

ULTRASTRUCTURAL FEATURES OF THE MIDGUT OF *Rhinocricus padbergi* (DIPLOPODA: SPIROBOLIDA)

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ABSTRACT

The midgut epithelium of the millipede *Rhinocricus padbergi* has been reported to be stratified or pseudostratified, and there is evidence that this region of the intestine is lined by a single layer of absorptive epithelial cells interspersed with smaller regenerative cells. In this work, transmission electron microscopy was used to study the structure and organization of the midgut of *R. padbergi*. The midgut was lined by a pseudostratified epithelium in which all of the cells were in contact with the basement membrane but did not necessarily reach the apical surface. The epithelium contained three cell types, namely, absorptive cells with apical microvilli that gave the midgut epithelium a "brush border" appearance, secretory cells that were interspersed with the absorptive cells and probably served to lubricate the epithelial surface, and regenerative cells located in the basal region of the epithelium. This organization of the gut cells in *R. padbergi* was similar to that of other arthropods.

Key words: Diplopods, midgut, *Rhinocricus padbergi*, ultrastructure

INTRODUCTION

Diplopods, commonly known as millipedes, occur in all zoogeographical zones, especially the tropics. According to Golovatch *et al.* [11], this group is the third largest class of the Arthropoda after the Insecta and Arachnida, and consists of about 80,000 species. However, only about 10% of these species have been formally described. Diplopods have a resistant exoskeleton and most live in dark, humid environments where they feed on detritus, decomposing organic matter, fruits, and mosses [17].

Most of the information concerning the internal morphology of diplopods dates from early reports and refers mainly to European and North American species [8]. Brazil is rich in diplopods, but only in recent years have some studies focused on Brazilian species, particularly in relation to the morphology of the reproductive organs [1,5-7,10] and the digestive tract [3,8,9]. However, this information is fragmentary and our understanding of the biology of these animals is still poor.

Previous histological studies of the midgut epithelium of *Rhinocricus padbergi* showed that this structure was either stratified or pseudostratified [4].

Recent work has shown that this region of the gut is lined by a single layer of absorptive epithelial cells interspersed with smaller regenerative cells [8].

In this work, we examined the cellular structure and organization of the midgut of *R. padbergi* using transmission electron microscopy (TEM).

MATERIAL AND METHODS

Specimens of *R. padbergi* were collected in the Bela Vista neighborhood in Rio Claro, São Paulo State, Brazil, from November to March of 1999 and 2000. The animals were anesthetized with sulfuric ether and dissected in Petri dishes containing physiological saline. The entire digestive tract was removed and the midgut was separated and processed for TEM. Initially the tissue was fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2 for 2 h and post-fixed in 1% OsO₄ for 1 h. The samples were then embedded in an Epon:Araldite resin mixture and polymerized at 60°C for 72 h. Ultrathin sections were contrasted with uranyl acetate and lead citrate, and were analyzed and photographed in a Philips CM 100 transmission electron microscope.

RESULTS

The midgut epithelium was organized as a pseudostratified epithelium that consisted of absorptive or principal cells, regenerative cells and secretory cells (Figs. 1-3). The absorptive cells had a well-developed brush-border (Fig. 2) and were interspersed with secretory cells (Fig. 3). The regenerative cells occurred in the basal region of the epithelium (Fig. 1).

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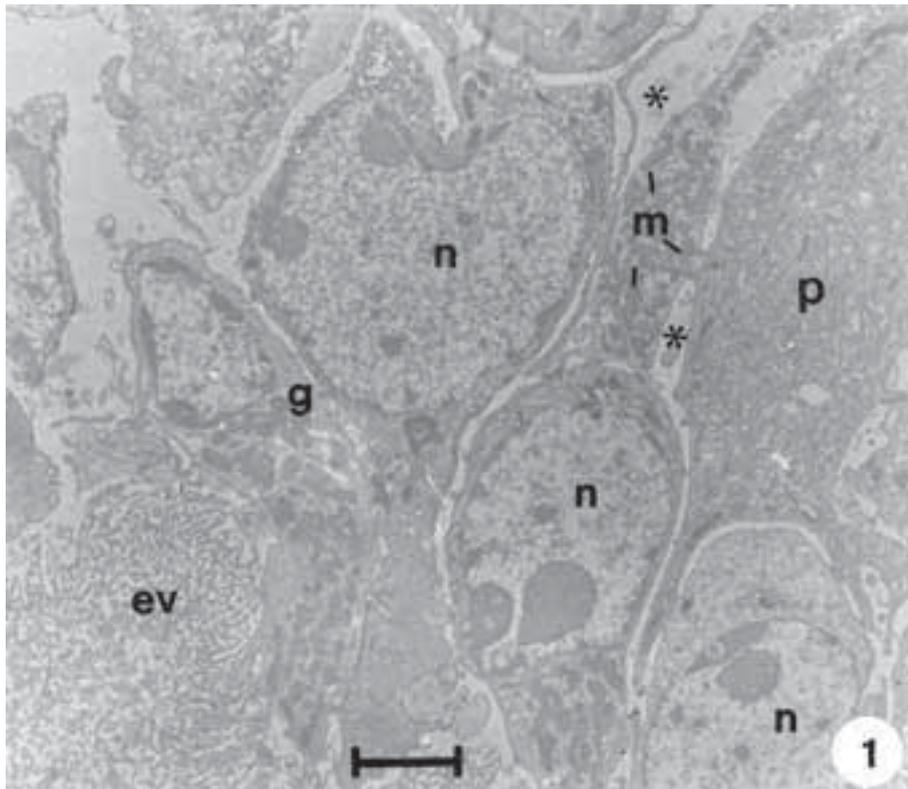


Figure 1. Electron micrograph of the basal portion of the midgut epithelium. **ev** - evaginations of the basal membrane in the basal region of the epithelial cells, **g** - regenerative cells, **m** - mitochondria; **n** - nucleus, **p** - principal cells, * - intercellular spaces. Bar = 2 μ m.

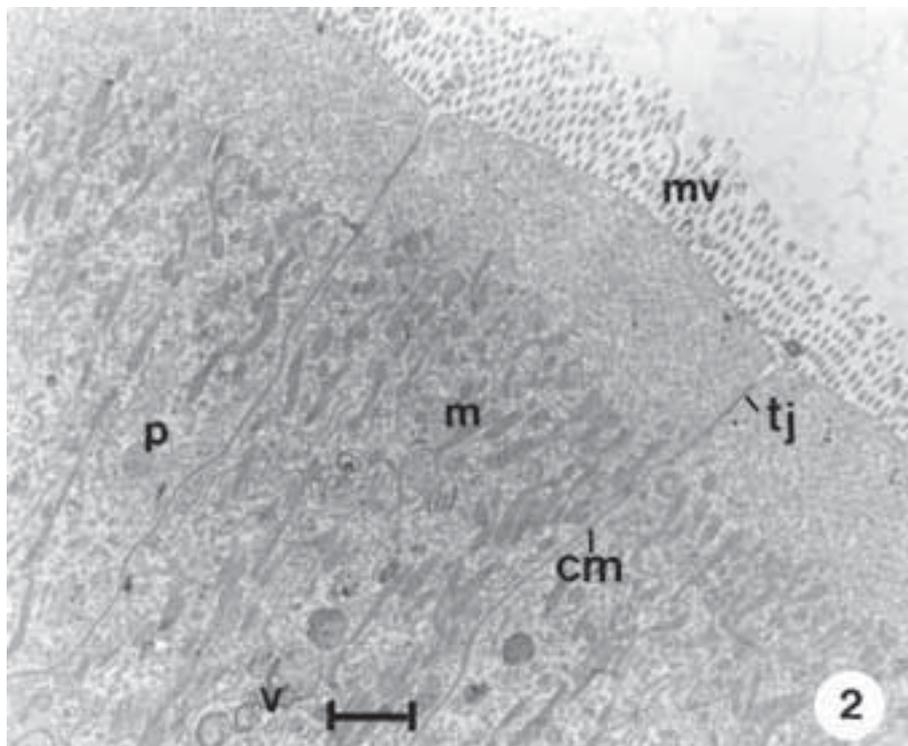


Figure 2. Electron micrograph of the apical portion of the midgut epithelium. **cm** - cell membrane, **m** - mitochondria, **mv** - microvilli, **p** - principal cells, **tj** - tight junction, **v** - vacuoles. Bar = 2 μ m.

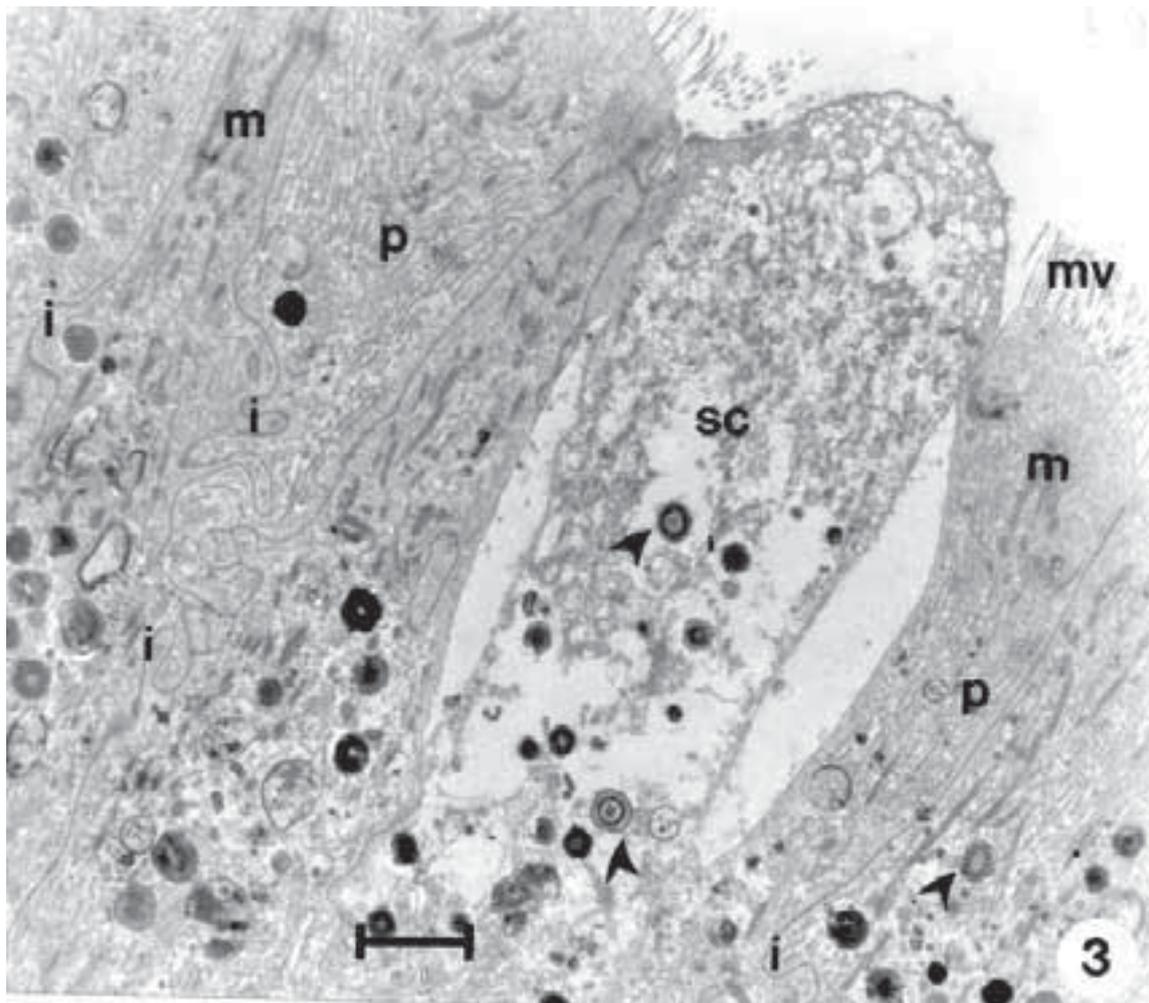


Figure 3. Electron micrographs of absorptive and secretory cells. **i** - interdigitations, **m** - mitochondria, **mv** - microvilli, **arrowhead** - calcium-like spheres, **p** - principal cell, **sc** - secretory cell. Bar = 2 μ m.

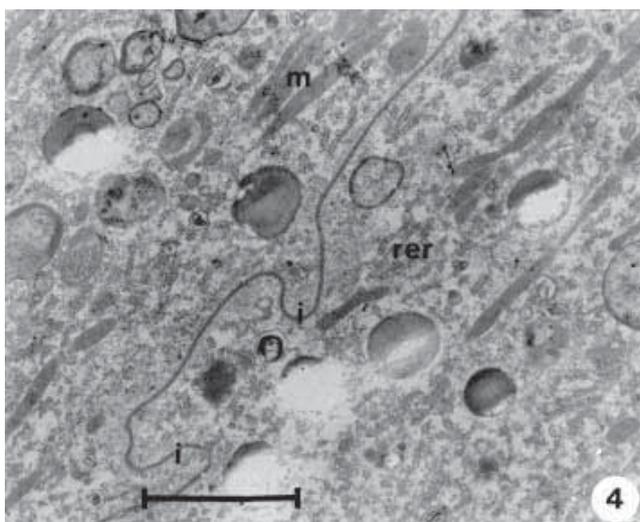


Figure 4. Detail of absorptive cells. **i** - interdigitation, **m** - mitochondria, **rer** - rough endoplasmic reticulum. Bar = 2 μ m.

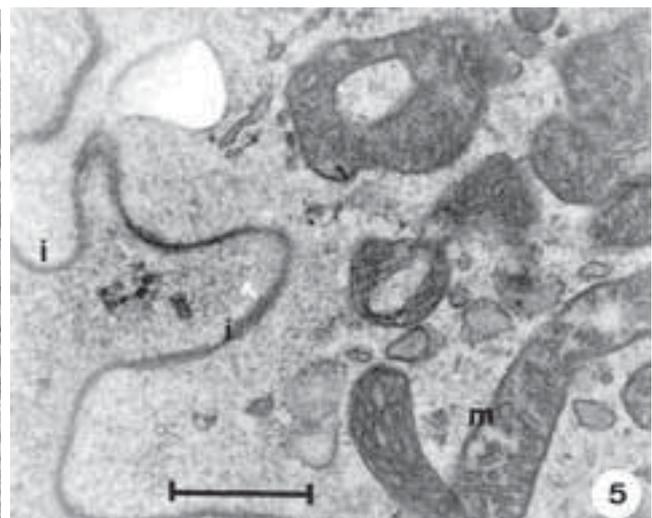


Figure 5. Detail of absorptive cells in the midgut of *R. padbergi*. **i** - interdigitations, **m** - mitochondria. Bar = 1 μ m.

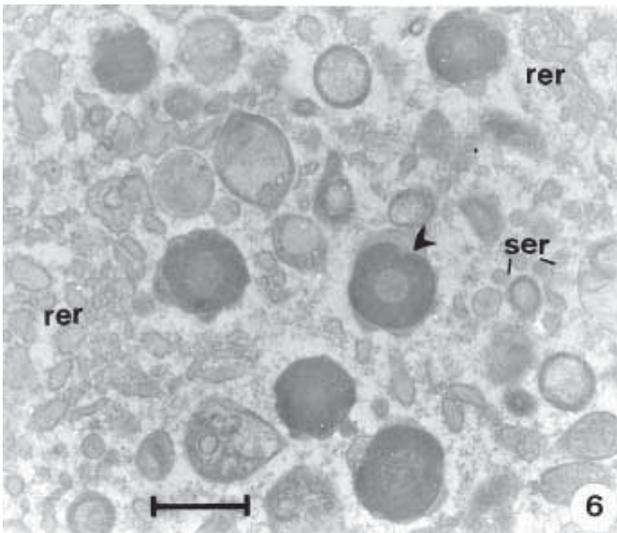


Figure 6. Detail of absorptive cells of the midgut of *R. padbergi*. **rer** - rough endoplasmic reticulum, **ser** - smooth endoplasmic reticulum, **arrowhead** - calcium-like spheres. Bar = 1 μ m.

The absorptive cells were joined to each other by interdigitations (Figs. 3-5), and tight junctions prevented the paracellular transport of substances (Fig. 2). The basal region of the epithelium had intercellular spaces (Figs. 1 and 7), that were absent in the apical portion (Figs. 2 and 8). This region also had a large number of evaginations towards the muscle layer (Figs. 7 and 11) and was supported by a thick basal lamina.

The cytoplasm of absorptive cells contained a large number of elongated mitochondria that were located mainly in the apical region (Figs. 3, 5 and 8) but also occurred in the basal portion, where they were fewer in number and smaller in size (Figs. 1, 7 and 11). The cytoplasm of these cells also had an abundant granular endoplasmic reticulum. Several Golgi complexes were seen releasing numerous secretory vesicles close to the nucleus (Figs. 8-10).

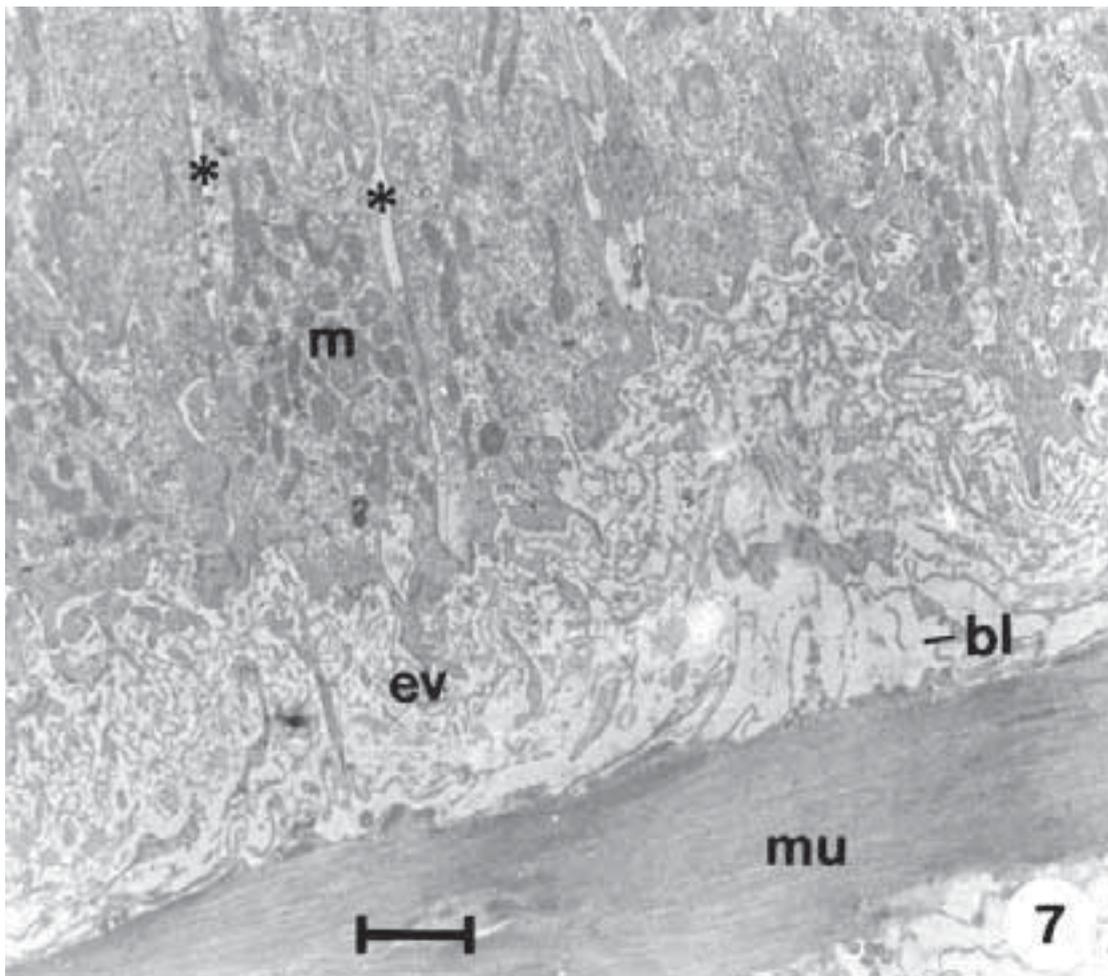


Figure 7. Basal portion of the epithelium. **ev** - evaginations of the basal cytoplasm of midgut epithelial cells, **bl** - basal lamina, **m** - mitochondria, **mu** - muscle layer, * - intercellular space. Bar = 2 μ m.

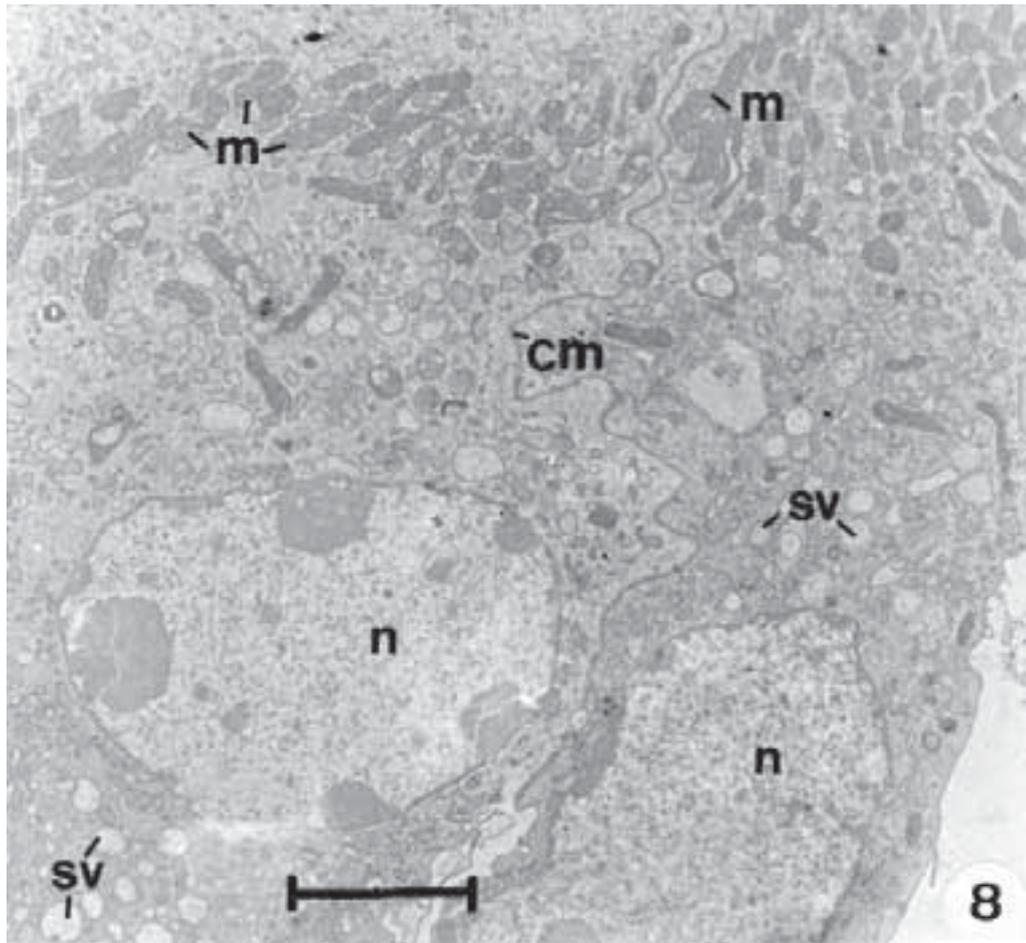
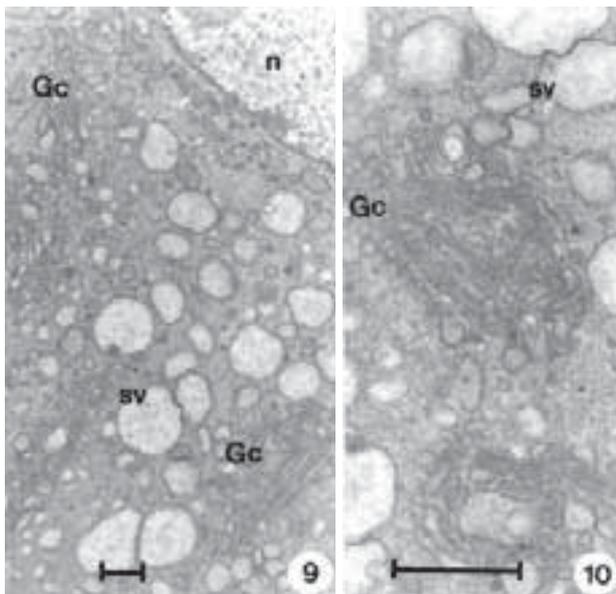


Figure 8. Electron micrograph of absorptive cells located in the mid-apical region of the midgut epithelium. **cm** - cell membrane, **m** - mitochondria, **n** - nuclei, **sv** - secretory vesicles. Bar = 2 μ m.



Figures 9 and 10. Electron micrographs of the absorptive cells of the midgut. **Gc** - Golgi complex, **n** - nucleus, **sv** - secretory vesicles. Bar = 1 μ m.

Several calcium spheres were seen in both absorptive and secretory cells (Figs. 3 and 6).

DISCUSSION

According to Chapman [2], the columnar cells of the insect digestive tract can secrete and absorb enzymes, although enzyme secretion has not been reported yet in diplopods. The microvilli seen in the apical portion of the gut cells probably increased the efficiency of absorption by providing a greater surface area.

The structural organization of the basal portion of the absorptive cells in the midgut of *R. padbergi* supports the idea that, in addition to absorbing compounds from food, these cells may also be involved in the transport of elements from the hemolymph. This morphology is similar to that of vertebrate renal tubule cells which have important ion transport functions. The large number of mitochondria seen in this region supports the hypothesis of a similar

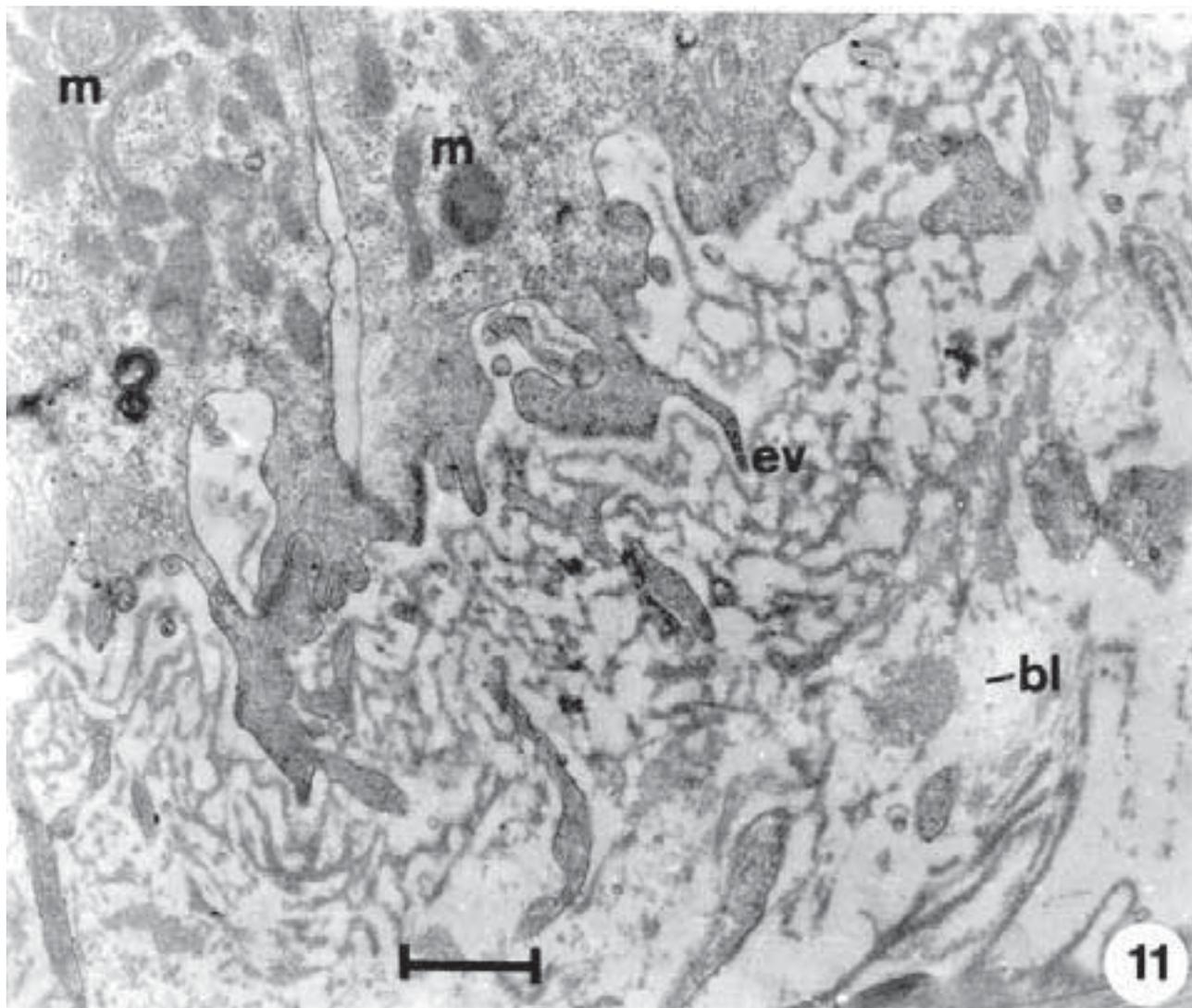


Figure 11. Detail of basal portion of absorptive cells. **bl** - basal lamina, **ev** - cell membrane evaginations, **m** - mitochondria. Bar = 1 μ m.

function for the absorptive cells.

Secretory cells are widespread in the gut of many animal species and serve to lubricate the epithelial surface and/or produce substances of a mucous nature that may help absorption.

Hopkin and Read [12] reported that the absorptive cells of Millipedes are joined basally to the surrounding hepatic cells through interdigitations. According to Seifert and Rosenberg [18], the hepatic cells do not form an epithelium and are not interconnected to one another in any way. However, hepatic cells do have a basal plasma membrane, that ramifies between the absorptive cells of the midgut. The presence of fusiform junctions among the hepatic and intestinal cells suggests that there is open transport between these two cell types [12,16]. Indeed, several studies have indicated that the intestinal and

hepatic cells perform complementary functions, with the intestinal cells being involved in the absorption of digestion products whereas hepatic cells are involved in mineral retention [13-15]. Although several evaginations were seen in the basal region of the absorptive cells in *Rhinocricus padbergi*, no communication was observed between the intestinal cells and other types of cells.

Hopkin and Read [12] noted numerous granules of calcium phosphate arranged concentrically within the intestinal cells of diplopods. Fantazzini *et al.* [4] described the same structures in the midgut of *R. padbergi*. According to Hubert [15], the midgut of iulids is an important site for mineral accumulation and has an important role in the ionic regulation of the organism. Consequently, the cyclic elimination of these granules would be a form of excretion. A

similar excretory process probably occurs in *R. padbergi* and would explain the excretory function of the absorptive cells.

The ultrastructural analysis of the intestinal cells of diplopods has revealed characteristics shared by other arthropods. Hubert [15] postulated that the differentiations of the apical and basal regions and the development of the endoplasmic reticulum and Golgi complexes, are analogous in diplopods and insects. The results presented here corroborate this similarity between the midgut cells of diplopods and insects.

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