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Midgut of the millipede, "*Rhinocricus*" *padbergi* Verhoeff, 1938 (Diplopoda: Spirobolida): Histology and histochemistry

Средний кишечник диплоподы "*Rhinocricus*" *padbergi* Verhoeff, 1938 (Diplopoda: Spirobolida): гистология и гистохимия

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КЛЮЧЕВЫЕ СЛОВА: Diplopoda, средний кишечник, пищеварительный тракт, гистохимия, апокринная секреция.

ABSTRACT: Both histological and histochemical analyses of the midgut of the Brazilian millipede "*Rhinocricus*" *padbergi* reveal the presence of lipids, carbohydrates, calcium and proteins in certain cell elements of this region of the digestive tract. The midgut epithelium might participate directly in the processes of digestion and/or synthesis of lipidic and proteic compounds, and is involved in the transport of calcium. It also takes part in the production of neutral polysaccharides, performed by mucus cells, as well as in the synthesis of digestive enzymes, as demonstrated by an apocrine release of the secretions.

РЕЗЮМЕ: Как гистологический, так и гистохимический анализ средней кишки бразильского кивсяка "*Rhinocricus*" *padbergi* выявили присутствие жиров, углеводов, кальция и белков в определенных клеточных элементах этого отдела пищеварительного тракта. Эпителий средней кишки, возможно, непосредственно участвует в процессах разложения и(или) синтеза жировых и белковых соединений, а также задействован в транспорте кальция. Кроме того, он принимает участие и в выработке нейтральных полисахаридов, осуществляемой слизистыми клетками, и в синтезе пищеварительных ферментов, как показывает апокринное высвобождение секретов.

Introduction

The digestive tract of millipedes (Diplopoda) consists of a straight tube extending from mouth to anus. Three major regions can be discerned: foregut, midgut, and hindgut. Flanking the foregut, a pair of salivary glands can be observed. At the junction between the midgut and hindgut, there is a pair of Malpighian tubules.

Studies on the anatomy and histology of the digestive tract of millipedes are scarce and, in most cases, limited to members of the orders Polydesmida and Julida. The works of Verhoeff [1914] (cited after Hubert [1978]), Randow [1924], Hefner [1929] and Miley [1930] are among the first on the subject. More recently, Nunez & Crawford [1977] discussed the anatomy and histology of the digestive tract of *Orthoporus ornatus* (Girard, 1853), Fontanetti & Camargo-Mathias [1997] of *Plusioporus setiger* (Brölemann, 1902), both these species representing the family Spirostreptidae, order Spirostreptida, and Fantazzini et al. [1998] studied the foregut of "*Rhinocricus*" *padbergi* Verhoeff, 1938 (Rhinocricidae, Spirobolida).

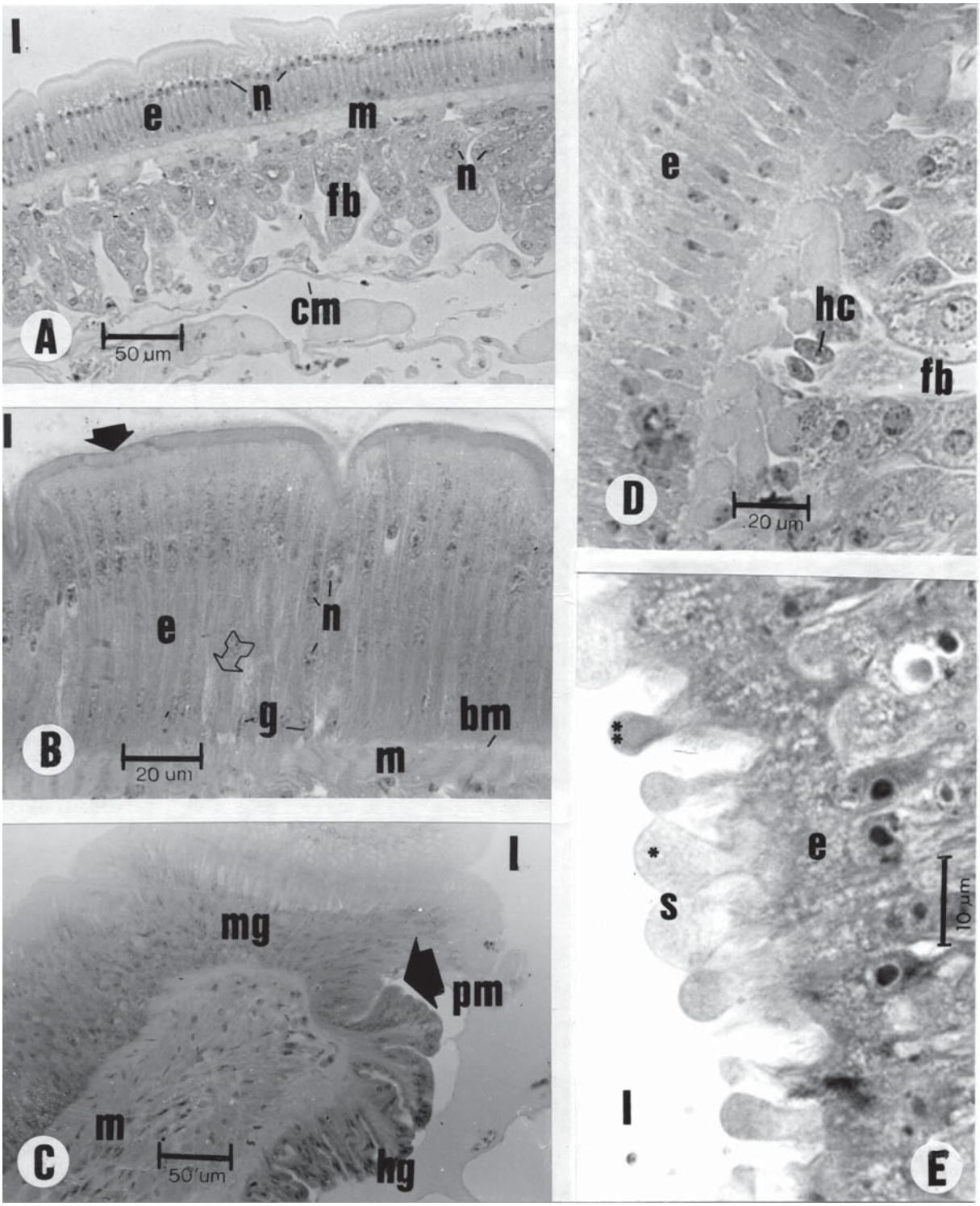
A few other papers focused on the morphology and histology of certain regions or cellular types that form the digestive tract of millipedes, such as the studies made by Bowen [1968], Schluter [1979], Crawford et al. [1983] and Schluter & Seifert [1985].

The present contribution shows the histology and histochemistry of the midgut of the Brazilian species, "*Rhinocricus*" *padbergi* Verhoeff, 1938. The quotation marks in the generic name reflect the fact that most of the South American rhinocricid species still assigned to *Rhinocricus* actually belong to other genera [Hoffman, 1999].

Materials and Methods

The specimens were collected by E. R. Fantazzini at the Instituto de Biociências of the UNESP Campus at Rio Claro, São Paulo State, Brazil, between November and March of 1999 and in 2000.

The individuals were anesthetised with ether and dissected in physiological solution in order to extract the digestive tract, which was fixed in 4% paraformaldehyde and/or Carnoy I. The material was then dehydrated in ethanol and embedded in JB4 resin during 24 h at 4ºC. The material was then transferred to plastic moulds previously filled with resin containing a catalyzer. After resin polymerisation, the material was sectioned using a Sorvall JB4 microtome (Bio Rad)



Figs 1A–E. Intestine of "*Rhinocricus*" *padbergi* stained with hematoxiline and eosin. A — general aspect; B — details of epithelial structure; C — transition between midgut and hindgut; D — region where the hepatic cells are located; E — release of an apocrine secretion.

e — epithelium; m — muscle fibers; hc — hepatic cells; cm — cell membrane surrounding the intestine externally; fb — fat body cells; l — lumen; mg — midgut; hg — hindgut; g — regenerative cell; n — nucleus, bm — basal membrane; pm — peritrophic membrane; s — secretion vesicle; Filled arrow in Fig. B indicating a brush-like border (microvilli); Filled arrow in Fig. C indicating the transition point between midgut and hindgut; Empty arrow in Fig. B indicating clear cells (mucus cells).

and stained with hematoxiline and eosin, following routine histological procedures.

Histochemical tests were applied in order to detect the presence of the following compounds: proteins (using Bromophenol Blue as proposed by Pearse [1985]); lipids (using Nile Blue as proposed by Lison [1985], and Sudan Black, according to Junqueira & Junqueira [1983]); polysaccharides (simultaneous staining with PAS/Alcian Blue, according to Junqueira & Junqueira [1983]); and calcium (following the method of Von Kossa as proposed by Junqueira & Junqueira [1983]).

Results and Discussion

1. Histology

The midgut of millipedes extends from the esopharyngeal valve to the hindgut, which is marked by the insertion of the Malpighian tubules. In *"R." padbergi*, the midgut represents about 39% total length of the digestive tract [Fantazzini et al., 1998].

The midgut is provided with a cylindrical epithelium, which folds forming numerous microvilli directed toward the lumen. Histologically, the midgut of *padbergi* consists of the epithelium, a basal lamina and a muscular layer surrounded by perivisceral fat body cells (Fig. 1A).

The epithelial cells show nuclei localised in the upper half, with shapes varying from rounded to oval (Figs 1A, B); the apical membrane of these cells shows numerous microvilli forming a well-defined brush border (Fig. 1B, arrow). These cells are commonly known as principal cells.

Located at the basal region and interspersed with the principal cells, smaller cells showing spherical and basal nuclei can be found. These cells are known as generative cells (Fig. 1B). This cellular arrangement suggests the presence of a pseudo-stratified epithelium. Among the principal cells, another cell type can be revealed in being much lighter than the principal cells and resembling mucus cells (Fig. 1B, empty arrow). The basal membrane can be observed being in direct contact with the epithelium, with muscular cells adhering to the latter (Figs 1A, B). Right underneath the basal membrane, there is a continuous layer of fat body cells. These cells are irregular in shape and possess spherical nuclei and a highly vacuolated cytoplasm (Figs 1A, D).

Strongly stained cells showing a more homogeneous cytoplasm when compared to the fat body cells are observed underneath the muscle layer, right beneath the epithelium (Fig. 1D); these cells are probably the hepatic cells as termed by Verhoeff (cited after Hubert [1978]). Due to their function and their similarity to the gastric coecae or hepatopancreas of other arthropods, these groups of cells are also known as "liver" and/or "hepatic tissue". According to Hubert [1978, 1988], these cells accumulate substances that are transported into the haemolymph to be excreted.

At the junction site between the midgut and hindgut, a higher muscular development and a thicker epithelium are observed (Fig. 1C, arrow).

The presence of a peritrophic membrane can be noticed throughout the midgut, being intimately associated with the intestinal cells. However, at the junction point with the hindgut this membrane is in poor contact with the epithelial cells of the hindgut (Fig. 1C). According to Nunez & Crawford [1977], the presence of this structure is well established in the digestive tract of Diplopoda. The fact that this membrane is in intimate association with the epithelial cells of the midgut of *padbergi* supports the hypothesis suggesting that this membrane originates from delamination of the brush border of the midgut epithelial cells [Mason & Gilbert, 1954]. This has also been documented in the Brazilian species, *Plusioporus setiger* [Fontanetti & Camargo-Mathias, 1997].

Numerous secretion vesicles can be observed being released toward the lumen (Fig. 1E). The vesicles show varying degrees of condensation of their content (* and ** in Fig. 1E), thus suggesting the presence of either maturation or a dehydration process of the secretions during their transport from the cytoplasm into the intestinal lumen. It is also possible that the vesicles contain different secretion materials.

The type of secretion release can be classified as apocrine, similar to the one described by Caetano et al. [1994] for ants of the genus *Pachycondyla*, and by Fontanetti et al. [2001] in the millipede, *Plusioporus setiger*. On the other hand, Hefner (1929) observed a merocrine type of secretion release by epithelial cells of the midgut in *Parajulus impressus* and suggested that, in other millipedes, the release should be of the holocrine type.

Bowen [1968] reported the presence of a pseudo-stratified columnar epithelium in the midgut of *Floridobolus penneri* Causey, 1957 and *Narceus gordanus* (Chamberlin, 1943) (both Spirobolida); all the other species of millipedes studied showed a simple prismatic epithelium. This was confirmed at the ultrastructural level by Hubert [1979] in *Cylindroiulus londinensis* (Leach, 1815), who described this epithelium as being constituted by principal cells and clear cells, interspersed with generative cells in the basal region.

In *"Rhinocricus" padbergi*, our light microscopy observations suggest that the midgut of this species is composed by a pseudo-stratified epithelium. Nevertheless, an ultrastructural analysis reveals the presence of a stratified epithelium [Fantazzini et al., unpublished data].

Рис. 1A–E. Кишечник "*Rhinocricus*" *padbergi*, окрашенный гематоксилином с эоэином. A — общий вид; B — детали строения эпителия; C — переход между средней и задней кишкой; D — район, где расположены клетки печени; E — высвобождение акокринного секрета.

e — эпителий; m — мышечные волокна; hc — клетки печени; cm — клеточная мнембрана, окружающая кишечник снаружи; fb — клетки жирового тела; l — просвет; mg — средняя кишка; hg — задняя кишка; g — регенеративная клетка; n — ядро, базальная мембрана; pm — перитрофическая мембрана; s — пузырек секрета; Заполненная стрелка на Рис. B указывает на щетковидный край (микроворсинки); Заполненная стрелка на Рис. C указывает на точку перехода средней кишки в заднюю; Незаполненная стрелка на Рис. B указывает на прозрачные клетки (слизистые клетки).

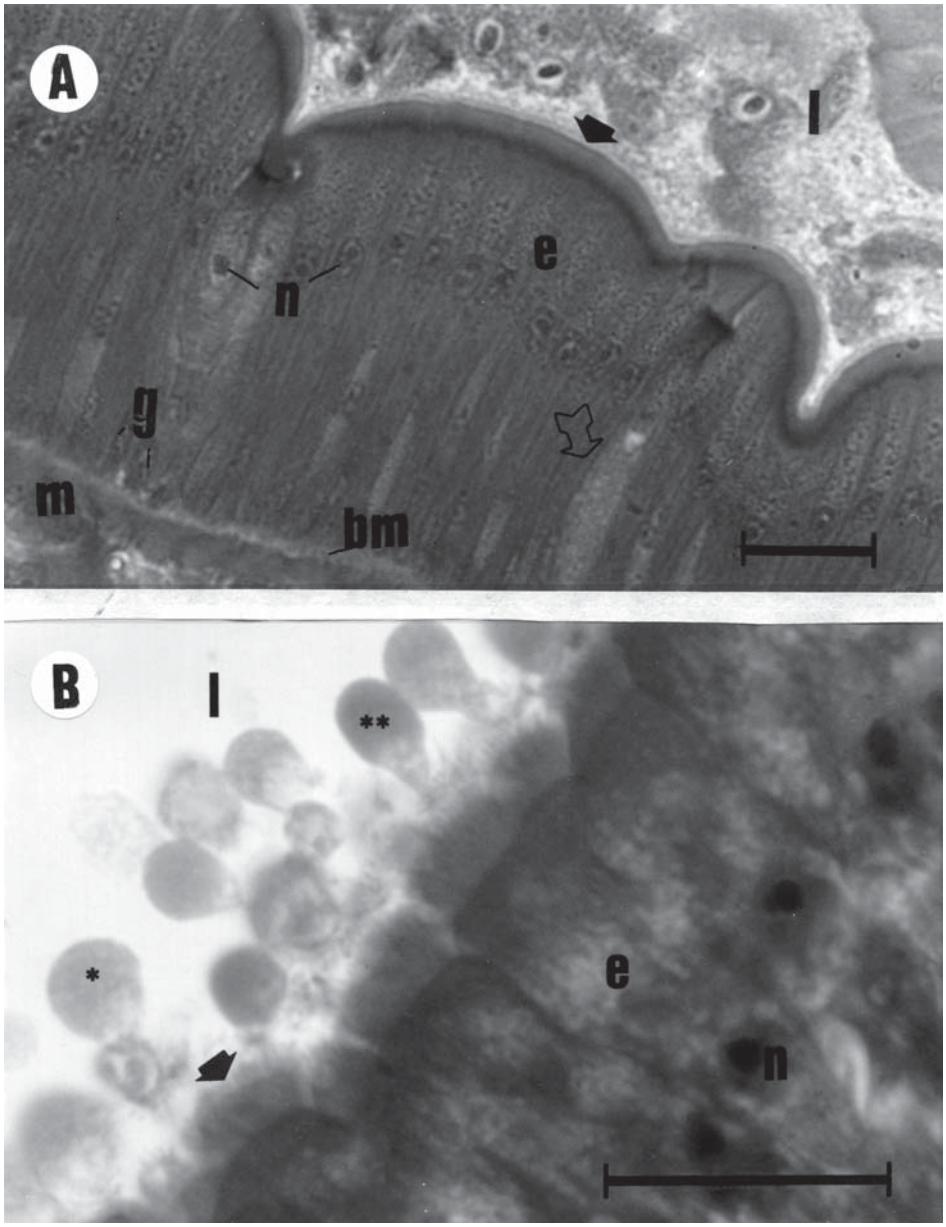


Fig. 2A & B. Midgut of *"R.." padbergi* subjected to tests for the detection of proteins. Bromophenol Blue.

g — generative cells; Filled arrows indicating microvilli; Empty arrow indicating clear cells (mucus cells); (*) = poorly compacted secretion vesicle; (**) = highly compacted secretion vesicles. Scale bars = 25mm. Other designations as in Fig. 1.

Рис. 2A и B. Средняя кишка *"R.." padbergi* после опытов на присутствие белков. Бромфенол синий.

g — генеративные клетки; Заполненные стрелки указвают на микроворсинки; Незаполненная стрелка указывает на прозрачные клетки (слизистые клетки); (*) = слабокомпактный пузырек с секретом; (**) = сильнокомпактные пузырьки с секретом. Масштаб = 25 mm. Прочие обозначения, как на Рис. 1.

2. Histochemistry

The histochemical analyses show a strong positive reaction for proteins in the epithelium and fat body, yet the basal membrane (Fig. 2A) and the clear (mucus) cells located at the epithelium (Fig. 2A, empty arrow) demonstrate a weak positive reaction. The hepatic cells and the secretion released by the intestinal cells also reveal a strong positive reaction, indicating the presence of high concentrations of proteins (Fig. 2B).

With the exception of the clear cells (Fig. 3A, empty arrow), the epithelium reacted weakly to the tests for neutral polysaccharides. The microvilli and the basal lamina show the same results. The strong positive reaction observed for the clear cells suggests that they take part in the production of polysaccharides. The fat body also shows a strong positive reaction to these tests and a large amount of small granules are observed in the cytoplasm, which probably represent aggregations of glycogen (Fig. 3A).

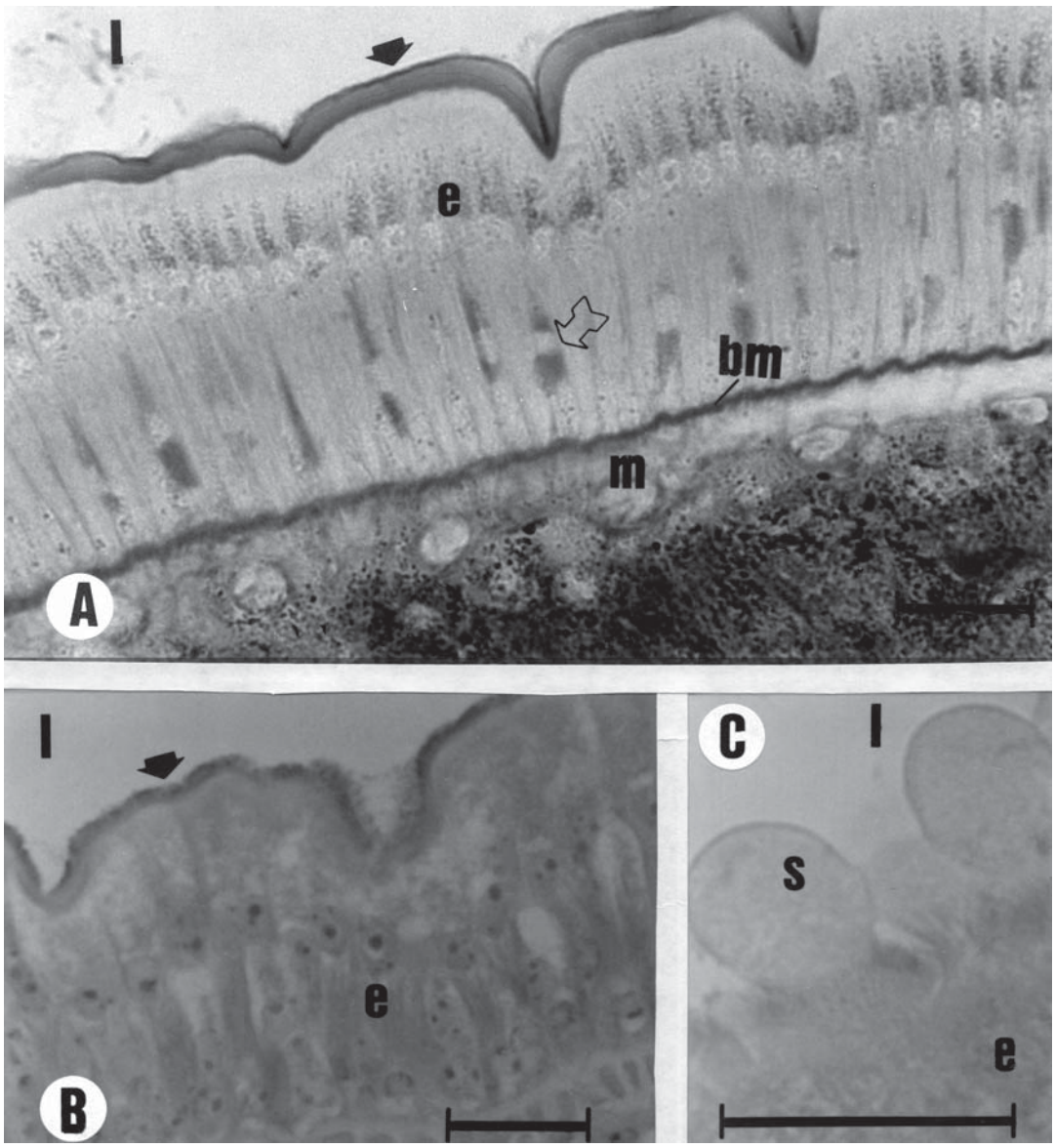


Fig. 3. Midgut of "*R.*" *padbergi* subjected to tests for the detection of polysaccharides. A. PAS; B & C. Simultaneous staining with PAS/Alcian Blue.

s — secretion; Filled arrows indicating microvilli; Empty arrows indicating clear cells reacting positive to PAS (mucus cells). Scale bars = 25 mm. Other designations as in Fig. 1.

Рис. 3. Средняя кишка "*R.*." *padbergi* после опытов на присутствие полисахаридов. A. PAS; B и C. Одновременное окрашивание PAS и Alcian Blue.

s — секрет; Заполненные стелки указвают на микроворсинки; Незаполненные стрелка указывают на прозначные клетки с положительной реакцией на PAS (слизистые клетки). Масштаб = 25 mm. Прочие обозначения, как на Рис. 1.

The histochemical tests reveal that, at the basal membrane, there is predominance of glycoproteic compounds in relation to proteins.

Acid polysaccharides are noted by the microvilli; such a reaction is probably related to the presence of amorphous material (glycoaminoglycanes of an acid nature) between the microvilli (Figs 3B & C). While studying the ventricle of ants, Caetano [1988] also showed the presence of polysaccharides in the microvilli of epithelial cells.

The tests performed for the detection of lipids demonstrate the presence of strongly positive droplets in the medio-apical cytoplasm of the principal cells (Figs 4A, B & C, arrows). This observation suggests that the lipids liberated in the lumen (* in Fig. 4B) might originate in principal cells.

The fat body appears moderately positive to the Nile Blue test and negative to the Sudan Black test (Fig. 4A), thus suggesting that the fat body contains lipids phospholipidic in nature.

The presence of calcium is detected in the midgut cells. In some individuals, this element is observed in the apical portion of principal cells (Fig. 5A, arrow). Yet in

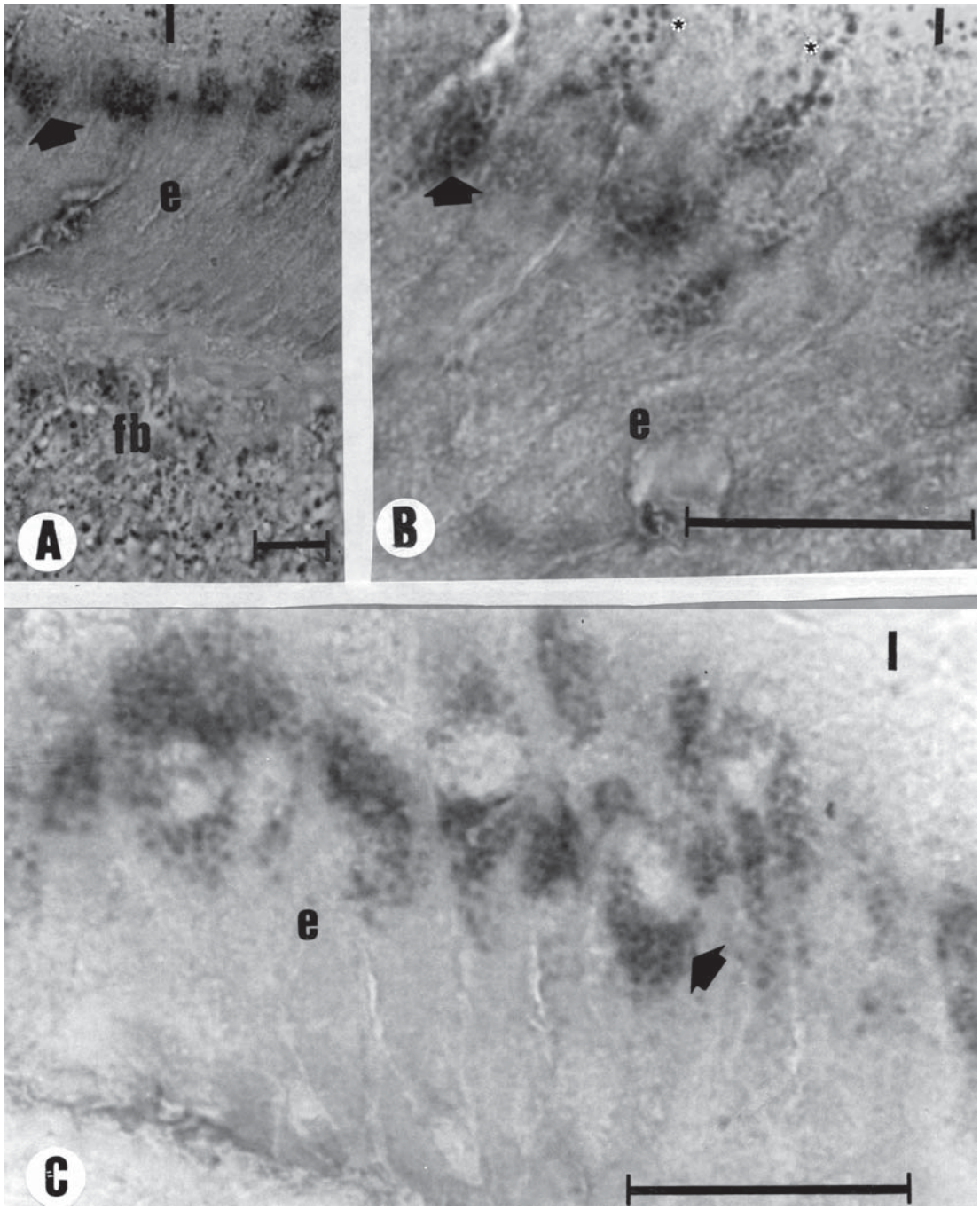


Fig. 4A–C. Midgut of *"R." padbergi* subjected to staining by Sudan Black (A & B) and Nile Blue (C) for the detection of lipids. Arrows = lipid droplets in the apical portion of the principal cells; (*) = release of these compounds into the lumen. Scale bars = 25 mm. Other designations as in Fig. 1.

Рис. 4A–C. Средняя кишка *"R." padbergi* при окрашивании Суданом черным (A и B) и Нилом голубым (C) на присутствие жиров. Стрелки = капельки жира в вершинной части главных клеток; (*) = высвобождение этих соединений в просвет кишечника. Масштаб = 25 мм. Прочие обозначения, как на Рис. 1.

other specimens, evidence of calcium is revealed in fat body cells, between the muscular fibers located underneath the epithelium (Fig. 5C), and in the mediobasal portion of epithelial cells (Fig. 5B, empty arrow). There is also a strong positive reaction in the microvilli (Fig. 5B, filled arrow). These results imply that the epithelium might be involved in the transport of this element.

The results of the histochemical tests are summarised in Table.

The observation of secretion vesicles being released from the epithelial cells towards the lumen of the digestive tract suggests that the midgut liberates proteic

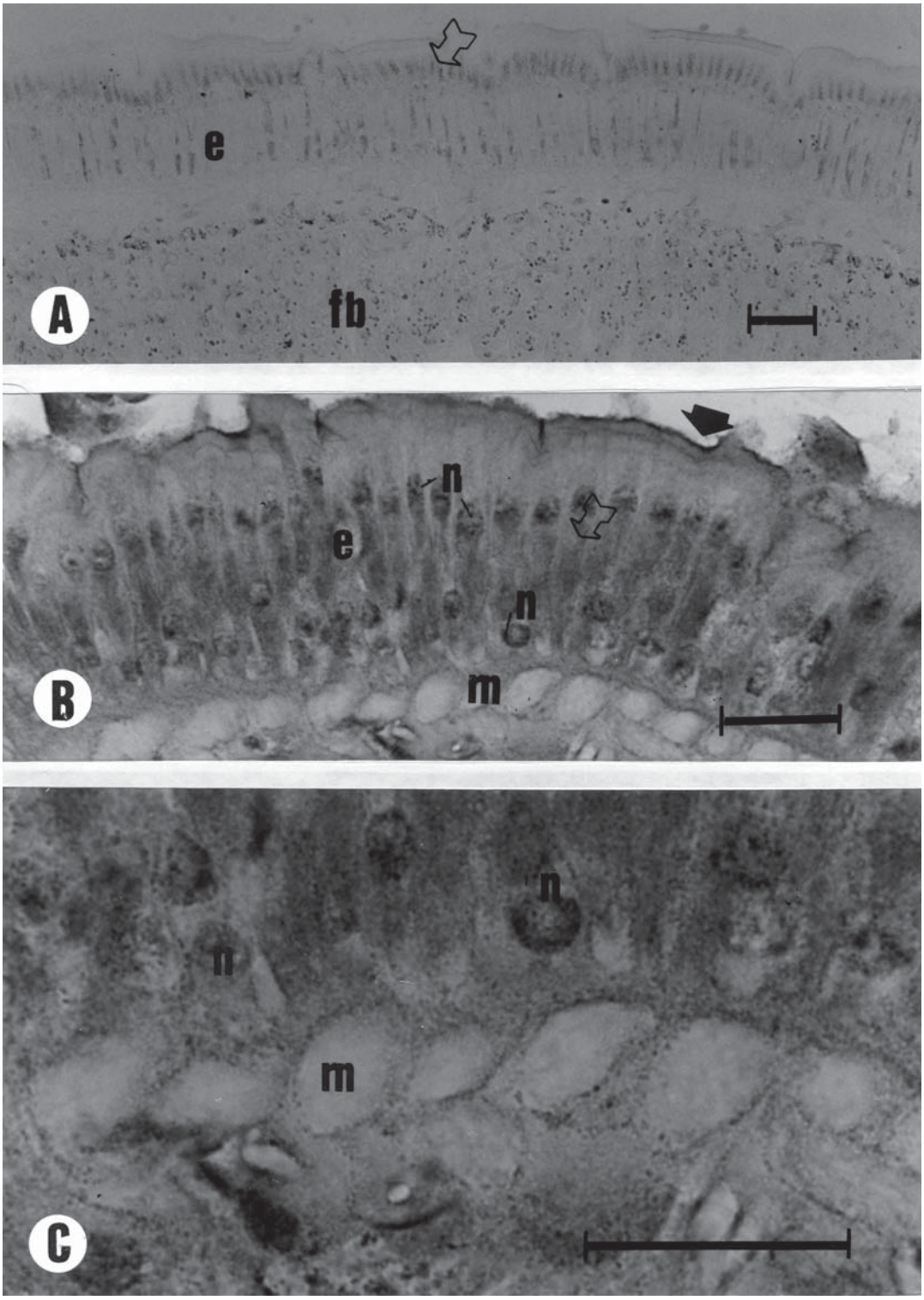


Fig. 5A–C. Midgut of *"R." padbergi* subjected to the Von Kossa technique and stained by hematoxiline for calcium detection. Empty arrow in Fig. B = calcium in the mediobasal portion of the epithelial cells; Filled arrow in Fig. B = calcium at the crush-like edge; Empty arrow in Fig. A = calcium in the apical portion of the intestinal cell. Scale = 25 mm. Other designations as in Fig. 1.

Рис. 5A–C. Средняя кишка *"R." padbergi* после обработки по методу Von Kossa и окрашивания гематоксилином на присутствие кальция.

Незаполненная стрелка на Рис. B = кальций в медиобазальной части эпителиальных клеток; Заполненная стрелка на Рис. B = кальций у похожего на разрушенный края; Незаполненная стрелка на Рис. A = кальций в апикальной части кишечной клетки. Масштаб = 25 mm. Прочие обозначения, как на Рис. 1.

Table. Results of histochemical tests applied to the midgut of the millipede, "*Rhinocricus*" *padbergi*.
Таблица. Результаты гистохимических опытов над средней кишкой кивсяка "*Rhinocricus*" *padbergi*.

Structure		Applied tests					
		Calcium	Polysaccharides		Proteins	Lipids	
		Von Kossa	Neutral	Acidic	Bromophenol Blue	Nile Blue	Sudan Black
Epithelium	Principal cells	+ +	+	–	+ + +	+ + +	+ + +
	Microvilli	+	+ + +	– (*)	+ + +	–	–
	Mucus cells	–	+ + +	–	+	–	–
Basal membrane		+ +	+ + +	–	–	–	–
Fat body cells		+ +	+ + +	–	+ + +	+ +	+
Muscle fibers		–	–	–	+ + +	–	–

+ + + strongly positive; + + moderately positive; + weakly positive; – negative; (*) positive in individuals, in which the presence of secretion was verified.

compounds, which are probably involved in the production of digestive enzymes. Nevertheless, this process is probably not continuous and might depend on the physiological moment the individual is undergoing.

The histochemical results suggest that the fat body in association with the midgut of *padbergi* might be an important site for the storage of neutral polysaccharides, proteic compounds and calcium.

The hepatic cells observed are probably associated with the digestion or synthesis of proteins but are involved in the metabolism of neither carbohydrates nor calcium. Hubert [1988] observed that these cells branch between the regenerative cells of the midgut. This author also noticed the presence of junctions with a structure suggesting an open transport between the two cellular types.

Our data indicate that the midgut epithelium of *padbergi* might participate directly in the processes of digestion and/or synthesis of lipidic and proteic compounds, and is involved in the transport of calcium. It also takes part in the production of neutral polysaccharides, performed by mucus cells, as well as in the synthesis of digestive enzymes, as demonstrated by an apocrine release of the secretions.

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