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THE HINDGUT ULTRASTRUCTURE, AND EXCRETORY PRODUCTS OF LARVAE OF THE IMPORTED FIRE ANT, *SOLENOPSIS INVICTA* BUREN *

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SUMMARY

The ultrastructure and excretions of the hindgut of larvæ of the imported fire ant, *Solenopsis invicta* Buren, is described. The anterior or base of the hindgut is closed and rooted (via the " dome ") in the posterior wall of the midgut, and is composed of basal cells of both the malpighian tubules and the small intestine (ileo-colon). The ileo-colon opens via a rectal valve into the rectum and transfers uric acid (white excretion) secreted by the malpighian tubules, into the rectum. The thin walled rectal epithelium has three rectal pads composed of large, cuboidal cells. The excretory product of larvæ consists of 2 components, a white precipitate composed of uric acid and a clear liquid consisting of water and salts. Ultrastructural and observational studies suggest that the rectal pads and malpighian tubules secrete the clear fluid from the hemolymph. Results of experiments with the waste products indicate that larval excretions are not ingested by other members of the colony except adults which under water stress do consume some of the clear liquid. The adults do participate in the removal of the excretory products from the brood chamber.

RESUME

L'ultrastructure de l'intestin postérieur et les matériaux excrétés chez la larve de la « Fourmi de feu » Solenopsis invicta **Buren**

Nous décrivons dans cet article l'ultrastructure et la fonction de l'intestin postérieur chez la « fourmi de feu » *Solenopsis invicta* Buren. La base de l'intestin postérieur est fermée et insérée (par le « dôme ») dans la paroi postérieure de l'intestin moyen ; elle est composée de cellules basales provenant à la fois des quatres tubes malpighiens et de

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l'intestin postérieur (ileo-colon). L'ileo-colon débouche, grâce à une valvule rectale, dans le rectum, et y déverse de l'acide urique (excrétion blanchâtre) sécrété par les tubes malipighiens. L'épithélium rectal à paroi mince possède trois papilles rectales composées de grosses cellules cuboïdes. Les matériaux excrétés par la larve consistent en deux éléments : un précipité blanc composé d'acide urique et un liquide clair composé d'eau et de sels. Les observations ultrastructurales suggèrent que les papilles rectales et les tubes malipighiens sécrètent le liquide clair à partir de l'hémolymphe ; les résultats d'expériences menées sur les excréments indiquent par ailleurs que les excrétions larvaires ne sont pas ingérées par les autres membres de la colonie, à l'exception des adultes qui mangent du liquide clair en cas de déficit en eau. Les adultes transportent les matériaux excrétés hors du nid.

INTRODUCTION

A control measure for the imported fire ant *Solenopsis invicta* Buren, a serious pest in the southeastern United States, may involve the use of food baits which are fed directly to the larvae (PETRALIA and VINSON, 1978). The success of this idea will require an adequate knowledge of the biology of the larvae, particularly nutrient cycling and the fate of various components in the food. To this end, the feeding behavior (PETRALIA and VINSON, 1978), external (PETRALIA and VINSON, 1979), and internal anatomy (PETRALIA and VINSON, 1980) of larvae have been described.

It is important to determine the excretory products of larvae and how these products are handled by adult workers. O'NEAL and MARKIN (1973) report that the excreta of the larvae consist of 2 separate fractions, a white precipitate and a clear watery excretion. They suggest that workers ingest the white precipitate and discard the clear excretion. This, in turn suggests that the white excretory product may contain nutrients recycled in the colony. In support of this, amino acids have been found in the excreta of larvae of other ants (MASCHWITZ, 1966 ; WÜST, 1973). However, no chemical analysis has been done on the excretion of imported fire ant larvae.

BONAVITA, COUGOURDAN and POVEDA (1972) report that the midgut may connect to the hindgut via a " filtering organ " which allows excess water to pass directly from the midgut into the hindgut to eventually be ingested again by the adult workers (LE MASNE, 1953) but ATHIAS-HENRIOT (1947) suggests that excess water might be removed from the hemolymph via the rectum.

Although the general anatomy of the hindgut of the larvae of fire ants has been described (PETRALIA and VINSON, 1980) as well as that of other ants (PÉREZ, 1902 ; ATHIAS-HENRIOT, 1947 ; LAPPANO, 1958 ; NITSCHMANN, 1959 ; BONAVITA-COUGOURDAN and POVEDA, 1972), no ultrastructural examination has been reported.

We report on the ultrastructure and physiology of the larval hindgut,

chemical analysis of the excretory products of larvae and behavior of the colony towards larval excretory products.

MATERIALS AND METHODS

Mature colonies of *S. invicta* were maintained according to PETRALIA and VINSON (1978). For anatomical studies, combined midguts and hindguts were dissected from 4th instar larvæ in cold Pringle's physiological saline (PRINGLE, 1938). Both excised digestive tracts and whole, live larvæ were placed in Reichard capsules and fixed under vacuum in 3 % glutaraldehyde-3 % acrolein in cacodylate buffer for 3-4 hours. Some live larvæ were fixed via phase-partition fixation, by placing larvæ in Reichard capsules and submersing these in the heptane phase (heptane : 25 % glutaraldehyde :: 5 : 1) for 2 minutes (ZALOKAR and ERK, 1977). These larvæ were then fixed as above. For both methods larvæ in Reichard capsules were post-fixed in 2 % osmium tetroxide for 2 hours, and then bulk stained in 2 % uranyl acetate overnight, both under vacuum. Specimens were then dehydrated, imbedded in Spurr's medium (SPURR, 1969) and sectioned with an ultramicrotome (using glass knives). Serial thick sections (0.5 — 2 μm) were examined to determine areas requiring ultrastructural study. Thin sections in these areas were then mounted on formvar coated single-grids (1 × 2 mm), stained with uranyl acetate and lead citrate, and examined at 50 kV in a Hitachi 8 transmission electron microscope.

The identification of the excretory products of larvæ was obtained from excrement collected from over 200 fourth instar larvæ. Gentle prodding of the mouthparts or ventral region usually caused the release of a small drop of excretion at the anus. The excretion was collected in a micropipette drawn to a fine point and secured to a 10 μl syringe. The secretion was separated into a white precipitate and a clear fluid in a microcentrifuge at 15,000 × G for 10 min.

IR spectra were recorded on a Beckman IR8 spectrophotometer on KBr pellets. Liquid chromatography was performed using Waters ALC 200 unit using 60 % distilled water — 40 % methanol or pure distilled water as solvent, flow rate 1 ml/min. Thin layer chromatography was performed on commercial microcrystalline cellulose plates with water buffered to pH 11 as solvent and iodine vapor as developing agent. NMR spectra were recorded on a JEOL FX-90Q FT-NMR in 5 mm tubes.

The behavior of the colony towards the larval excretory products was ascertained from the excretory products removed from larvæ as described for the product identification portion of this study. White precipitate, clear excretory product, and a mixture of the two were applied to small circles of filter paper (about 2 mm). Paper circles soaked in a saturated solution of allantoic acid, uric acid, combination of allantoic acid and uric acid, and distilled water were used as controls. The filter paper discs as well as 2 μl droplets of the above solution placed on a small piece of plastic were presented to colonies and the adults' behavior towards them was noted. In addition, 5 hours of worker-larval interactions with regard to larval excretion was recorded.

Methylene blue was added to some samples of the larval excretion consisting of both the clear and precipitate portions, and 2 μl of the dyed excretion was presented to adult ants applied as a small drop to a piece of plastic. Adults that appeared to ingest the dyed excretory product were collected and dissected to determine the location of the dye and uric acid granules within the digestive system. Uric acid crystals were placed in several colonies and the ants' response was noted.

The density of the hemolymph and clear excretion was determined by the method of PATTON (1962), using a 50 ml graduated cylinder containing bromobenzene-kerosene gradients held in a constant temperature bath at 25°. Copper sulfate solutions for standardization were prepared by the method of HAWK *et al.* (1947). Reproductive larvæ were used in

these studies due to their larger size. The anus of a larva dilated with forceps gave a drop of clear excreta which was collected in a one microliter (Microcap ®) capillary tube. The cuticle was then punctured dorsolaterally with a minuten pin, pressure was applied to the body and hemolymph was collected in a one microliter capillary tube. Densities were taken at three and five minutes after addition of the sample to the gradient. Larvæ from a dry environment were prepared for this experiment by removal from a colony and storage overnight in a dry plastic dish. Some larvæ exposed to dry conditions by the above method were placed in a petri dish between layers of well-wetted filter paper and allowed to stand for four hours to prepare larvæ exposed to moist conditions for comparison. In another experiment larvæ used were freshly floated out of a colony by the method of JOUVENAZ *et al.* (1977), and others were allowed to remain with workers in a dry environment for 5 additional hours before being used in density measurements.

It appeared impractical to determine the specific osmotic pressure of the hemolymph and excretory material due to the small volume available. We thus determined the relative osmotic pressure of the hemolymph and excretory material as follows. One half microliter of excretory fluid was collected with a micropipette and placed in a 1 µl capillary tube (Microcap ®). The fluid containing end was then sealed with a small amount of wax. The procedure was repeated with hemolymph obtained via a small puncture near the posterior heart. The open air containing ends of the two capillary tubes were sealed together so the air space between the two tubes were joined. The linear space occupied by the hemolymph and excretory fluid at the opposite ends of the sealed tube were measured with the aid of an ocular micrometer and were measured 24 h. later after the two solutions had a chance to come to equilibrium via the connecting air space. Reproductive larvæ used in the study consisted of those held in a humid environment and dry environment as described above.

Dyes were fed and injected into the hemocoel to aid in ascertaining the movement of liquids from the midgut into the rectum. One percent solutions of neutral red, nile blue or methylene blue in distilled water or 10 % sugar solution were fed to fourth instar larvæ by placing a drop of the solution on the food basket (PETRALIA and VINSON, 1978). These solutions were injected into the hemocoel with glass micro-needles. The needles were fashioned by drawing out capillary tubes on an electrode puller and the needles were attached to a 10 µl syringe. To obtain adequate pressure, it was necessary to fill the syringe and glass needle with mercury. The modified syringe was attached to a micro-applicator (Instrumentation Specialties Co., Lincoln, Nebraska, Model M) at a setting of 1. Several microliters of dye solution were drawn into the needle and 0.01 µl was injected into the hemocoel of fourth instar larvæ dorsally just behind the head capsule. Larvæ fed or injected with dye were observed every half hour and the location of dye noted in the midgut, hemocoel, malpighian tubules and rectum.

RESULTS AND DISCUSSION

Anatomy and ultrastructural studies

The base (anterior end) of the hindgut is rooted between the midgut epithelial cells, which completely seal off the connection between the lumina of the midgut and hindgut *(fig. 1, 2, 3)* until pupation when the lumen of the digestive system becomes continuous. In some specimens, the midgut epithelial cells directly anterior to the base of the hindgut were less electron dense after staining than adjacent midgut cells.

The hindgut is composed of 3 major cavities *(fig. 1, 2)*: the " superior

cavity" of BONAVITA-COUGOURDAN and POVEDA (1972); the ileo-colon (or small intestine); and the rectal cavity. The "inferior cavity" *(op. cite.)* is a distinct structure in the larvae of some ants, but appears to be fused with and indistinguishable from the ileo-colon in *S. invicta.* Thus, PETRALIA and VINSON (1980) use the terms "small intestine" (i.e., ileo-colon) and "inferior cavity" synonymously. The superior cavity is formed by the intersections of the 4 malpighian tubules, and the cavity continues posteriorly to open into the lumen of the ileo-colon. The superior cavity is bounded on the anterior side by several layers of dome cells *(op. cite.)* which are imbedded between the posterior portion of the midgut and the basal cells of the malpighian tubules *(fig. 1 b, 3).* A ring of muscle cells surround the base of the hindgut just posterior to the dome cells. The tapered basal ends of the dome cells at their junction with the ring of muscle cells contain thick, palmately-bran-

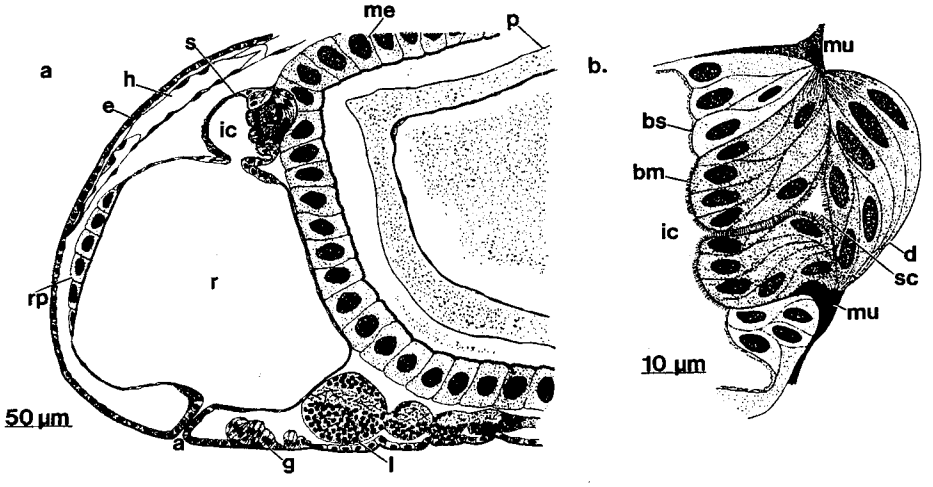

Fig. 1. — a) Drawing based on thick posterolongitudinal sections of the fourth instar worker larvæ. Anus (a), epidermis (e), gonopodal discs (g), heart (h), ileo-colon cavity (ic), last abdominal ganglion (1), midgut epithelium (me), peritrophic membranes (p), rectum (r), rectal pad (rp), small intestine (= ileo-colon) (s).
b) Drawing based on longitudinal sections of the base of the hindgut, magnified from *figure 1a.* Basal cells of the malpighian tubules (bm), basal epithelial cells of the ileo-colon (bs), dome (d), ileo-colon cavity (ic), muscular tissue (mu), superior cavity (sc).

Fig. 1. — a) Dessin basé sur d'épaisses sections postéro-longitudinales de la larve d'ouvrière après la quatrième mue. (a) anus, (e) hypoderme, (g) disques gonopodes, (h) cœur, (ic) cavité de l'ileo-colon, (l) dernier ganglion abdominal, (me) épithélium de l'intestin moyen, (p) membranes péritrophiques, (r) rectum, (rp) papilles rectales, (s) ileo-colon.
b) Dessin basé sur des sections longitudinales de la base de l'intestin moyen, agrandissement obtenu à partir de l'illustration 1a. (bm) cellules basales des tubes malpighiens, (bs) cellules basales et épithéliales de l'ileo-colon, (d) dôme, (ic) cavité de l'ileo-colon, (mu) tissus musculaires, (sc) cavité supérieure.

ching double membranes (infoldings of the basal plasma membrane) arranged perpendicular to the long axis of the superior cavity of the hindgut and midgut lumen. Between these infoldings are numerous microtubules *(fig. 4-6)*. There is no cytological evidence for the filtering of water from the midgut directly to the hindgut as suggested by BONAVITA-COUGOURDAN and POVEDA (1972). The midgut epithelium is continuous even anterior to the dome cells of the hindgut. However, in late fourth instar larvae entering the prepupal stage, the midgut epithelium anterior to the dome cells has enlarged intercellular spaces possibly in preparation for the passing of the meconium. The most posterior dome cells appear to be continuous with the basal cells of the malpighian tubules which have infoldings of the plasma membrane near their base in contact with the muscle cells. The basal cells of the malpighian

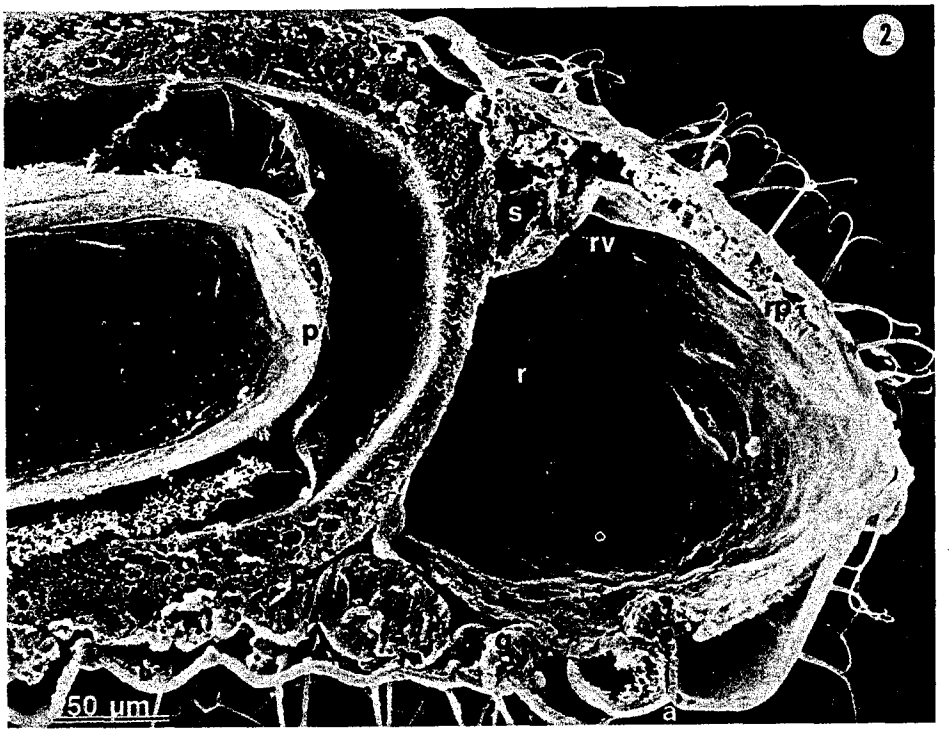

Fig. 2. — Scanning electron micrograph of posterolongitudinal section of a fourth instar worker larva. Anus (a), gonopodal discs (g), last abdominal ganglion (1), midgut epithelium (me), peritrophic membranes (p), rectum (r), rectal pad (rp), rectal valve (rv), small intestine (=ileo-colon) (s).

Fig. 2. — Photographie au microscope à balayage d'une section postéro-longitudinale d'une larve d'ouvrière après la quatrième mue. (a) anus, (g) disques gonopodes, (l) dernier ganglion abdominal, (me) épithélium de l'intestin moyen, (p) membranes péritrophiques, (r) rectum, (rp) papilles rectales, (rv) valvule rectale, (s) ileo-colon.

tubules have a thin microvillar border continuous with that of the malpighian tubule cells.

Lateral to and surrounding the basal cells of the malpighian tubules are the basal epithelial cells of the ileo-colon which lack a microvillar border but bear a thin, convoluted, loosely attached lining continuous with the cuticular lining of the ileo-colon *(fig. 1 b, 3)*. The nuclei of the basal epithelial cells of the ileo-colon stain less dense than the nuclei of the basal cells of the malpighian tubules.

The ileo-colon opens via a muscular rectal valve into a thin walled rectum composed mostly of flat epithelial cells *(fig. 1 a, 2)*. Large, cuboidal, epithelial cells make up the 3 rectal pads (1 dorsal and 2 lateral) *(fig. 1 a, 2, 7, 8)*.

Rectal pad anatomy is similar to that found in larvae of the ant, *Myrmica ruginodis* Nyl (NITSCHMANN, 1959). Rectal pad ultrastructure is similar to that found in other insects (SMITH, 1968 ; WALL and OSCHMANN, 1973 ; LEADER and GREEN, 1978), and analogous to the " primary folds " in the hindgut of Protura (DALLAI, 1977). It is less complex than the anatomy of rectal pads of adult blowflies (BERRIDGE and GUPTA, 1968).

The intercellular junctional membranes of rectal pad cells have septate desmosomes near the apical and basal ends, and a desmosome (macula adhaerens) on the apical end. The cellular membrane of the apical border is deeply infolded forming numerous canaliculi with numerous elongate mitochondria between them *(fig. 7, 8)*.

As in other insects (SMITH, 1968) intercellular junctional complexes (septate desmosomes and macula adhaerens) are found near both the apical and basal borders of the rectal pad cells, and probably function to prevent passive movement of material between cells from the lumen to the hemolymph and the reverse. The apical infoldings and elongate mitochondria probably function as an active transport mechanism. This mechanism serves to absorb water from the rectal lumen in other insects (BERRIDGE and GUPTA, 1967 ; SMITH, 1968 ; WALL and OSCHMAN, 1973 ; LEADER and GREEN, 1978). However, larvae of most ants are faced with the problem of excreting excess water, due to excessive amounts of liquid in their diet and to their humid environment (ATHIAS-HENRIOT, 1947 ; BONAVITA-COUGOURDAN and POVEDA, 1972). ATHIAS-HENRIOT (1947) suggests that excess water is removed from the hemolymph via the rectal wall.

The rectal pads of larvae of the imported fire ant have apical infoldings somewhat smaller than but similar in structure to those of the salt-water mosquito, *Aedes campestris* Dyer and Knab. These mosquitoes actively secrete ions into the rectum to maintain a rectal fluid which is hyperosmotic to the hemolymph (PHILLIPS, 1977).

The rectum opens via a long narrow anal valve passing a thickened cuticular intima continuous to the anus *(fig. 1 a, 2)*.

Excretory product identification

The white excretory precipitate was found to be crystalline and highly insoluble in water and methanol but soluble in pH 11 buffer. A sample dissolved in buffer was subjected to thin layer chromatography on cellulose plates and produced a single spot which had the same Rf value as a sample of commercial (Aldrich) uric acid.

A sample of the white excretory precipitate was allowed to equilibrate with water and the saturated supernatant solution was injected onto a C18 reverse phase liquid chromatography column (25 cm × 4.7 mm, 10 m). With solvents of 60 : 40 : : water : methanol and water, retention time of the only major peak was identical to that of commercial uric acid. An infrared spectrum of the ant excretion was very similar to that of commercial uric acid, although some peak broadening due to impurities was noted. Thus the white precipitate appears to be uric acid.

The clear excretion was freeze-dried and a NMR spectrum taken to test for the presence of organic materials. In both CD_3CN and D_2O, no significant peaks due to organic material were noted, although in CD_3CN, material containing an acidic proton was noted. Since this peak, along with some others at 2-3 ppm, was not seen in D_2O, due to solvent exchange, a sample was subjected to high pressure liquid chromatography as before. As expected we detected the presence of a small amount of uric acid along with an even smaller amount of material with a retention time of allantoic acid, a breakdown product of uric acid. From the small size of the NMR peaks relative to the mass (100 μg) of material subjected to NMR analysis it was concluded that the major part of the dried clear excretion consist of inorganic salts.

General physiology and behavior of adults towards larval excretory products

When larvae were exposed to water by their placement between sheets of wet filter paper after being placed for 12 hr in dry conditions, they increased in weight by 4.7 to 7.8 percent. The density of the hemolymph of larvae

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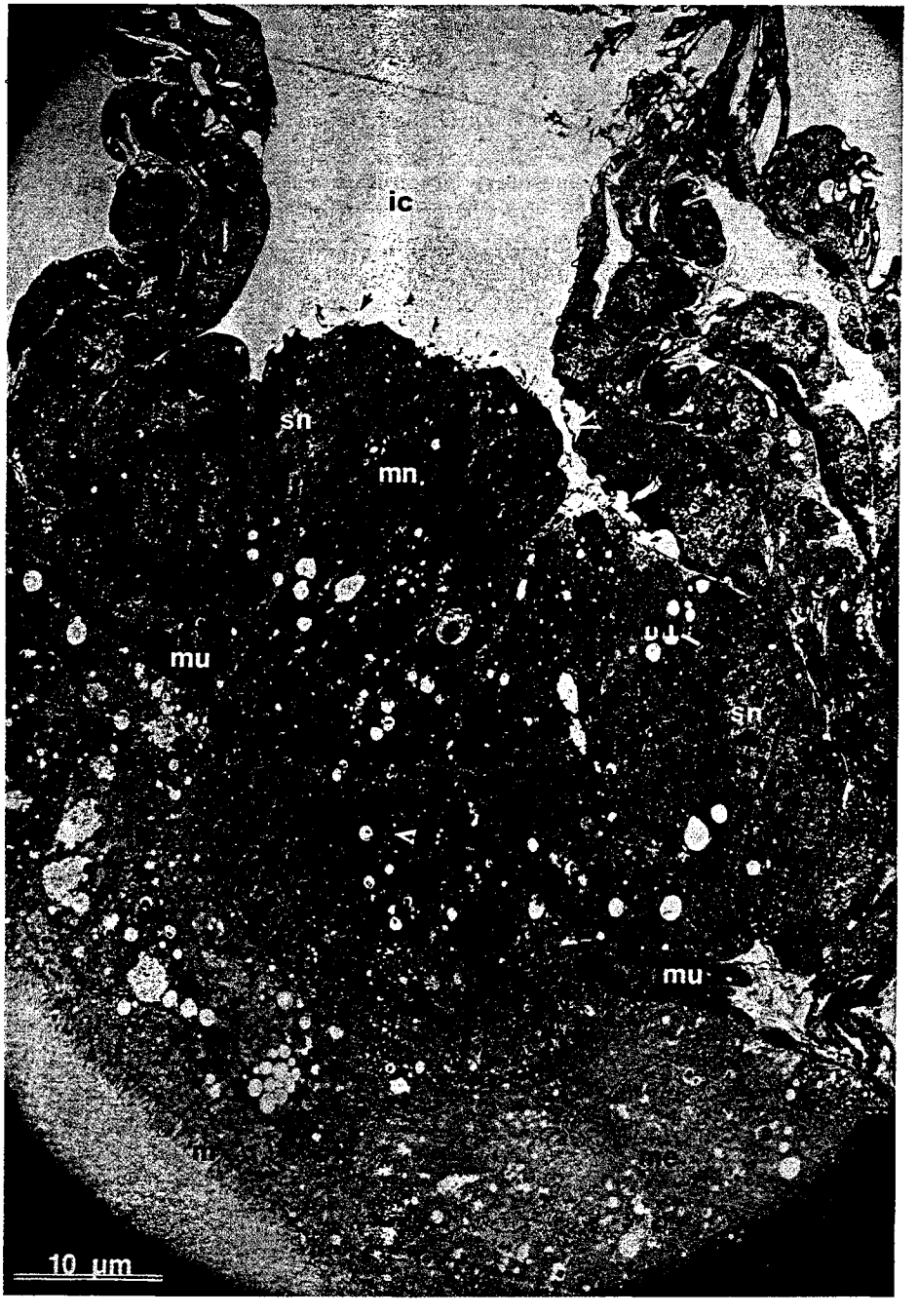
Fig. 3. — Longitudinal section through the base of the gut. Note the dense staining nuclei (mn) and microvillar border (small arrow) of the basal cells of the malpighian tubules, compared to the lighter staining nuclei (sn) and cuticular border (larger arrow) of the basal epithelial cells of the ileo-colon. Dome (d), ileo-colon cavity (ic), microvillar border (m) of midgut epithelial cells (me), muscular tissue (mu), superior cavity (sc).

Fig. 3. — Coupe longitudinale de la base de l'intestin moyen ; noter le noyau fortement coloré (mn) et la bordure microvillaire (petite flèche) des cellules basales des tubes malpighiens, comparé avec le noyau moins fortement coloré (sn), et la bordure cuticulaire (grande flèche) des cellules basales et épithéliales de l'ileo-colon. (d) dôme, (ic) cavité de l'ileo-colon, (m) bordure micro-villaire des cellules épithéliales de l'intestin moyen (me), (mu) tissus musculaires, (sc) cavité supérieure.


ic
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exposed to dry conditions for 24 hr (ave. 1.0191, N = 11) was greater than the clear rectal excretion (ave. 1.0081, N = 11). Upon exposure of larvae to wet conditions in the absence of workers the density of the hemolymph decreased (ave. 1.0165, N = 11) as did the rectal excretion (ave. 1.0077, N =11). When larvae were floated from a colony (thus exposed to water) the density of the hemolymph (ave. 1.0149, N = 14) was again greater than the rectal excretion (ave. 1.0093, N = 4). When these larvae were exposed to dry conditions the hemolymph increased in density (ave. 1.0188, N = 12) as did the rectal excretion (1.0158, N = 12). However, when larvae were present with workers the difference between the hemolymph and rectal excretion was less than in isolated larvae. Larvae held without workers appeared to retain excrement longer while larvae held with workers appeared to be stimulated by workers to release their excrement which the workers then removed.

Studies comparing the relative osmolarity of the rectal excretion and hemolymph revealed no difference in larvae held under dry conditions. However, in the small capillary tubes the rectal fluid equilibrium volume from larvae exposed to humid conditions decreased while the hemolymph equilibrium volume increased. While the changes were small the results indicate the rectal fluid from larvae exposed to humid conditions is slightly hyposmotic with respect to the hemolymph. The lower density and hyposmolarity of the clear rectal excretion in these studies compared to the hemolymph suggests that water is actively secreted into the rectum. While neither this study nor the density study alone is conclusive, their combined results strongly support this function of the hindgut.

The uric acid precipitate could readily be seen through the thin transparent cuticle to move down the malpighian tubules and through the small intestine, accumulating in the rectum. The uric acid was heavier than the clear fluid and readily settled to the bottom of the hindgut.

The dyes fed to larvae were readily visible in the midgut within 1/2 hour after feeding and the neutral red and nile blue were observed in the hemolymph within an hour and in the rectum in 2 hours. Methylene blue was

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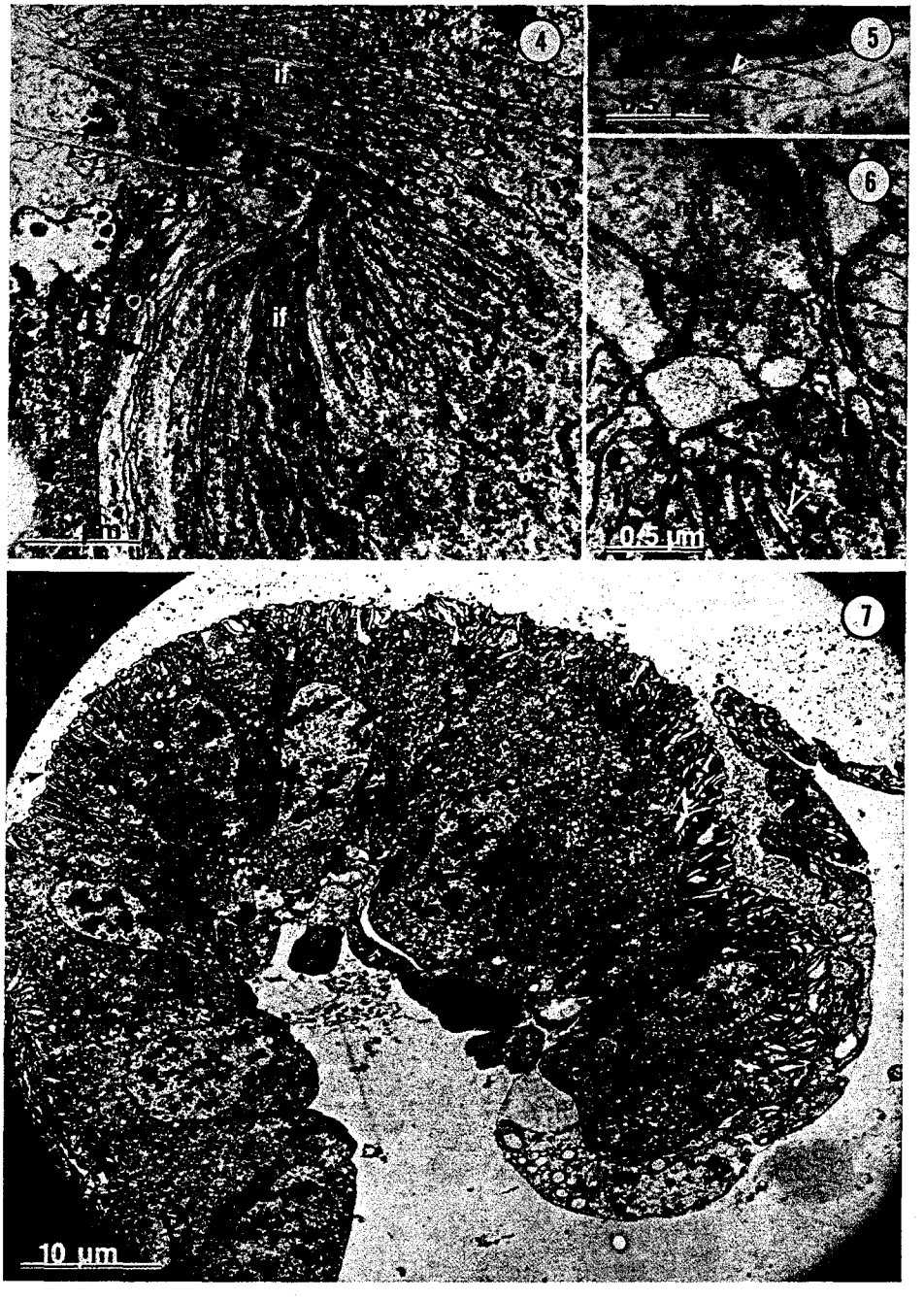
Fig. 4-6. — High magnifications of the basal infoldings (if) in the cells of the base of the hindgut. Note microtubules (arrows) and muscle cells (mu).

Fig. 7. — Section through a rectal pad dissected out of a fourth instar larva. Note apical infoldings and elongate mitochondria (arrows). Rectal epithelium (re).

Fig. 4-6. — Agrandissements poussés des plis de base (if) dans les cellules de la base de l'intestin postérieur. Noter les micro-tubules (flèches) et les cellules musculaires (mu).

Fig. 7. — Coupe d'une papille rectale prélevée sur une larve après la quatrième mue. Noter les plis apicaux et la mitochondrie qui va en s'amincissant (flèches). (re) épithélium rectal.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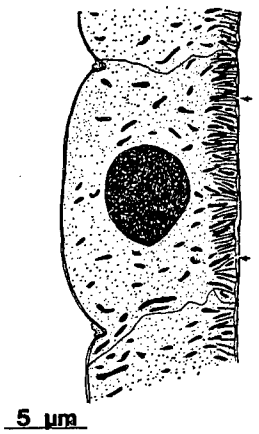

Fig. 8. — Drawing of a rectal pad cell from a fourth instar larva. Note apical infoldings and elongate mitochondria (arrows).

Fig. 8. — Dessin d'une papille rectale de larve après la quatrième mue. Noter les plis apicaux et la mitochondrie qui va en s'amincissant (flèches).

not observed in the hemocoel but was present in the rectum in 2 hours. Nile blue concentrated in the malpighian tubules indicating that these organs were involved in the excretion of nile blue ; the other 2 dyes were not observed in the malpighian tubules.

Injection of nile blue and neutral red resulted in coloration of the hemocoel and the presence of these dyes was noted in the rectum in 1 hour. Again nile blue was first observed in the malpighian tubules. Methylene blue decolorized upon injection into the hemocoel but was visible in the rectum within 1 hour. It is of interest to note that methylene blue is decolorized in reducing environments and regains its color in oxidizing environments (MICHAELIS and FLEXNER, 1930). The results reported here indicate that the hemocoel of the imported fire ant is a reducing environment while the midgut and hindgut are oxidizing.

The presence of dye in the hemocoel prior to its presence in the rectum after feeding and the more rapid appearance of dye in the rectum when injected than when fed suggests that the dye moves from the midgut to the hemocoel and then into the rectum. The dye studies do not support the hypothesis that chemicals move from the midgut into the rectum by way of the base (anterior end) of the hindgut.

When workers were presented the white precipitate or clear excretory products as well as uric acid or allantoic acid on filter paper discs, they ignored them. Loose uric acid crystals were picked up by workers but were carried off and stuffed into cracks in the plaster containers. Anal excretions dyed with methylene blue, and placed on plastic, appeared to be ingested by a few ants, and adult workers were observed to elicit the anal excretion from larvae. Some of these adults were collected and dissected. The uric acid granules, which are about 1 µm in diameter were found in the buccal cavity of adult workers that removed the anal excretion from larvae or from the plastic, but were not found in the crop or midgut. The observation of GLAN-

CEY *et al.* (In press) that adult workers rarely ingest particles larger than 0.9 μm may preclude the ingestion of significant amounts of uric acid other than that dissolved in the clear excretion. A blue dye solution was found in the crop of some adults that ingested the methylene blue dyed excretion. The addition of methylene blue to the diet of larvae resulted in blue dye in the clear excretion which was subsequently found in the crop of adult workers which had been deprived of water. Dye was not found in adult workers who had access to a water source. It thus appears that the clear excreta may be consumed in response to water needs of the adult. In contrast to O'NEIL and MARKIN (1973), who reported that adults fed on the white precipitate ; we found that larval excretions, whether clear or milky, generally appeared to be carried out of the colony and rejected rather than being ingested.

We conclude that uric acid (white excretory product) is excreted via the malpighian tubules into the ileo-colon and finally into the rectum while waste water and salts are excreted via the rectal pads and the malpighian tubules. The excretion of larvae of the imported fire ant appears to offer little in the way of nutrient cycling, as it is probably rarely ingested by adult members of the colony unless they are subjected to water stress.

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