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Histopathology of the Tropical Fire Ant, *Solenopsis geminata*, Infected with *Burenella dimorpha* (Microspora: Microsporida)

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Pupae of the tropical fire ant, *Solenopsis geminata*, infected by the microsporidium *Burenella dimorpha* develop clear, blister-like areas in the vertex of the head and in the petiole. In sexual pupae, clearing may also develop in the dorsal thorax. The developing eyes of both worker and sexual pupae appear sunken and irregular in outline, with deranged facits. These pathognomonic signs are the result of destruction or inhibition of development of the cuticle. The clear areas result from tissue fluids seeping into the space between the denuded hypodermal tissue and the pupal sheath (old larval integrument). The fat body is progressively diminished and the brain shrinks as the disease progresses. These organs, which are covered by a layer of hypodermal tissues, decrease in mass and recede from the pupal sheath, which is kept extended by tissue fluids. The malformation of the eyes is also the result of destruction of the cuticle. The lenses, which are cuticular, are destroyed, leaving the ommatidia unanchored distally. In advanced infection, the pupae rupture and are cannibalized by adult workers. The workers do not ingest the spores into the crop, but divert them (with other particulate matter) to the infrabuccal cavity. An infrabuccal pellet is formed which is subsequently expelled and fed to fourth instar larvae only. The intracolonial dissemination of spores is thus facilitated by the destruction of the cuticle of infected pupae.

KEY WORDS: *Burenella dimorpha;* microsporida; *Solenopsis geminata;* fire ants; histopathology; insect eye; insect cuticle.

Very few protozoan diseases of insects may be diagnosed by simple observation of pathognomonic signs. Pebrine (infection of the silkworm, *Bombyx mori,* by the microsporidium *Nosema bombycis*) provides a classic example of pathognomonic signs in insects, being characterized by the appearance of dark, pepper-like spots on the integument. Similarly, infection of the tropical fire ant, *Solenopsis geminata,* by the neogregarine *Mattesia geminata,* described by Jouvenaz and Anthony (1979), may be readily diagnosed by eye teratology and a distinctive pattern of abnormal melanization in pupae. In general, however, specific manifestations of protozoan infections, if present at all, may be detected only by histological examination (Brooks, 1974).

Insects infected by protozoa may exhibit nonspecific signs such as loss of appetite, diarrhea, sluggishness, irregular growth, stunted or malformed adults, reduced fecundity, and premature death of immatures or adults. Often, however, there are no in-

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dications of disease other than premature death (Brooks, 1974). Individual red imported fire ant workers, *Solenopsis invicta*, infected with *Thelohania solenopsae* (see Knell et al., 1977), for example, cannot be differentiated from healthy specimens by either appearance or behavior. Not only are bees, *Aphis* spp., infected by *Nosema apis* completely devoid of outward signs of disease, but histological diagnosis is also difficult (Bailey, 1981).

Pupae of *S. geminata* infected by *Burenella dimorpha* exhibit pathological manifestations which are, to the best of our knowledge, unique. Jouvenaz and Hazard (1978) describe these manifestations and attribute them to destruction of hypodermal tissues. The first noticeable change is the appearance of a clear area in the vertex of the head at about the time the developing eyes become prominent. Later, similar clear areas appear in the petiole and gaster, and the eyes become irregular in outline and appear sunken. Pupae having such changes do not mature or even melanize. Instead, the clear areas increase in size, and the cuticle eventually ruptures. Jouvenaz and Hazard (1978) also noted that the binucleate free spores (spores not bound by a pansporoblast membrane) of *B. dimorpha* develop in the hypodermal tissues, and the uninucleate octospores (spores bound by a pansporoblast membrane) develop in the fat body.

The above brief description of the appearance of diseased worker pupae and notation of tissue specificity of the spore types is the only published information on the pathology of *B. dimorpha* infection. In this paper we describe the histopathological basis of these unique signs and discuss their role in the transmission of infection. Also, the appearance of infected sexual pupae is described for the first time.

MATERIALS AND METHODS

Specimens for both light and transmission electron microscopy were embedded in epoxy resin. Tissue specimens (heads, gasters, whole pupae, etc.) were prefixed in buffered 1% osmium tetroxide (0.1 M sodium cacodylate buffer, *p*H 7.5) for 30–60 min at room temperature, rinsed in the same buffer, and partially hardened in buffered 2.5% gluteraldehyde–1% acrolein (same buffer). Specimens were washed in buffer and usually stored in Histocon (polyvinylpyrrolidone, Tris–HCl, 2% chlorhexidine, distilled water; Polysciences, Warrington, Pa.) in the refrigerator overnight or for several days prior to postfixation. The specimens were then washed in buffer, postfixed in buffered 1% osmium tetroxide for 2 hr at room temperature, washed in deionized water, and stained overnight en bloc in 0.5% aqueous uranyl acetate. Specimens were dehydrated with acidified 2,2-dimethoxypropane (Lin et al., 1977) and infiltrated and embedded in a Spurr–Quetol 651 resin (Ringo et al., 1979).

Blocks were sectioned with a LKB Huxley ultramicrotome. For light microscopy, 2- to 4-μm sections were cut on dry glass knives, spread in a drop of 10% acetone, mounted in immersion oil, and studied by phase-contrast microscopy. For transmission electron microscopy, gold sections were poststained with 2% aqueous uranyl acetate followed by lead citrate (Reynolds, 1963). Grids were examined and photographed at an accelerating voltage of 75 kV in a Hitachi H-600 electron microscope.

The laboratory colony of *S. geminata* from which diseased specimens were obtained was maintained as described by Banks et al. (1981).

RESULTS

Portraits of healthy and diseased worker, male, and female pupae are presented in Figures 1–3. The clearing which occurs in the dorsal thorax of the sexual pupae does not occur in worker pupae, and occasionally is reduced or absent in sexual pupae. The eyes of the sexual pupae appear to be less affected than the eyes of the worker pupa; this may be an artifact due to the larger (ca. 16–20× in area) eyes of sexual

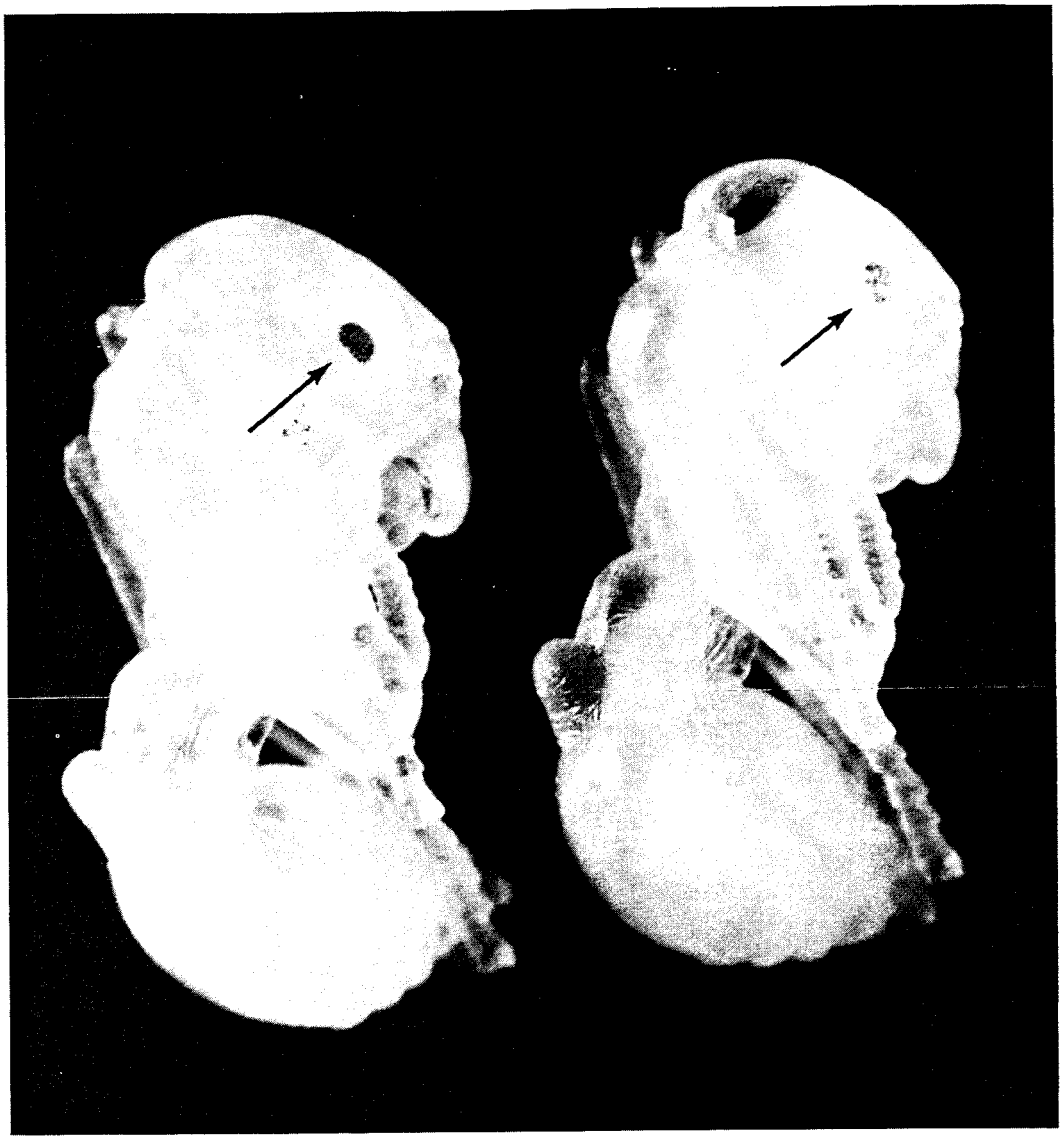


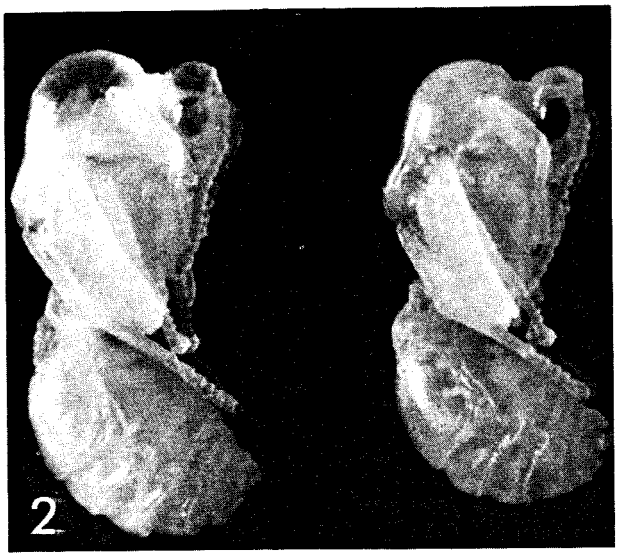
FIG. 1. Healthy (left) and diseased (right) worker pupae of *Solenopsis geminata*. Note eye pathology and clearing in the vertex of the head and in the petiole of the diseased specimen. ×40.

pupae. The diseased worker pupa is in an advanced stage of infection and exhibits maximal development of pathognomonic signs. Note the irregular outline of the eye, the derangement of the facets, and the faint coloration.

The destruction or inhibition of development of the cuticle in infected pupae is shown in Figures 4–7. The healthy, developing cuticle with its trophic microvilli (Fig. 4) is completely absent in the diseased specimen (Fig. 5). The microvilli of the latter extend randomly into a fluid space. Details of the microvilli are presented at higher magnification in Figures 6 and 7. The tissues shown in these electron micrographs are from the vertex of the head where the clearing develops. In tissues where little or no clearing occurs, some cuticle is present (Fig. 16).

The structures of a healthy, developing pupa eye and a diseased eye of similar age are compared at increasing magnifications in Figures 8–13. The lenses, composed of cuticle, are absent in the diseased eye and the remaining components of the ommatidia are twisted, tangled masses. Also, the basement membrane is absent, and only remnants of the lamina ganglionaris may be seen. Pigment granules are present in the pigment cells of the diseased eye.

The fat body is diminished in infected



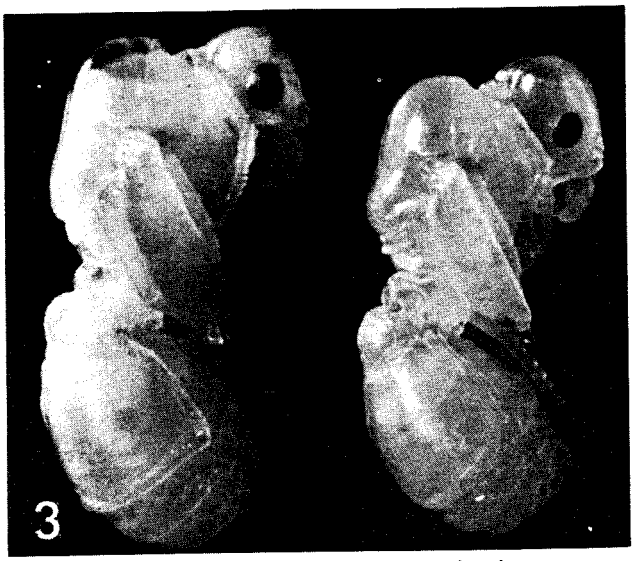


FIG. 2. Diseased (left) and healthy (right) male pupae of *Solenopsis geminata*. Note the clearing in the head and dorsal thorax. The light color of the eye is due to destruction of the lenses and the fluid space over the eye. ×10.

FIG. 3. Diseased (left) and healthy (right) female pupae of *Solenopsis geminata*.

pupae (Figs. 14, 15). Several octospores may be seen in the latter electron micrograph.

The tissue specificities of the two spore types are show in Figure 16. The octospores are confined to the fat body; free spores are confined to hypodermal and connective tissues, including those in deeper parts of the body such as the tissue enveloping the brain.

DISCUSSION

The pathognomonic signs of *B. dimorpha* infection in *S. geminata* reflect damage or destruction of the developing adult cuticle. In the pupal stage of development, the integument of the adult ant forms under the integument of the fourth instar larva. During this period, the larval integument is transformed into a protective sheath that adheres closely to the developing adult integument and is molted when the development of the latter is complete (eclosion). Except in the vertex of the head and in the petiole, the cuticle of pupae infected by *B. dimorpha* appears to be damaged or inhibited only to the extent that tanning is inhibited (Fig. 16; also, the adult morphology is essentially developed). However, in the vertex and petiole the cuticle is completely destroyed (Figs. 4–7). In these areas, tissue fluids seep into the space between the denuded hypodermis and the pupal sheath. As the infection progresses, the fat body is diminished and the brain shrinks. These organs, which are covered by hypodermal tissue, decrease in mass and recede from the pupal sheath, which is kept extended by fluid. Jouvenaz and Hazard (1978) attributed the development of clear areas in the head and petiole to destruction of the hypodermis. More correctly, infection of hypodermal tissue results in destruction or inhibition of formation of the cuticle.

The malformation of the eyes is also due to destruction of the cuticle. The lenses are part of the cuticle, and the remaining components of the ommatidium extend between the lens and a basement membrane. The lenses are nonexistent in the eyes of pupae infected by *B. dimorpha* and the ommatidia are distally unanchored, thus becoming tangled, twisted masses. Perhaps the interstitial matrix is also destroyed. The eyes of pupae infected by *B. dimorpha* develop after cuticle destruction has begun, and are abnormal from the time they first become visible. Thus, the derangement of the eyes is teratologic in nature.

The intracolonial dissemination of *B. di-*

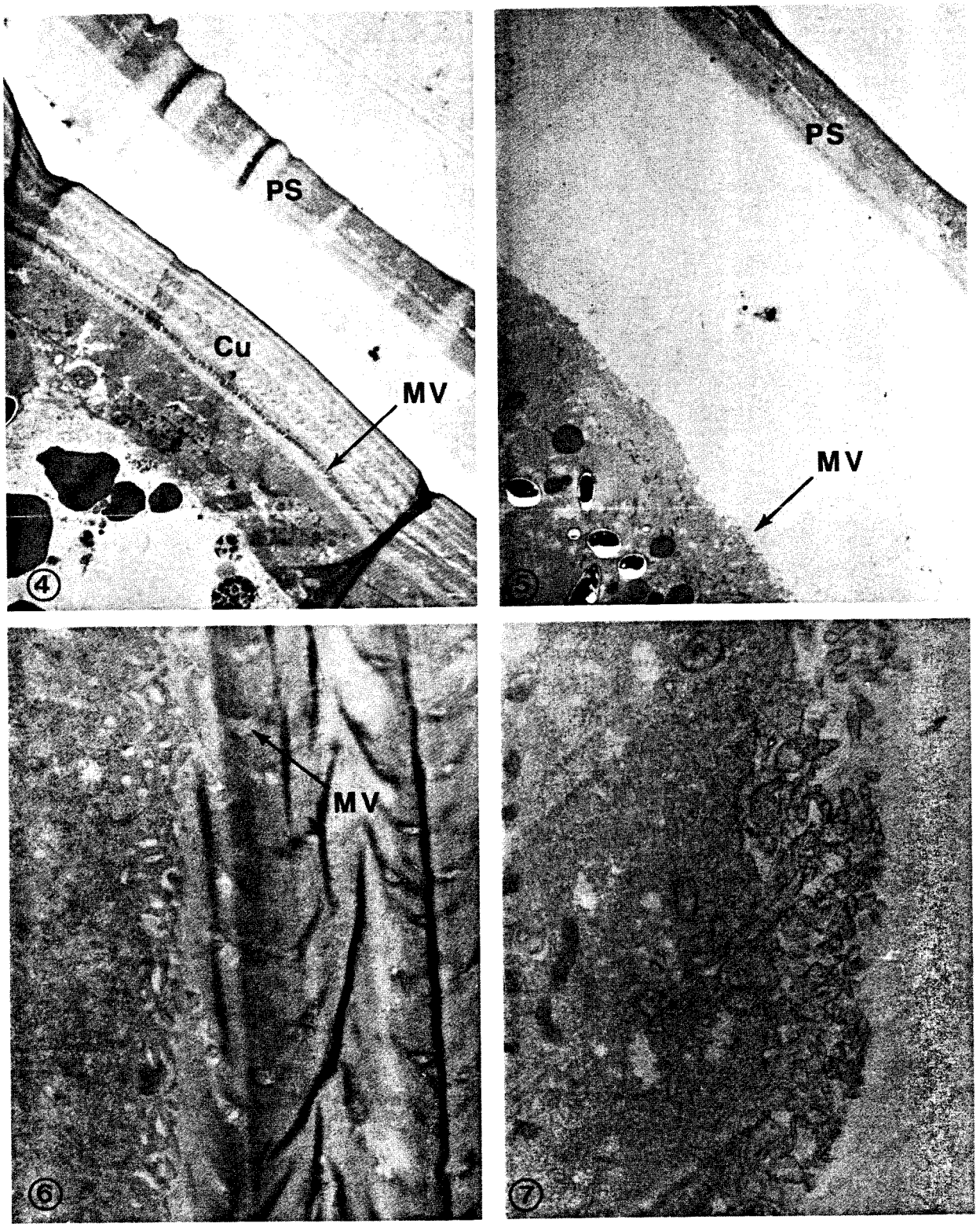


FIG. 4. Cuticle of a healthy pupa. Note the nourishing microvilli. PS = pupal sheath; MV = microvilli; Cu = cuticle. ×1500.

FIG. 5. Body surface of a diseased pupa. There is no cuticle, and the microvilli that normally penetrate the cuticle extend randomly into the fluid space. PS = pupal sheath; MV = microvilli. ×1500.

FIG. 6. Detail of the cuticle of a healthy pupa. Note the microvilli penetrating the cuticle. MV = microvilli. ×12,500.

FIG. 7. Detail of microvilli extending into the fluid space of a diseased pupa. ×12,500.

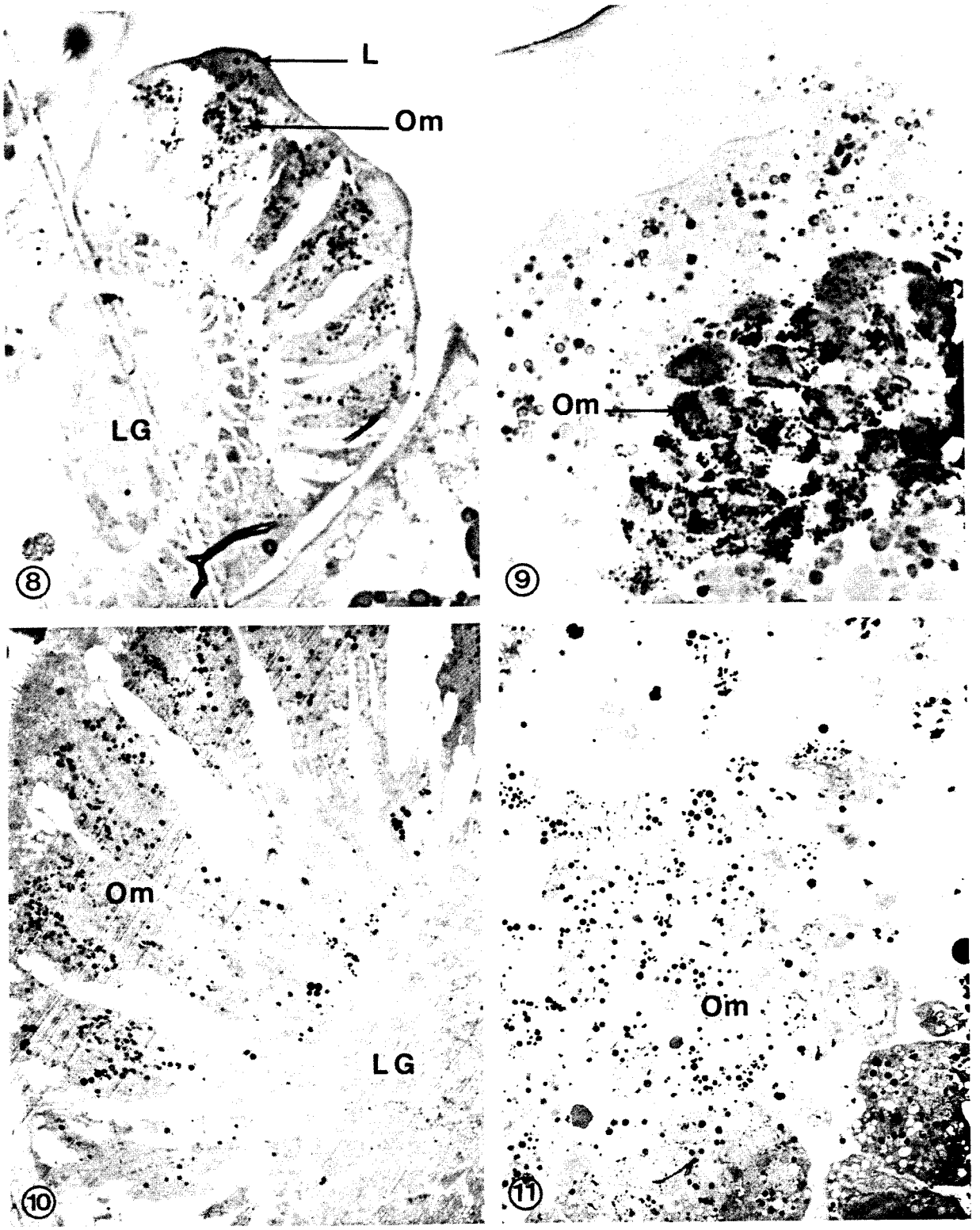
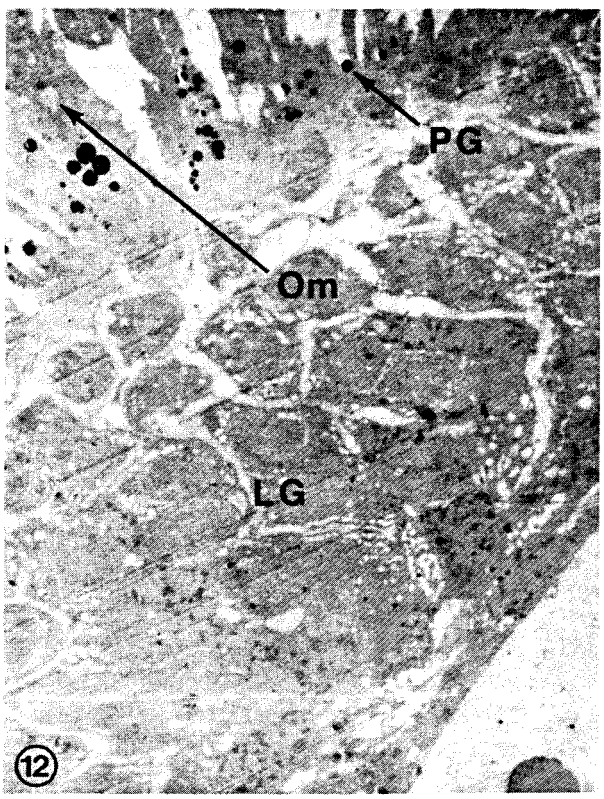


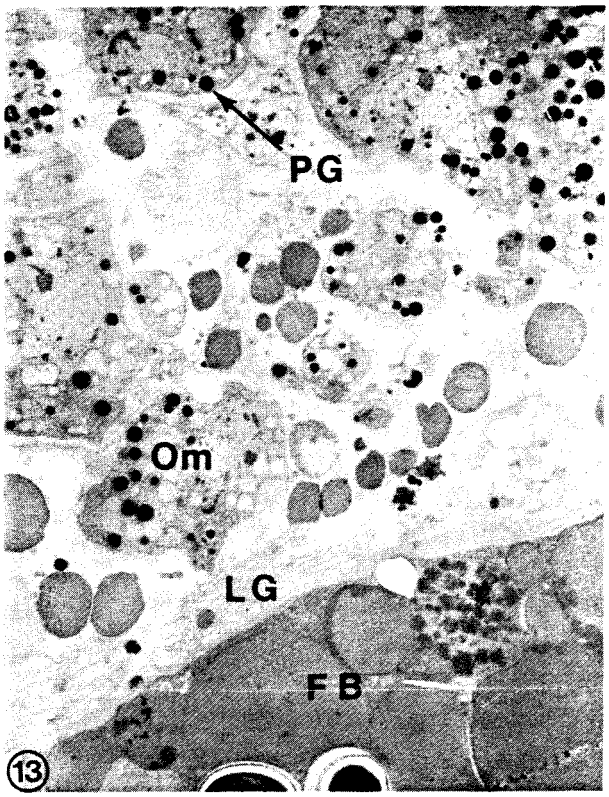
FIG. 8. Sagittal section through the developing eye of a healthy pupa. L = lens; Om = ommatidium; LG = lamina ganglionaris. ×450.

FIG. 9. Sagittal section through the eye of a diseased pupa. There is no lens and the ommatidia are twisted and tangled in an amorphous mass. ×450.

FIG. 10. Sagittal section through the eye of a healthy pupa. ×1500.

FIG. 11. Sagittal section through the eye of a diseased pupa. ×1500.





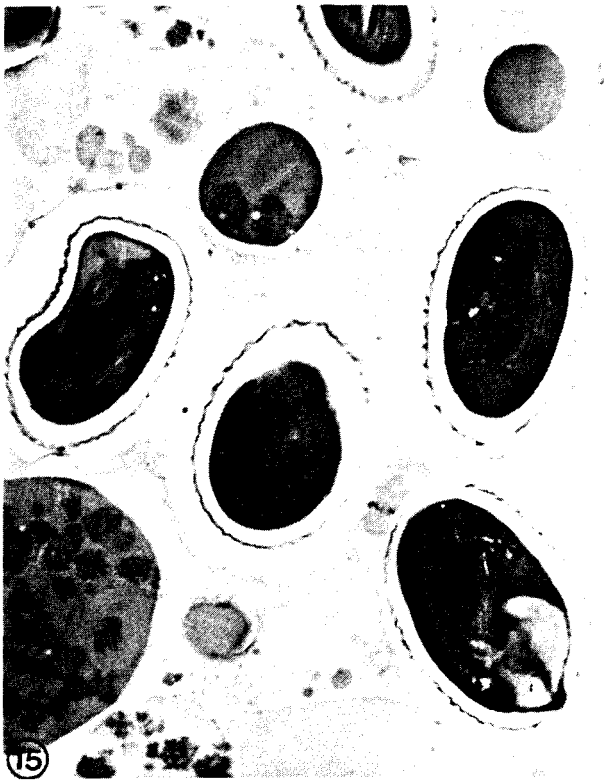


FIG. 12. Sagittal section through the developing eye of a healthy pupa showing detail of lamina ganglionaris and base of the developing ommatidium. OM = ommatidium; LG = lamina ganglionaris; PG = pigment granules. ×3000.

FIG. 13. Sagittal section through the eye of a diseased pupa. Only remnants of the lamina ganglionaris may be seen. The basement area is destroyed. FB = fat body; LG = lamina ganglionaris; PG = pigment granules. ×3000.

FIG. 14. Fat body of a healthy pupa. ×7500.

FIG. 15. Fat body of a diseased pupa. Note the depleted appearance of the fat body and the presence of several octospores. ×7500.

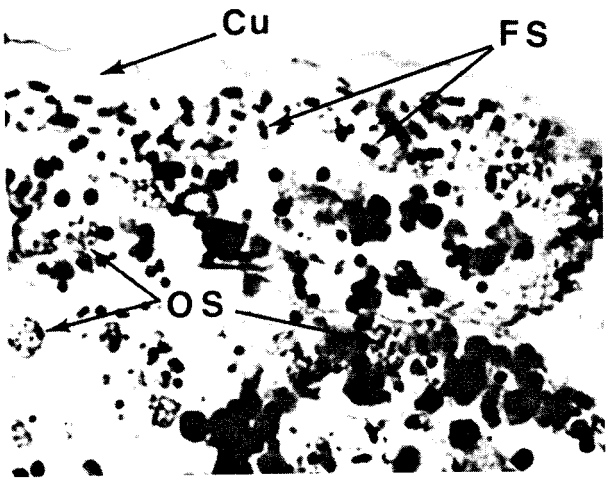


FIG. 16. Tissue specificities of spores of *B. dimorpha*. Free spores are confined to the hypodermal tissues. Octospores are confined to the fat body. Some developing cuticle is seen in this section from the gaster. Cu = cuticle; FS = free spores; OS = octospores. ×230.

morpha spores is facilitated by the destruction of the host cuticle. In advanced infections, the integument (pupal sheath only) which covers the now extensive clear areas becomes extremely fragile and eventually ruptures. The adult worker ants cannibalize these ruptured pupae, which harbor maximal numbers of mature spores. However, the spores are not ingested into the crops of the workers, but are filtered, retained in the infrabuccal cavity, and formed into a pellet with other particulate matter (Figs. 17, 18). (See Glancey et al. 1981 for a discussion of the filtration apparatus of *Solenopsis* spp.). The infrabuccal pellets are expelled and fed to fourth instar larvae only; younger larvae are fed liquids. Thus, the intracolonial cycle of infection is from ruptured, diseased pupae to fourth instar larvae via adults who act as mechanical vectors (Jouvenaz et al., 1981).

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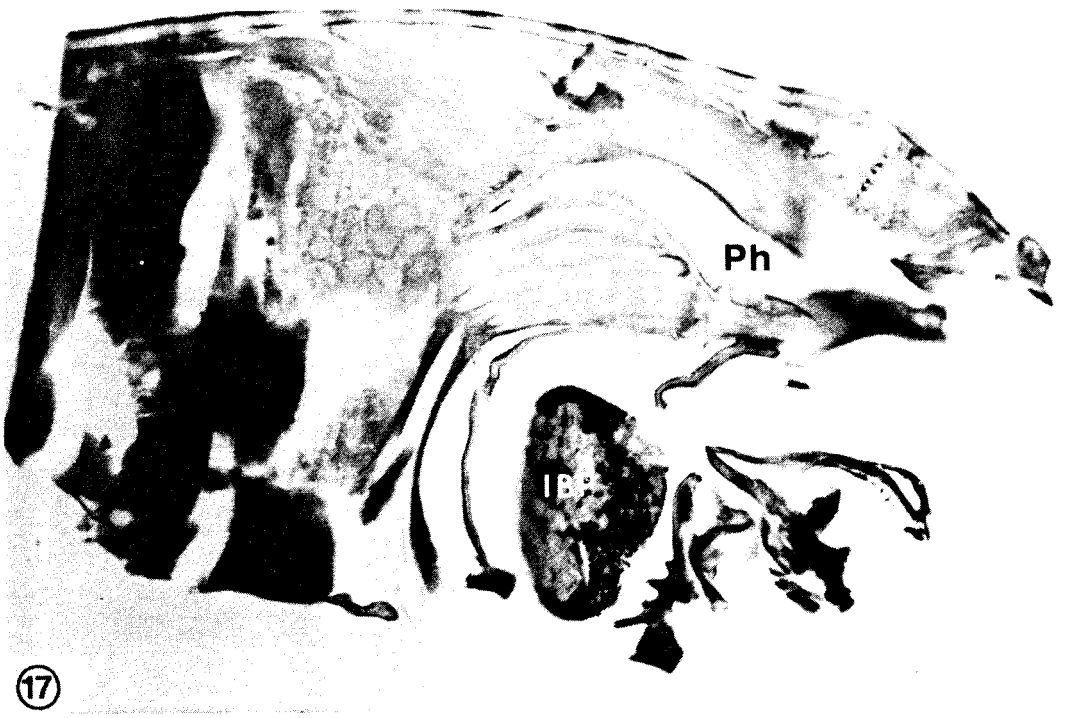

FIG. 17. Infrabuccal cavity. Medial section of an adult fire ant worker head containing an infrabuccal pellet in the infrabuccal cavity. Ph = pharynx; IBP = infrabuccal pellet. ×140.

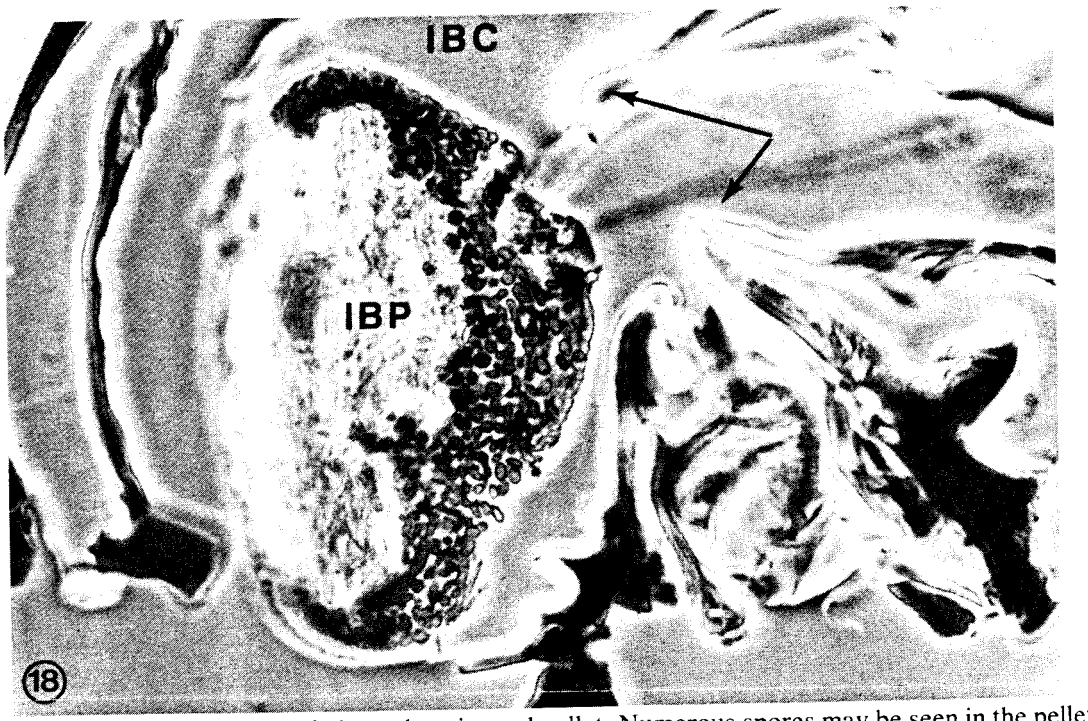

FIG. 18. Enlarged view of infrabuccal cavity and pellet. Numerous spores may be seen in the pellet. Arrows point to valves of the orifice of the infrabuccal cavity. ×350.

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