low-magnification electron micrograph from the epithelium of the hindgut where virus-like particles appear within lysosomes (arrowheads). In, intima; m, mitochondrion. $\times 7500$.

FIG. 17. A viroplasm in the epithelium of the midgut, close to the nucleus (N). Note a few empty virus-like particles in the viroplasma's matrix. $\times 52,000$.

FIG. 18. Epidermal cell from a larva, with a single virus-like particle (arrowhead). Q, cuticle. $\times 48,000$.



The tracheolar element is surrounded by the tracheocyte, and VLPs were commonly seen within lysosomes (Fig. 24).

Muscles. Muscle fibers are present in most of the beetle body either forming massive groupings as in the flight muscle, heart, and moving parts, or surrounding internal organs such as the intestine and reproductive organs. The muscle fibers exhibit syncytial organization with large nuclei. The VLPs were commonly found in muscle cells within lysosomes or scattered in the sarcoplasm, sometimes interspersed among the myofibrils (Fig. 25). Some nuclei contained VLPs embedded in the chromatin material (Fig. 26).

Some attempts were made to observe the VLPs in vitro in extracts of beetles or isolated germaria by homogenizing in 0.1 M Tris buffer, pH 7.2, and centrifuging the extract at low (8000g/10 min) and high (50,000g/40 min) speeds. The final pellet was resuspended in a drop of buffer, negatively stained with 2% phosphotungstic acid, pH 5.4, and examined with the electron microscope. A few particles of the expected size and exhibiting ultrastructural features resembling known reoviruses were found. These particles usually appeared to be penetrated by the stain and had a hollow center ca. 40 nm in diameter surrounded by a shell that had radial projections ca. 15 nm long (Fig. 27).

DISCUSSION

The virus-like particles found ubiquitously in the tissues of all *E. varivestis* examined from the colony kept at the Vi-

rology and Biocontrol Laboratory of the University of Arkansas are believed to represent a latent and vertically transmitted virus and will be referred to as the Mexican bean beetle virus (MBBV) hereafter. Although critical evidence demonstrating their infectivity is lacking, the morphology and intracellular behavior of the particles suggest that they are viral in nature. This suggestion is strengthened by the fact that in many cells the MBBV particles are associated with viroplasmic structures which have been known in many cases, including reoviruses, as the sites of virus replication and/or assembly (Maramorosch, 1977; Matthews, 1982; Kim, 1980). The absence of any external or behavioral manifestation resembling a pathological condition and the lack of major cytological changes associated with the presence of the particles suggests that this is a latent virus. The presence of the MBBV within the gametes, either oocyte or sperm, is evidence that the virus is transmitted through them from one generation to the next, and also provides a reasonable explanation for the 100% infection in this particular colony. Another line of evidence for vertical transmission is the detection of the MBBV in larvae raised from eggs completely separated from the main colony. Certainly beetles collected from the field and also from colonies kept in other laboratories must be examined to verify whether or not the infection by MBBV is a general phenomenon. Apparently a colony kept in Germany (Schluter et al., 1982), as well as specimens collected in Maryland (Adams et al., 1979) are

FIG. 19. Part of the cortical region of the brain, formed by neurons. A large lysosome (L) is packed with virus-like particles. Some of the virus-like particles also appear in the cytoplasm (arrowhead). $\times 30,000$.

FIG. 20. Tightly packed axons and dendrites in the central region of the brain. A single virus-like particle is indicated by an arrowhead. $\times 32,000$.

FIG. 21. Cytoplasm of a cell from the fat body. Note a large lipid granule with two virus-like particles at its periphery (arrowheads). $\times 80,000$.

FIG. 22. Cytoplasm of an oenocyte, with virus-like particles within a lysosome (L) or scattered in the ground cytoplasm (arrowhead). $\times 30,000$.

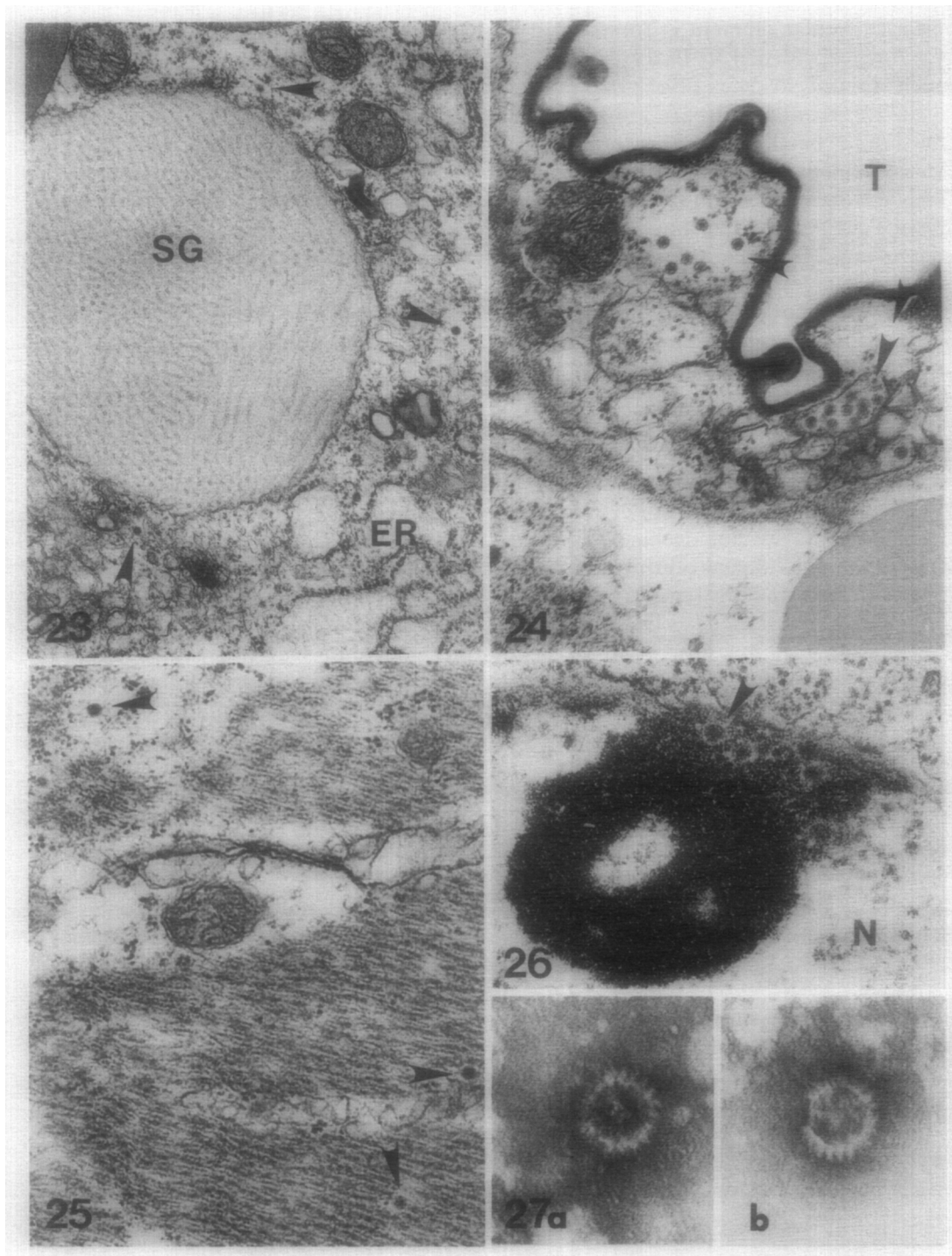


FIG. 23. A structured granule (SG) in a granulocyte-type hemocyte. Few virus-like particles appear scattered in the cytoplasm (arrowheads). $\times 30,000$.

FIG. 24. Cross section of a tracheocyte, showing virus-like particles within lysosomes (arrowheads). T, tracheal lumen. $\times 40,000$.

MBBV-free as judged by examination of the published electron micrographs.

The MBBV particles strongly resemble those of the family Reoviridae both in situ and in vitro. Some members of Reoviridae, such as the cytoplasmic polyhedrosis subgroup, orbivirus and plant reovirus subgroups 1 and 2, infect insects. Since the MBBV seems to be restricted to *E. varivestis* and possibly some other beetles such as bean leaf beetle and spotted cucumber beetles (Kim and Scott, 1978; Kim, 1980), and since the particles are not occluded, it does not fit properly into either of these subgroups. Nevertheless, reovirus-like particles have been described in several insects, and in some cases their infectivity was demonstrated. These are leafhopper A virus, found in the leafhopper, *Cicadulina bimaculata*, possibly associated with the wallaby ear disease of maize, but apparently not multiplying in this plant host (Boccardo et al., 1980); a latent virus described in the planthopper, *Peregrinus maidis*, in Venezuela (Herold and Munz, 1967); and a virus responsible for high mortality rates in a housefly (*Musca domestica*) colony (Moussa, 1978a). In the Queensland fruitfly (*Dacus tryoni*) colony which showed a high mortality rate, Moussa (1978b) observed two types of particles, one smaller ca. 30 nm in diameter which could be extracted, and another ca. 70 nm considered to be the 30-nm particles surrounded by an envelope. The published micrographs of the larger particles closely resemble reovirus, and these fruitflies might have been infected by two different viruses. Transovarially transmitted reovirus-like particles were observed in thrips, *Frankliniella fusca*, collected in Ontario (Paliwal, 1979). In Coleoptera, particles similar to

those of MBBV were described in the bean leaf and spotted cucumber beetles, and cytopathic effects were observed in the latter (Kim and Scott, 1978; Kim, 1980).

These virus and virus-like particles resembling reovirus in insects are tentatively grouped separately from established subgroups of the family Reoviridae (Matthews, 1982), and the MBBV might well be among them.

The dense cytoplasmic inclusions, referred to as viroplasms, seem to be the site of the MBBV synthesis and/or assembly. Such a role has been assigned and was eventually demonstrated in some of the Reoviridae (Favali et al., 1974). The abundance of these inclusions in reproductive organs, particularly in nurse cells, suggests that these organs are the main sites for MBBV replication. Very few particles were found in the oocyte, however, despite the connection with the nurse cells through the trophic chord. Apparently only a few particles manage to migrate from nurse cells to the oocytes, and the dramatic increase in the volume of the oocyte during its development must result in a very large dilution factor, thus making their detection difficult. In the testes, spermatocytes might have viroplasms, but not so often as in the nurse cells of the germarium. However, MBBV particles were consistently found in the primary and secondary spermatocytes as well as in the differentiating spermatids. The large number of particles found in the lysosomes of the cyst cells in harboring spermatids at the final stages of differentiation indicated that there must be an appreciable production of MBBV in the testes. Relatively few, however, are found in the sperm, most of them being eliminated with the cytoplasmic blebs during differentia-

FIG. 25. Muscular mass in the body cavity of a larva. The virus-like particles are in the sarcoplasm or interspersed among the myofibrils (arrowheads). $\times 40,000$

FIG. 26. Nucleus of a flight muscle cell in which a few virus-like particles appear associated with the chromatin material (arrowhead). $\times 60,000$.

FIG. 27. (a) and (b) Negatively stained virus-like particles in extract from ovariole. $\times 225,000$.

tion. In the spermatids MBBV was occasionally present in the cytoplasm but were more commonly embedded in the condensing nucleus. The particles persist in the sperm nucleus even within female organs after copulation. Longitudinal sections clearly show that relatively large numbers of MBBV particles were trapped in the mature sperm nucleus. This is reminiscent of the description of the transmission of a rickettsia-like organism through sperm in some Homoptera (Maillet and Folliot, 1967). In the dipteran, *Coelopa frigida*, isometric virus-like particles were found in the nucleus of developing spermatids, but their fate in the mature sperm is unreported (Schrankel and Schwalm, 1975). Sigma virus of *Drosophila*, a rhabdovirus, is known to be sperm transmitted, but the particles appear to bud from the developing spermatid membrane and are not located in the nucleus (Tennings, 1972). Occasionally MBBV particles are found associated with the chromatin material in the nucleus of some muscle cells and neurons. A similar finding was reported for the reovirus-like particle found in spotted cucumber beetle hemocytes (Kim, 1980). It is not known exactly how the MBBV gets into the nucleus. It might be trapped during cell division or assembled within the nucleus.

In most of the cells MBBV appeared within cytoplasmic structures that were classified as lysosomes on the basis of their granules, droplets, and membranes. For many viruses, including reoviruses, incorporation into phagosomes after viroplasm is considered an essential step in the infection process. It is here that the protein coat is stripped off enzymatically and the viral nucleic acid gains access to the transcribing or translating machinery of the cell (Silverstein and Dales, 1968). In the present case, however, the MBBV-containing lysosomes could represent the process of eliminating the particles from the cell by reverse pinocytosis (exocytosis), thus resulting in an equilibrium state between virus replication

and elimination, which may establish a latent infection. In this connection, it is worthwhile to mention that the particles within lysosomes are often more uniformly dense, without the electron-lucent layer between the outer shell and the core, indicating some change in the particle structure.

The MBBV particles, either in the cytoplasm or in the nucleus, commonly have an electron-lucent halo 50- to 100-nm thick separating them from the adjacent structures. This might result from shrinkage during fixation or, alternatively, from the presence of some outer projections in the particles. Isolated particles did not possess such projections as have been reported in plant reoviruses (Milne et al., 1973), cytoplasmic polyhedrosis virus (Hosaka and Aizawa, 1964), and the leafhopper A virus (Boccardo et al., 1980). They did, however, appear somewhat disrupted, resembling rota- or orbiviruses without the external shell (Murphy et al., 1971; Palmer et al., 1977). Careful purification procedures might eventually reveal the presence of outer spikes.

REFERENCES

- ADAMS, J. R., TOMPKINS, G. J., AND WILCOX, T. A. 1979. Two viruslike particles in the Mexican bean beetle, *Epilachna varivestis*. *J. Invertebr. Pathol.*, **33**, 111-114.
- BERNHARD, W. 1969. A new staining procedure for electron microscopical cytology. *J. Ultrastruct. Res.*, **27**, 250-265.
- BOCCARDO, G., HATTA, T., FRANCKI, R. I. B., AND GRIVELL, C. J. 1980. Purification and some properties of Reovirus-like particles from leafhoppers and their possible involvement in Wallaby ear diseases of maize. *Virology*, **100**, 300-313.
- BÜNING, J. 1978. Development of telotrophic-meristic ovarioles of polyphage beetles with special reference to the formation of nutritive cords. *J. Morphol.*, **156**, 237-256.
- BURGESS, E. D. 1932. A comparison of the alimentary canals of the active and hibernating adults of the Mexican bean beetle, *Epilachna corrupta* Muls. *Ohio J. Sci.*, **32**, 249-261.
- DOUGLAS, J. R., AND PORTMAN, R. W. 1965. "The Mexican bean beetle in Idaho and the West." Idaho Agric. Extension Serv. Bull. No. 443.

- FAVALI, M. A., BASSI, M., AND APPIANO, A. 1974. Synthesis and migration of maize rough dwarf virus in host cell: An autoradiographic study. *J. Gen. Virol.*, **24**, 563-565.
- FELDMAN, J. 1979. "Ultrastructural features during blastoderm formation in the Mexican bean beetle, *Epilachna varivestis* (Mulsant) (Coleoptera: Coccinellidae)." M.S. Thesis, Univ. of Arkansas.
- FULTON, J. P., AND SCOTT, H. A. 1974. Virus vectoring efficiencies of two species of leaf feeding beetles. *Proc. Amer. Phytopathol. Soc.*, **1**, 159 (Abst.).
- GERGERICH, R. C., SCOTT, H. A., AND FULTON, J. P. 1983. Regurgitant as a determinant of specificity in the transmission of plant viruses by beetles. *Phytopathology*, **73**, 936-938.
- HAPP, G. M., AND EISNER, T. 1961. Hemorrhage in a Coccinellid beetle and its repellent effect on ants. *Science (Washington, D.C.)*, **134**, 329-331.
- HEROLD, F., AND MUNZ, K. 1967. Virus particles in apparently healthy *Peregrinus maidis*. *J. Virol.*, **1**, 1028-1036.
- HOSAKA, Y., AND AIZAWA, K. 1964. The fine structure of the cytoplasmic polyhedrosis virus of the silkworm, *Bombyx mori*. *J. Insect Pathol.*, **6**, 53-77.
- JANSEN, W. P., AND STAPLES, R. 1970. Transmission of cowpea mosaic virus by the Mexican bean beetle. *J. Econ. Entomol.*, **63**, 1719-1720.
- KIM, K. S. 1980. Cytopathology of spotted cucumber beetle hemocytes containing virus-like particles. *J. Invertebr. Pathol.*, **36**, 292-301.
- KIM, K. S., AND SCOTT, H. A. 1978. Two types of virus-like particles in the bean leaf beetle, *Cerotoma trifurcata*. *J. Invertebr. Pathol.*, **31**, 77-83.
- MAILLET, P. L., AND FOLLIOT, R. 1967. Nouvelles observations sur le transport de microorganismes intranuclearies, appeles particules Phi, par les spermatoides chez des insectes Homopteres. *C.R. Acad. Sci. Ser. D.*, **264**, 965-968.
- MARAMOROSCH, K. 1977. "The Atlas of Insect and Plant Viruses." Academic Press, New York.
- MATTHEWS, R. E. F. 1982. Classification and nomenclature of viruses. *Intervirolgy*, **17**, 1-200.
- MEINERS, J. P., WATERWORTH, H. E., LAWSON, R. H., AND SMITH, F. F. 1977. Curly dwarf mosaic disease of beans from El Salvador. *Phytopathology*, **67**, 163-168.
- MILNE, R. G., CONTI, M., AND LISA, V. 1973. Partial purification, structure and infectivity of complete maize rough dwarf virus particles. *Virology*, **53**, 130-141.
- MOUSSA, A. Y. 1978a. A new virus disease in the housefly, *Musca domestica* (Diptera). *J. Invertebr. Pathol.*, **31**, 204-216.
- MOUSSA, A. Y. 1978b. A new cytoplasmic inclusion virus from diptera in the Queensland fruitfly, *Dacus tryoni* (Frogg) (Diptera:Tephritidae). *J. Invertebr. Pathol.*, **32**, 77-87.
- MURPHY, F. A., BORDEN, E. C., SHOPE, R. E., AND HARRISON, A. 1971. Physicochemical and morphological relationships of some arthropod-borne viruses to bluetongue virus-a new taxonomic group. Electron microscopic studies. *J. Gen. Virol.*, **13**, 273-288.
- OSSIG, E. 1926. "Insects of Western North America." MacMillan, New York.
- PALIWAL, Y. C. 1979. Occurrence and localization of spherical viruslike particles in tissues of apparently healthy tobacco thrips *Frankliniella fusca*, a vector of the tomato spotted wilt virus. *J. Invertebr. Pathol.*, **33**, 307-315.
- PALMER, E. W., MARTIN, M. L., AND MURPHY, F. A. 1977. Morphology and stability of infantile gastroenteritis virus: A comparison with Reoviruses and blue tongue virus. *J. Gen. Virol.*, **35**, 403-414.
- SCHLUTER, U., HOLST, H., AND RUNNAU, K. C. 1982. Ultrastructural changes in the gut and the Malpighian tubules of *Epilachna varivestis* after application of the new antibiotic Nikkomycin involve osmoregulatory effect. *J. Invertebr. Pathol.*, **39**, 362-372.
- SCHRANKEL, K. R., AND SCHWALM, F. E. 1975. Virus-like particles in spermatids of *Coelopa frigida* (Diptera). *J. Invertebr. Pathol.*, **26**, 265-268.
- SHIKATA, E. 1977. Plant reovirus group. In "The Atlas of Insect and Plant Viruses" (Maramorosch, K., ed.), pp. 377-403. Academic Press, New York.
- SILVERSTEIN, S. C., AND DALES, S. 1968. The penetration of reovirus RNA and initiation of its genetic function in L-strain fibroblasts. *J. Cell Biol.*, **36**, 197-230.
- SRIVASTAVA, V. S. 1959. The maxillary glands of some Coleoptera. *Proc. Roy. Ent. Soc. Lond. (A)*, **34**, 57-62.
- TELFER, W. H. 1975. Development and physiology of the oocytes-nurse cell syncytium. *Adv. Insect. Physiol.*, **11**, 223-319.
- TENNINGS, D. 1972. Etude de la localisation du virus Sigma au cours de la differenciation des cellules germinales de Drosophiles males stabilisees. *Abn. Inst. Pasteur*, **122**, 541-567.
- WATERWORTH, H. E., MEINERS, J. P., LAWSON, R. H., AND SMITH, F. F. 1977. Purification and properties of a virus from El Salvador that causes mild mosaic in bean cultivars. *Phytopathology*, **67**, 169-173.