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A COMPARATIVE STUDY OF THE ULTRASTRUCTURE OF INTESTINE IN PROTOSTRONGYLUS COMMUTATUS AND PROTOSTRONGYLUS RUFESCENS (PROTOSTRONGYLIDAE: NEMATODA)

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Abstract. A comparison of the ultrastructure of intestinal cells of two taxonomically very close nematode species, *Protostrongylus commutatus* and *Protostrongylus rufescens*, revealed many interesting facts. The concentration of mitochondria in the basal part of intestinal cells, which is conspicuous particularly in *P. rufescens*, indicates that the nutrients penetrate also through the cuticle. An enormous occurrence of granular endoplasmic reticulum in the intestinal cells of *P. commutatus* shows an excessive protein synthesis. It may be supposed that the proteins will be used mainly for the construction of sex products, which are extremely abundant in *P. commutatus*. This phenomenon will be related with the conditions of egg development and localization of the worm in the vicinity of trachea bifurcation in the lungs of hare.

Protostrongylosis is an important disease of both domestic and wild animals. It is caused, among other helminths, also by *Protostrongylus commutatus* parasitic in hare and *Protostrongylus rufescens* obtained by us from sheep lungs. Although these two species have been studied from many aspects by several authors (Kotrlý and Ryšavý 1952, Erhardová et al. 1953, Dobšinský 1955, Sitko et al. 1970, Pajerský 1983), little attention has been paid to their ultrastructure. The ultrastructure of the intestinal cells of *Protostrongylus commutatus* is described in the present study. The results are compared with the data concerning *Protostrongylus rufescens* (Zmoray and Gutteková 1974). The comparative study was performed in order to assess whether the different ecological conditions under which the hosts of these nematodes (hare and sheep) live and the localization and mode of feeding of the worms are reflected in the ultrastructural arrangement of intestinal cells in these taxonomically very close species.

MATERIALS AND METHODS

The thin, hair-like biohelminth *Protostrongylus commutatus* is about 50—60 mm long and 0.15—0.12 mm wide and of red-brown colour. According to Železník (1939), the brown colour is caused by the contents of the intestine. The nematodes were collected during occasional dissections of lungs of *Lepus europaeus europaeus*. Adult worms were found in the bronchi, trachea bifurcation and sometimes even in trachea. They were lying on the mucosa of respiratory system, usually in clusters enveloped by phlegm.

The worms were fixed in 3 % glutaraldehyde in cacodylate buffer (pH 7.3) for about 4 h. After washing and post fixation in 1 % OsO_4 in cacodylate buffer the material was dehydrated through a graded alcohol series, saturated and embedded into Durcupan ACM. Ultrathin sections were prepared using Tesla BS 490 A ultramicrotome and LKB ultratome III. After staining with uranylacetate and lead citrate the sections were observed in a Tesla BS 613 electron microscope.

OBSERVATIONS

Ultrastructure of the intestinal wall of *Protostrongylus commutatus*

As it is evident from the transverse sections through the body of this nematode, its intestine consists of two epithelial cells. The ultrastructure of these cells is very interesting. There is a markedly large amount of endoplasmic reticulum, particularly of the granular one (GER, ER). The endoplasmic reticulum occurs in the basal, central and apical parts of the intestinal cell (Plate I, Figs. 1, 2, 3). As a dominant component of intestinal cells of *P. commutatus*, the reticulum is developed to such an extent that it fills all spaces of the cell between other ultrastructural organelles. Anastomosing channels of the granular endoplasmic reticulum are pressed together in the shape of a three-dimensional net. In the sections through the intestinal cells it may be observed in form of areas of different sizes (Plate I, Fig. 3). The agranular endoplasmic reticulum, on the other hand, forms a system of relatively large channels and cisterns communicating with one another also in the form of a three-dimensional net. In the sections through the intestinal cells, this reticulum is visible in form of light bands of different lengths running between the areas of granular endoplasmic reticulum (Plate I, Figs. 1, 2, 3). Inconspicuous mitochondria (M) occur throughout the intestinal cell between the endoplasmic reticulum (Plate I, Figs. 1, 2, 3).

A large heterochromatin nucleus (N) is situated more basally. Chromatin is dispersed throughout the nucleoplasm in form of dark clods. A reticular nucleolus lies slightly concentrically inside the nucleus (Plate I, Fig. 1). Large drops of non-saturated lipids (L) are dispersed throughout the intestinal cell (Plate I, Figs. 1, 2, 3).

The apical part of intestinal cells is provided with few short microvilli (Mv). They are about 0.4 μm long and their length : width ratio is about 5 : 1. They are visible in form of a thin, strongly osmiophilic stripe of pith surrounded by a layer of lighter plasma (Plate I, Fig. 2). They adhere to a relatively thin layer of terminal network. The substance of the terminal network coalesces with the layer surrounding the pith of microvilli. The surface of the microvilli, as well as the whole intestinal cell, is covered by a cellular plasma membrane to which adheres a layer of glycocalyx.

The lumen of intestinal cells (Lu) of *P. commutatus* is usually filled with a detritus. Besides a rather homogeneous substance, different membranous structures may be observed inside it (Plate I, Fig. 2).

Lamina basalis (B) measuring about 0.2 μm and surrounding the whole intestine from the outside adheres to the basal part of the plasma membrane. It forms a border between the intestinal cells and pseudocoelom (Co) of the worm, or between the worm and muscle cells or gonads (Plate I, Fig. 1).

Ultrastructure of the intestinal wall of *Protostrongylus rufescens*

A characteristic feature of the intestinal cells of this nematode is the large number of ultrastructural elements. The mitochondria (M) are concentrated in a relatively thick layer in the basal part of intestinal cell (Plate II, Fig. 1), but single mitochondria occur sporadically throughout the cell (Plate II, Figs. 2 and 3). The cytoplasm of intestinal cell contains a large amount of both granular and agranular endoplasmic reticulum (GER, ER) and glycogen (G), as well as secretory granules (S) and lipids (L) (Plate II, Figs. 1 and 2). The nucleus with reticular nucleolus and dispersed clods of chromatin is situated more basally (Plate II, Fig. 1). A thin layer of terminal network and microvilli (Mv) projecting into the lumen (Lu) of intestine are visible at the apical ends of intestinal cells (Plate II, Fig. 2). A thin lamina basalis (B) adhering to the basal part of the plasma membrane surrounds the intestine and forms a border between the intestinal cells and pseudocoeloma (Plate II, Fig. 1). The ultrastructure of the intestine of *P. rufescens* was dealt with in detail in a previous paper (Zmoray and Gutteková 1974).

Comparison of the ultrastructure of intestinal wall of *Protostrongylus commutatus* and *Protostrongylus refescens*

The thickness of the intestinal wall differs only slightly in the two nematode species, being about 25 μm in *P. commutatus* and 23 μm in *P. rufescens*. The representation of individual ultrastructural elements, however, is quite different.

In *P. rufescens*, the basal part of intestinal cells is characterized by a layer of polymorphous mitochondria localized immediately below the basal membrane. Channels of agranular endoplasmic reticulum and glycogen granules are visible between them. The mitochondria occur also in other parts of intestinal cells, but only occasionally. In *P. commutatus*, the mitochondria are dispersed throughout the intestinal cell. They are numerous, but do not form the even layer at the base, as it is visible in *P. rufescens*.

The central zone of intestinal cell in *P. commutatus* contains an enormous amount of granular endoplasmic reticulum in which channels and cisterns of agranular endoplasmic reticulum and dispersed drops of non-saturated lipids and mitochondria are visible. The amount of endoplasmic reticulum is so large that no free cytoplasm can be observed in the sections through the intestinal cell. The apical part of intestinal cell in *P. commutatus* is organized in a similar manner. The intestinal cells of both species contain also a large amount of both saturated and non-saturated lipids and a large number of pigment granules; those of *P. rufescens* contain also a large number of secretory granules. The terminal network of both species is a poorly developed, thin, dark layer to which the microvilli adhere.

The microvilli in the two nematode species are not very numerous and measure about 0.4 μm in length and 0.06 μm in width. Their ultrastructure is well developed. Their pith is conspicuous and their distal ends are blunt. Destructed microvilli are often observed in *P. rufescens*. Either whole microvilli or their distal ends are widened in form of a sac and filled with a granular substance. In *P. commutatus*, there often occur granules at the apical end of cell, even between microvilli, which indicates a secretion. The lumen of intestine in both species is filled with a detritus and variously formed structures.

DISCUSSION

The comparative study of the intestinal ultrastructure in *Protostrongylus commutatus* and *P. rufescens* revealed many interesting facts. Primarily, there is a marked concentration of mitochondria in the basal part of cells, particularly in *P. rufescens*, and the granular endoplasmic reticulum occurs in an enormous amount, particularly in *P. commutatus*. This different ultrastructural organization of intestinal cells in these two taxonomically closely related species is not incidental and is certainly substantiated. Our studies of the ultrastructure of model nematodes demonstrated that all peculiarities in the ultrastructural arrangement, be it in muscle cells or in intestinal cells, are in fact a reflection of the specific conditions of parasitism of the respective helminth species (Gutteková and Zmoray 1974, 1977, 1979, 1982).

From this aspect we shall try to explain the unusual localization of mitochondria and the development of granular endoplasmic reticulum in the intestinal cells of the two species of *Protostrongylus*.

Both *P. commutatus* and *P. rufescens* parasitize in the upper parts of respiratory systems of their hosts. Since they are not provided with any attaching organs, they lie freely on the mucosa in a layer of phlegm. It may be supposed that they will hardly move on the mucosa, since the transverse striation of cuticle enabling other worms to move dorso-ventrally (Wisse and Deams 1968) is lacking in Protostrongylidae.

As to the source of food and mode of feeding, it is probable that they use the slime and phlegm surrounding them. Their small buccal capsule (Boev 1975, Pajerský 1983) indicates that the mucosa of the respiratory system could not serve as a source of food (Lee and Atkinson 1976). It seems, however, that the slime and phlegm are not the only sources of food, since glandular hypodermal cells occur in the body wall in lateral chords in both species. The occurrence of these cells is usually considered to be associated with the secretory processes (Wright 1963). It is very probable that the secretions in *Protostrongylus*, like in *Trichostrongylus* and *Nippostrongylus* (Lee 1970, Ogilvie 1973), or as supposed in *Dictyocaulus* (Gutteková 1985), will inhibit the movement of villi and prevent the elimination of worms from the host respiratory system on the one hand and change the permeability of plasma membranes of epithelial cells lining the respiratory system on the other hand. This action would result in a release of nutrients from the host cells into the environment and their assimilation by the parasites. It is supposed that the released nutrients penetrate into the worm body per integumentum. This assumption is supported by the fact that the cuticle is so thin that it cannot represent any barrier for the passage of nutrients from the environment into the worm body. Moreover, the cortical layer, which is biochemically very active (Lešťan and Zmoray 1964, Lešťan and Brežná 1965, Lee 1966, 1972, Bird 1971), is well developed in the cuticle of both species. The hypodermis, which is considered to be a site of great metabolical activity (Roggen et al. 1967), contains an unusually large number of mitochondria. However, the principle evidence of feeding per integumentum is the high concentration of mitochondria in the basal part of intestinal cells. This concentration is particularly high in *P. rufescens*. A similar concentration of mitochondria in the basal part of intestinal cells was observed also in *Cystocaulus ocreatus* (Zmoray and Gutteková 1972) and *Dictyocaulus* (Zmoray and Gutteková 1970, Gutteková 1985a). Lee (1969) described this phenomenon in *Nippostrongylus brasiliensis* and assumed that the concentration of mitochondria at the periphery of intestine may be associated with an active transport of nutrients through the lamina basalis (Lee and Atkinson 1976). In addition to feeding per integumentum, these nematodes are likely to feed also per os, as it is evidenced by the presence of single mitochondria in the central and especially apical parts of intestinal cells. The small number of microvilli, however, does not enlarge the absorption surface, but the occurrence of glycocalyx around the microvilli suggests that a part of food penetrates into the parasite body also per os.

The intestinal cells of *Protostrongylus* contain also a large amount of agranular and particularly granular endoplasmic reticulum. This indicates that the intestinal epithelium of *Protostrongylus* is a tissue which plays an important role in synthetic processes of the organism and not only a barrier through which the nutrients are transported. Especially the enormous development of the granular endoplasmic reticulum in the intestinal cells of *P. commutatus* is surprising. The presence of such a large amount of granular endoplasmic reticulum in the cells shows that a large number of proteins even for extracellular use are synthesized. It is supposed that in *P. commutatus*, the proteins are used mainly for the structure of sexual products. The high sexual production of *P. commutatus* females fully supports this assumption. This phenomenon is substantiated in *P. commutatus*, since it is closely related with the localization of the worms at the sites of trachea bifurcation in the lungs of hare. Due to its mode of living and the frequency and intensity of respiration, the hare differs significantly from the host of *P. rufescens* — the sheep. Moreover, the eggs of *Protostrongylus* develop up to the first larval stage within the host organism. The lung anatomy in hare enables the eggs of *P. commutatus* to be aspirated through the bronchi up to alveoli, where they occur in large numbers (Pajerský 1983). It is highly probable, however, that the same numbers, if not higher, are expired into the environment as a result of the strongly frequented and intensive respiration of the hare. In the outer environment, the eggs cannot develop and die. Consequently, the continuous reproduction of *P. commutatus* must be assured by an increased production of sexual products. On the other hand, the high reproduction activity is reflected in the ultrastructural organization of intestinal cells of the worm, i.e. in the amount of the granular endoplasmic reticulum.

The above results show that the ultrastructural organization of intestinal cells in individual nematodes is influenced by the environment in which the nematodes live, as well as by the mode of living of the host organism. This means that the bionomy of nematodes and their ultrastructure is affected by the environment of the first, as well as of the second order (Zmoray 1973).

СРАВНЕНИЕ УЛЬТРАСТРУКТУРЫ КИШКИ НЕМАТОД *PROTOSTRONGYLUS COMMUTATUS* И *PROTOSTRONGYLUS RUFESCENS* (PROTOSTRONCYLIDAE: NEMATODA)

А. Гуттекова

Резюме. Из сравнения ультраструктуры клеток кишки двух таксономически очень близких видов нематод — *Protostrongylus commutatus* и *Protostrongylus rufescens* — вытекает ряд интересных данных. Концентрация митохондрий в базальной части клеток кишки, которая значительна особенно у *P. rufescens*, свидетельствует о принимании питательных веществ также через кутикулу. Громадное встречание гранулярной эндоплазматической сети в клетках кишки *P. commutatus* свидетельствует о чрезмерном синтезе белков. Можно полагать, что они будут использоваться главным образом для строения половых продуктов, так как у *P. commutatus* их продукция очень высока. Это явление, по видимому, соединено с условиями развития яиц и с локализацией черва в окрестности бифуркации трахеи в легких заяца.

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A. P. Markevich (Ed.): Parazitotsenologiya. Teoreticheskie i prikladnye problemy. (Parasitocenology. Theoretical and applied problems.)

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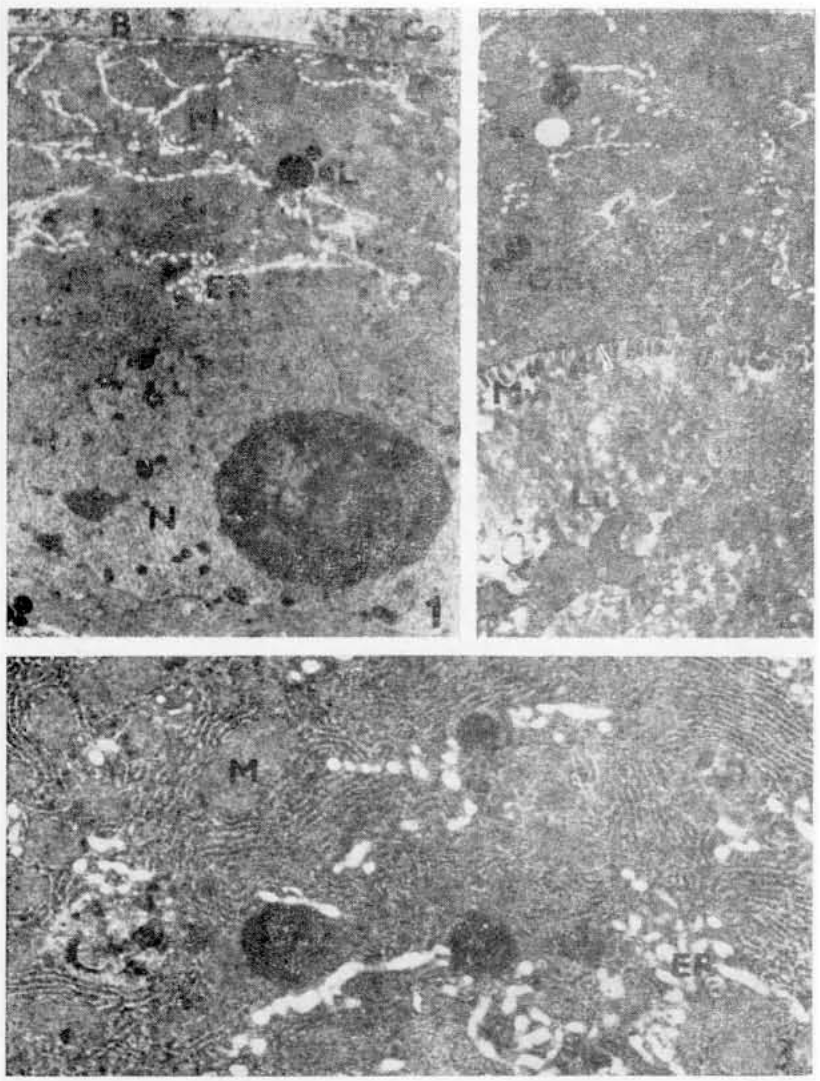
Parasitocenology — a new science — develops intensively in the last years. It may be defined as a complex theoretical and applied medical-, veterinary, phytopathological and biocenological science on ecoparasitic systems which include parasitic and conditionally pathogenic organisms, associations of their free-living generations and host environment. Its theoretical basis is a synthesis of general laws governing the different forms of parasitism studied by individual branches such as virology, medical, veterinary and phytopathological microbiology, myco-, phyto- and zooparasitology. The final aim of these investigations is the scientific management of the natural processes in the ecoparasitic systems directed to the control of diseases caused by parasitic organisms of all kind.

A group of 34 outstanding specialists headed by academician A. P. Markevich attempted in this monograph to analyse the broad spectrum of problems and aspects of this scientific branch. The text is divided into 9 chapters. 1—The world of parasites and some problems of the theory of parasitism, 2—Content of parasitocenology, theoretical and applied problems, 3—Host-parasite interrelations, 4—Physiological-biochemical research, 5—Molecular-genetic mechanisms of microparasitocenosis, 6—Importance of parasitocenology for epidemiological investigations, 7—Microparasitocenoses and their importance in the pathology of infections, 8—Mixed infectious diseases of man and animals, 9—Mathematical models of multicomponent parasitocenoses. The references include 552 citations.

The monograph represents the first book in the Soviet literature devoted to this subject. Many topics are discussed here and numerous fruitful ideas presented. It is a very stimulating reading. The authors of this publication are to be warmly congratulated.

Dr. V. Černý, C.Sc.



Figs. 1, 2, 3. Transverse sections through the intestinal cell of *P. commutatus* showing its basal part (Fig. 1), apical part (Fig. 2) and detail of the central part (Fig. 3). Note the large amount of granular and agranular endoplasmic reticulum (GER, ER) in all parts of the cell, mitochondria (M), lipids (L), lamina basalis (B), pseudocoelom (Co), microvilli (Mv), and lumen (Lu). (× 700; × 10 000; × 20 000).

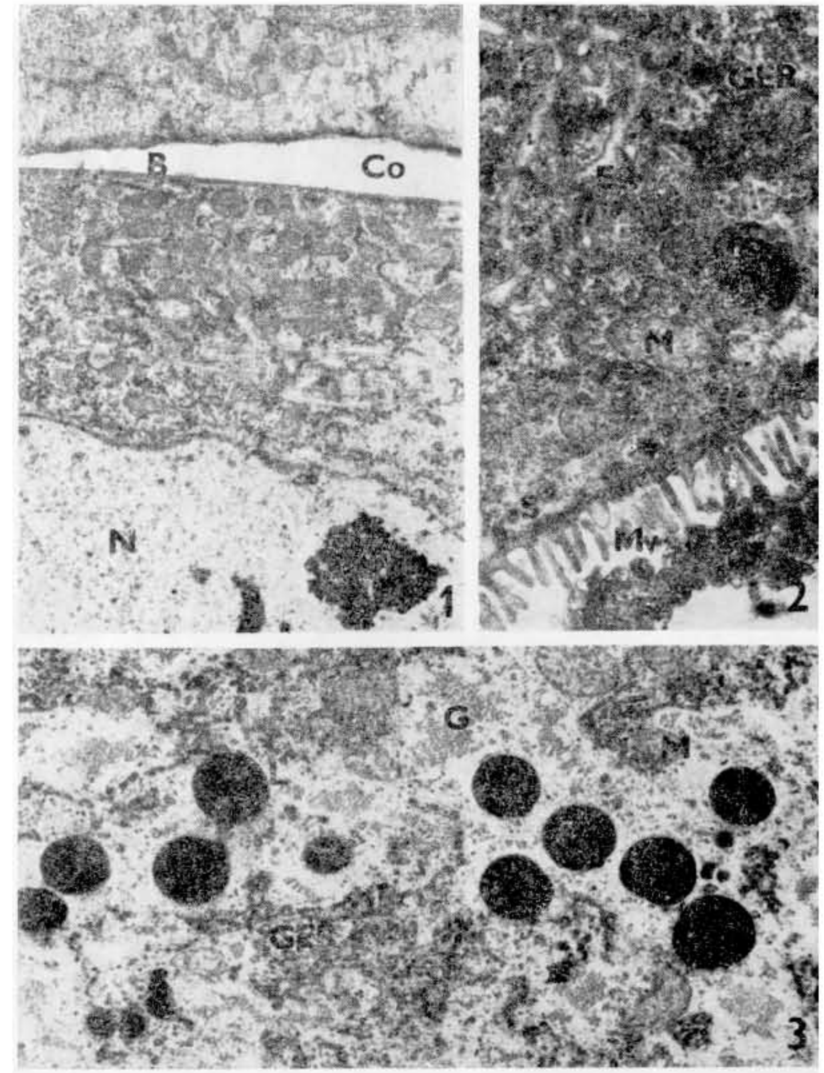


Fig. 1. Transverse section through the apical part of intestinal cell of *P. rufescens* showing a layer of mitochondria (M) localized under lamina basalis (B), nucleus (N), and pseudocoelom (Co). (× 7 000). **Fig. 2.** Detail of the transverse section through the apical part of intestinal cell of *P. rufescens*. Note the granular and agranular endoplasmic reticulum (GER, ER), mitochondria (M), secretory granules (S), and microvilli (Mv) protruding into the lumen of intestine (Lu) (× 21 000). **Fig. 3.** Detail of a transverse section through the central part of intestinal cell of *P. rufescens* showing granular endoplasmic reticulum (GER), mitochondria (M), lipids (L), and glycogen (G) (× 15 500).