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Morphology of the Nuclei, Some Cytoplasmic Organelles and Inclusions in the Body Walls of *Paragonimus westermani* in Final Hosts

FUSANORI HAMAJIMA, KOICHI FUKUDA AND TAKEMI OGUMA

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Key words: dog, rat, *Paragonimus westermani* (triploid type), body wall, development, ultrastructure

Introduction

The lung fluke, *Paragonimus westermani*, has three different kinds of hosts and different aerobic habitats during its life cycle. It is interesting to get knowledge of morphological changes in the nuclei, other organelles and inclusions of the body wall during the development from the metacercariae to adults in association with biosynthesis and energy metabolism. It has been known that dogs and some other carnivorous mammals are suitable hosts for the worm (Yokogawa *et al.*, 1960), while rats and mice are unfavorable (Ando, 1920 a, b; Miyazaki, 1946; Yokogawa *et al.*, 1962; Takizawa, 1964). However, ultrastructural changes of the body wall of *P. westermani* during the development within rats and dogs are not known. Therefore, the present study was carried out to elucidate the ultrastructure of the body walls, concentrating on nuclei, such organelles as ribosomes, endoplasmic reticulum, mitochondria and glycogen granules during the development of the worms within rats and dogs.

Materials and Methods

Encysted metacercariae of *Paragonimus westermani* (triploid type) were taken from the crab, *Eriocheir japonicus*. The metacercariae were excysted *in vitro* at 37°C in a solution of artificial gastric juice and intestinal juice following the method of Yokogawa *et al.* (1960). In rats, five-day-old juveniles were obtained from the abdominal cavities, 10-day-old juveniles from the abdominal and intrapleural walls, and 15-, 25- and 100-day-old worms from the abdominal and intrapleural walls, diaphragms and pleural cavities. In dogs, five-day-old juveniles were recovered in the abdominal cavities, 10-day-old juveniles in the livers, and 15- and 25-day-old juveniles in the abdominal cavities, livers, diaphragms and pleural cavities. In addition, 100-day-old adults were removed from the worms cysts in the lungs.

For electron microscopy, the recovered worms of each age were fixed for two hours in 2.5 % glutaraldehyde buffered with 0.1 M phosphate buffer, pH 7.4, and postfixed for two hours in 1 % osmium tetroxide in 0.1 M phosphate buffer, pH 7.4. The specimens were then dehydrated in an ethanol or acetone series and embedded in Epon 812. Thin sections were cut with diamond or glass knives on an LKB 8800 ultramicrotome, double-stained with uranyl acetate and lead citrate and observed with a JEOL JEM-100C electron microscope operated at 80 kV.

Observations

Newly excysted metacercariae

The body walls of newly excysted metacercariae were composed of tegument, subtegumental cells, muscle and parenchymal cells, and were essentially similar to those of the adult worms. The chromatin was dispersed

Department of Parasitology, National Defense Medical College, Tokorozawa 359, Japan.

throughout the nuclei in the subtegumental cells (Fig. 1). In contrast, the nuclei of the parenchymal cells contained heterochromatin stained as clumps with an irregular outline. There were no ribosomes nor endoplasmic reticulum in the tegument. However, many ribosomes were contained in the subtegumental cells (Fig. 2). On the other hand, few ribosomes were scattered throughout the cytoplasm of the parenchymal cells (Fig. 3), whereas the endoplasmic reticulum was sparse in the cytoplasms of both types of cells. Moderate numbers of mitochondria with few cristae were found near the basal area of the tegument of the juveniles. The majority of the mitochondria were ovoid in appearance, with the short cristae that seldom projected more than halfway across the width of the mitochondria, and the matrix was low in density. The mitochondria of the subtegumental cells were similar to those of the tegument. Mitochondria with few short cristae were also seen in the muscle and parenchymal cells. Numerous glycogen granules were found in the muscle and parenchymal cells (Fig. 3).

Five-day-old worms

No differences were found between the newly excysted metacercariae and five-day-old juveniles in rats except that the heterochromatin was begun to condense in the nuclei of the subtegumental cells of the latter (Fig. 4). In the body wall of the juveniles recovered from dogs, endoplasmic reticulum in the subtegumental cells was slightly more extensive than that of the excysted metacercariae. A few ribosomes were observed at the periphery of the nucleus in the parenchymal cells (Fig. 5), while the other appearance of the nuclei, cytoplasmic organelles and inclusions in the body wall of the worms was similar to those in the excysted metacercariae.

Ten-day-old worms

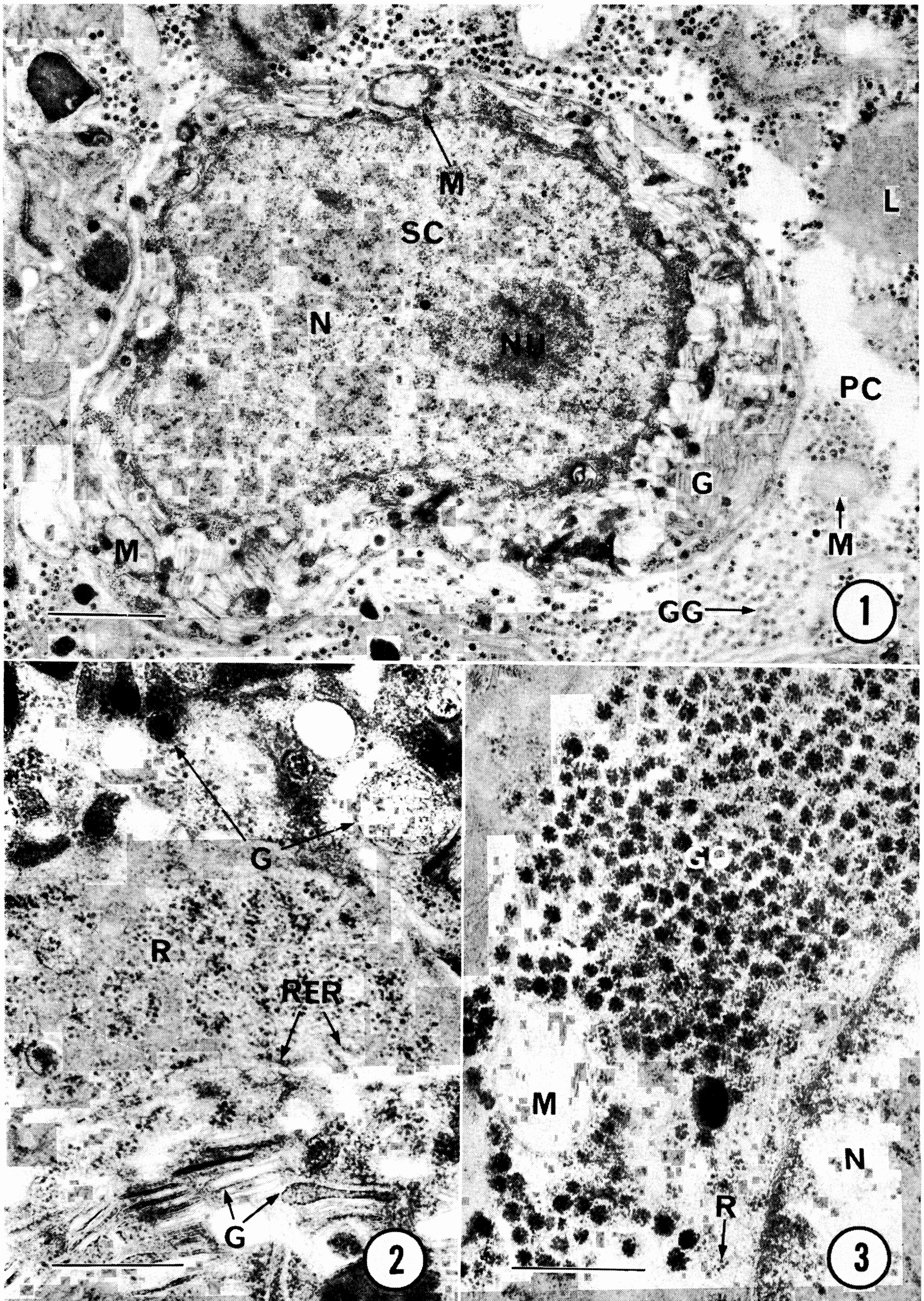
The nuclei, ribosomes and endoplasmic reticulum in the subtegumental and parenchymal cells and mitochondria in the tegument and subtegumental cells of the 10-day-old juveniles in rats generally retained the structure observed in five-day-old worms (Fig. 6).

The chromatin was heterochromatic in the majority of the subtegumental cells of the worms recovered from dogs. And the heterochromatin occurred in irregular clumps adjacent to the nuclear envelope (Fig. 7). In contrast, the heterochromatin of the nuclei of the parenchymal cells was dispersed as clumps throughout the nucleoplasm (Fig. 8). Differences were observed between the five- and 10-day-old worms in distribution of visible chromatin of the subtegumental cells. Furthermore, in the subtegumental cell, the nucleoplasm appeared to pass actively through the nuclear pores into the cytoplasmic matrix (Fig. 7). Numerous ribosomes and developed endoplasmic reticulum were contained in the subtegumental cells of these worms. On the other hand, a few ribosomes were scattered through the cytoplasm of the parenchymal cells. In addition to this, rough endoplasmic reticulum was sparse at the periphery of the nucleus (Fig. 8). Moreover, mitochondria increased in number in the tegument, subtegumental cells, muscle and parenchymal cells of the worms. The mitochondria with very few short cristae were found in the parenchymal cells. Glycogen granules were numerous in parenchymal cells.

Fifteen-, 25- and 100-day-old worms

The nuclei, cytoplasmic organelles and inclusions in the body wall of the 15-day-old juveniles from rats were essentially similar in shape to those of five-day-old worms (Fig. 9).

In the worms recovered from dogs, the euchromatin occurred in the nuclei of the majority of the subtegumental cells, and was dispersed throughout the nucleoplasm (Fig. 10). On the other hand, the heterochromatin occurred in the parenchymal cells. The subtegumental cells contained numerous ribosomes and well-developed endoplasmic reticulum (Fig. 11). Mitochondria with well-developed cristae were numerous in the tegument, subtegumental cells and muscle in these ages, although the mitochondria increased in number gradually from the 15-day-old to the 100-day-old worms. A few ribosomes and endoplasmic reticulum were present near the nuclei of the parenchymal cells. The parenchy-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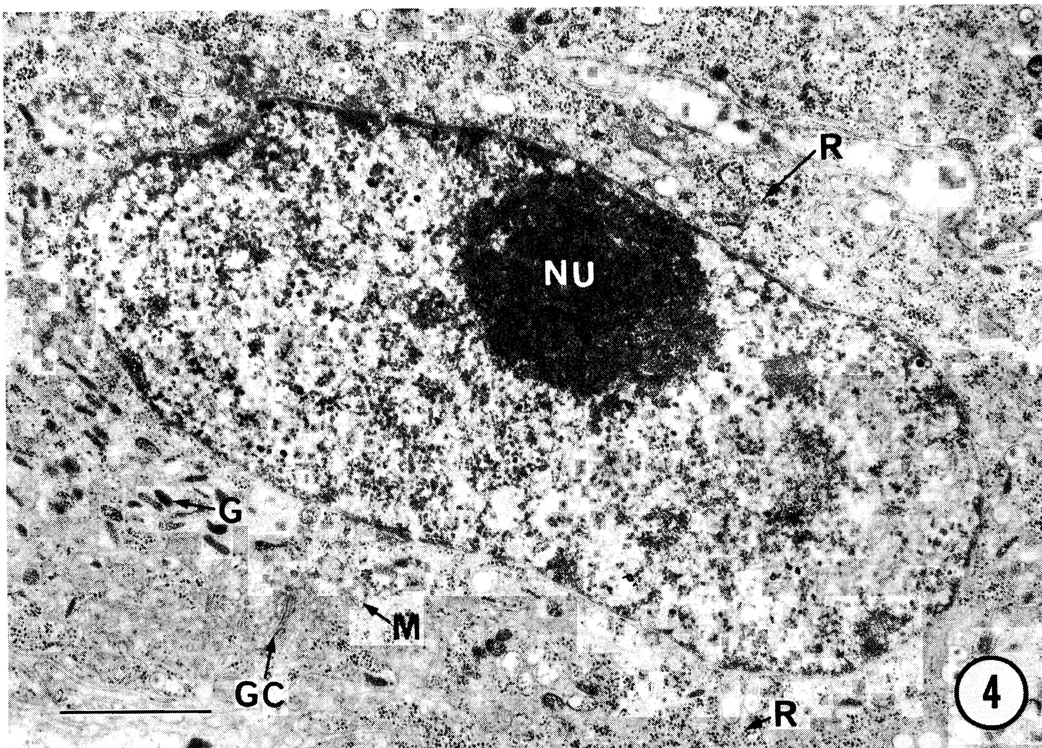
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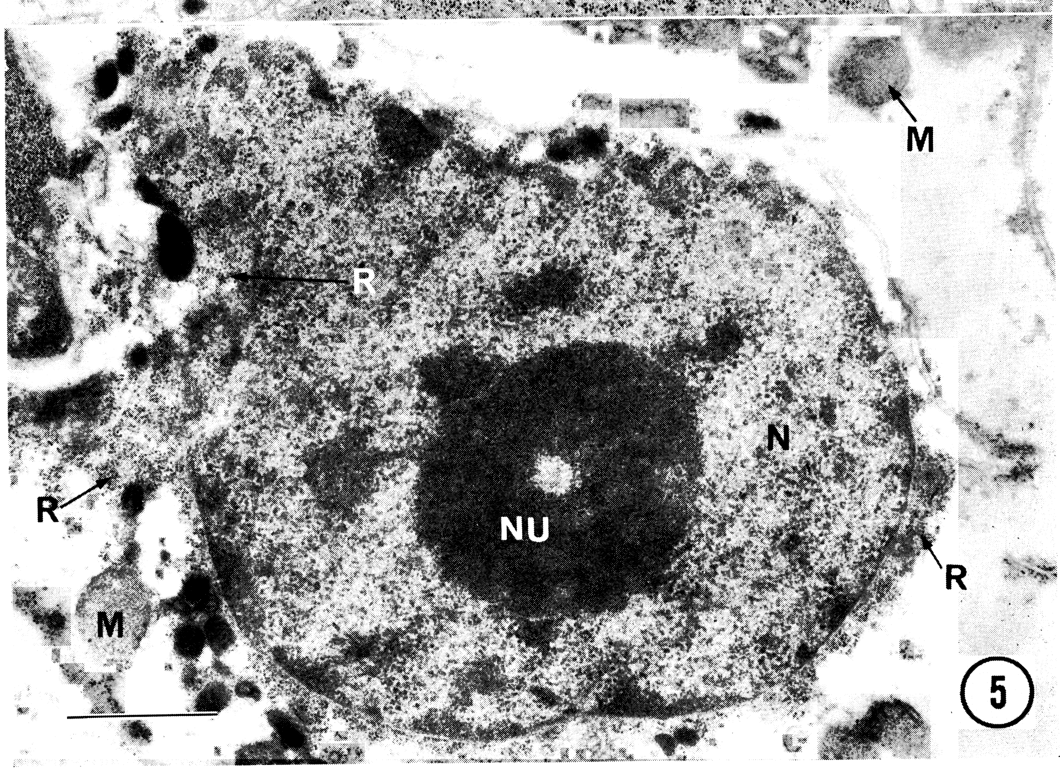
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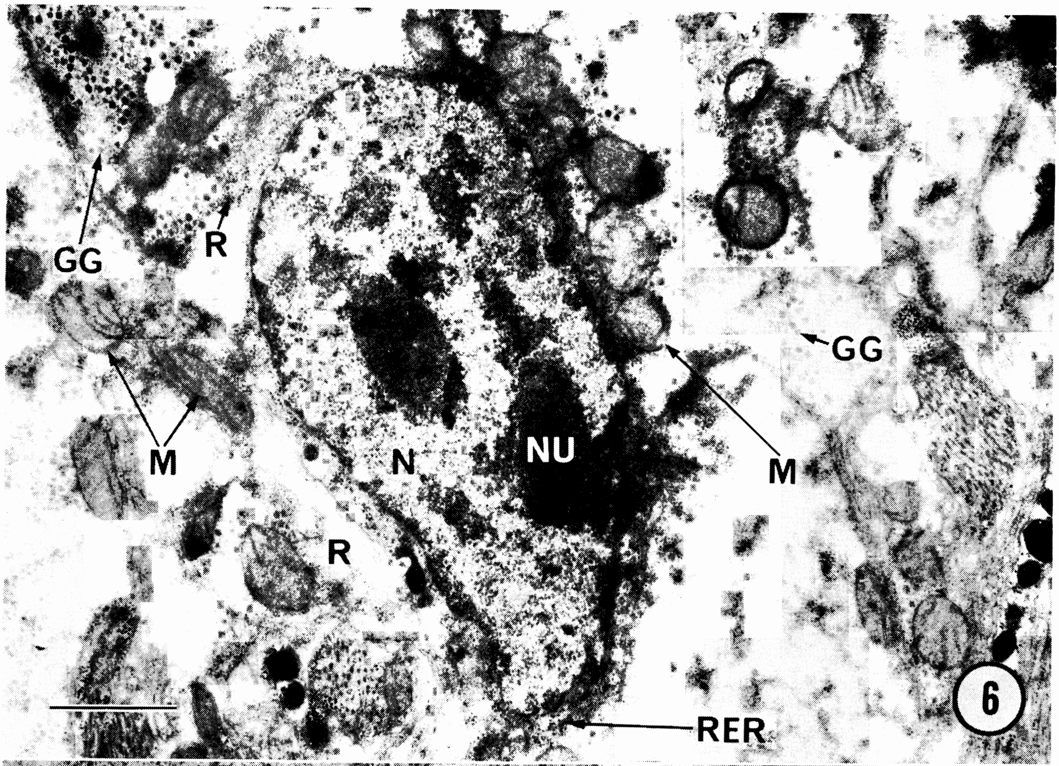
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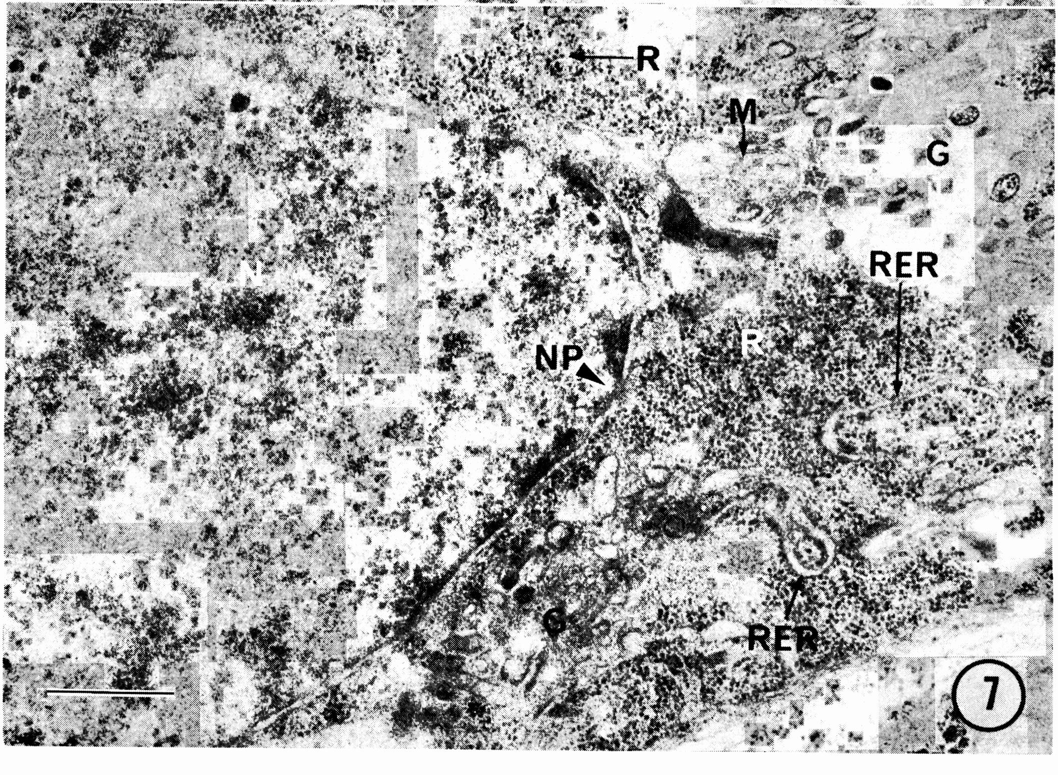
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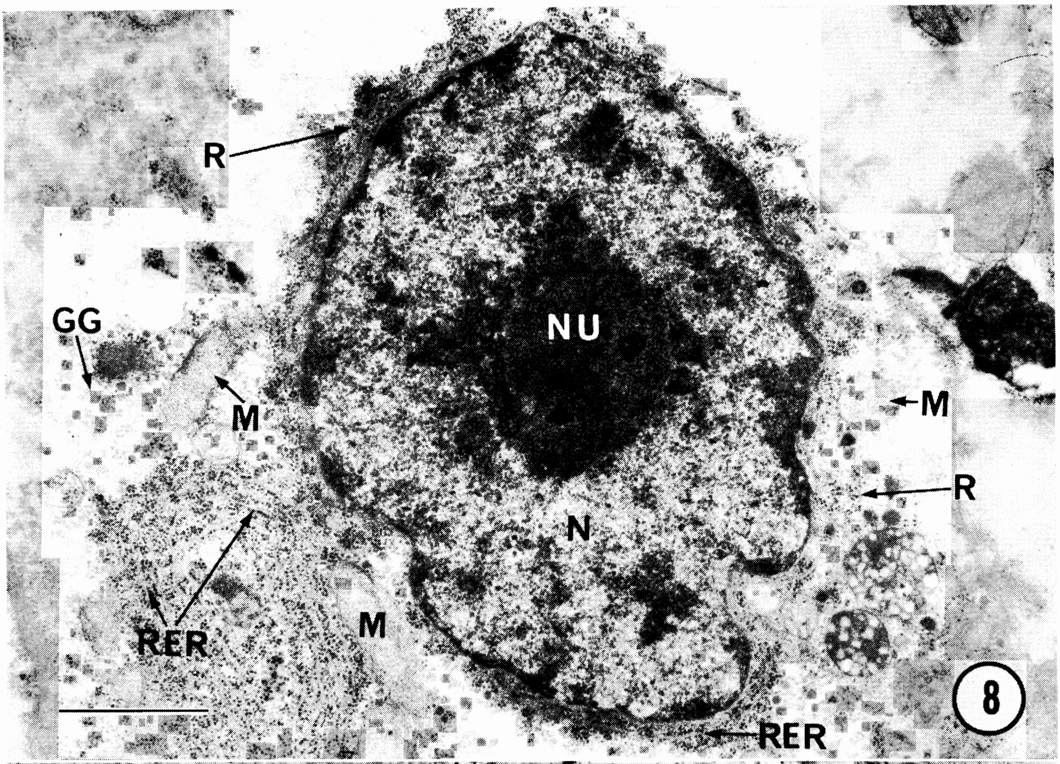
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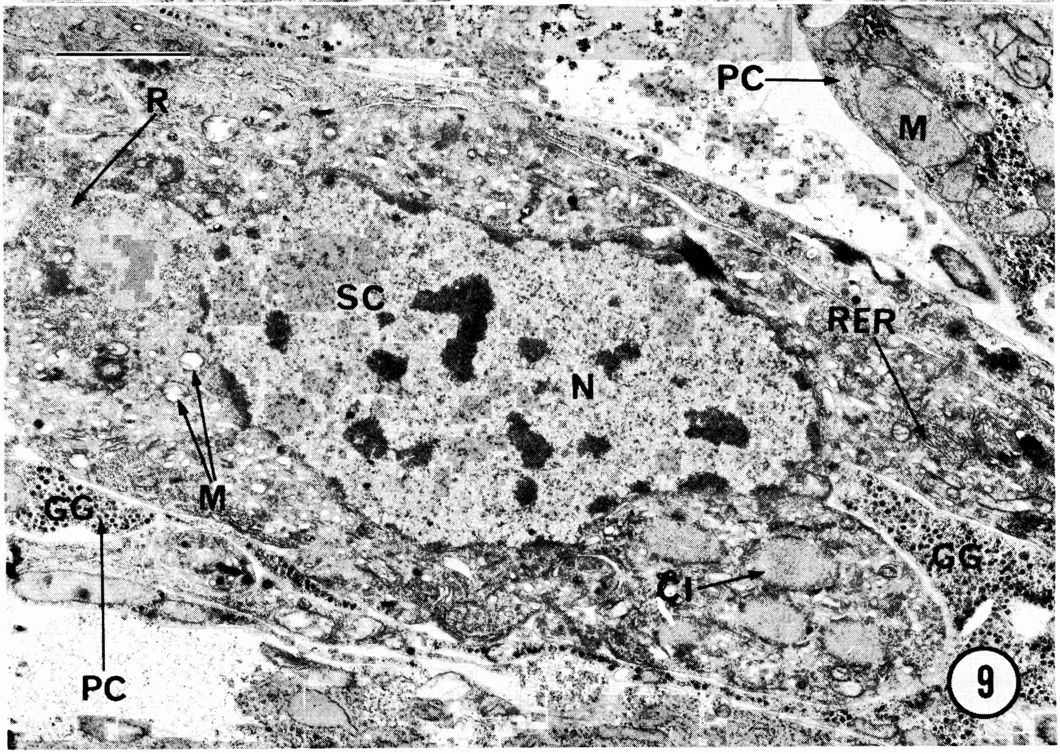
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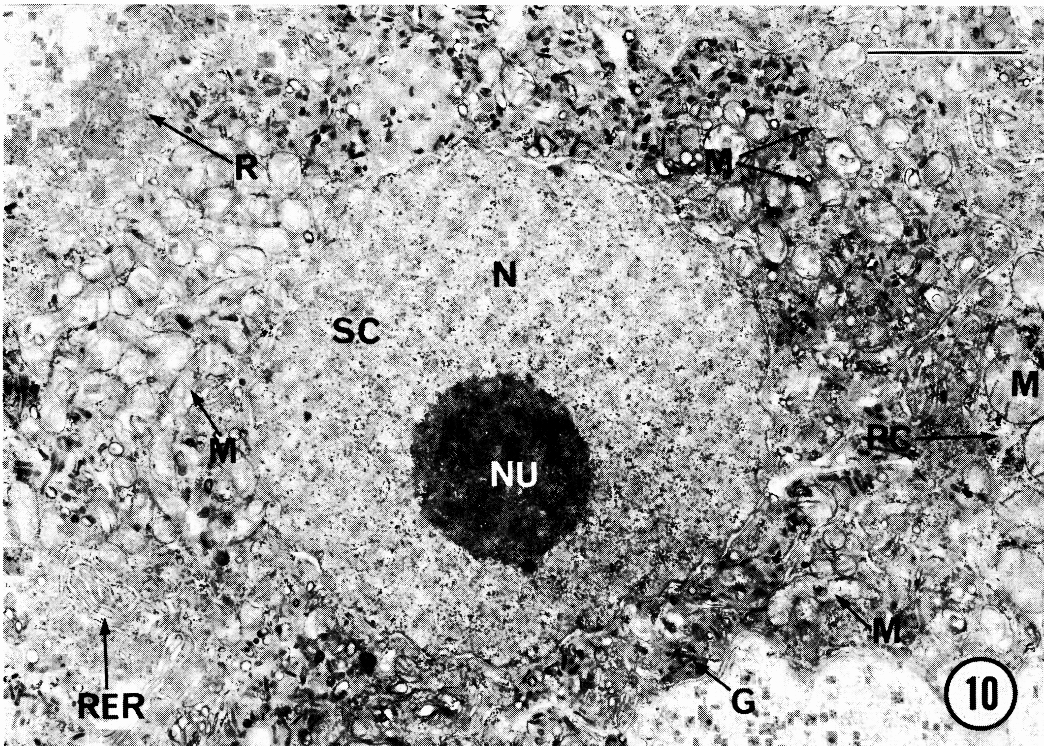
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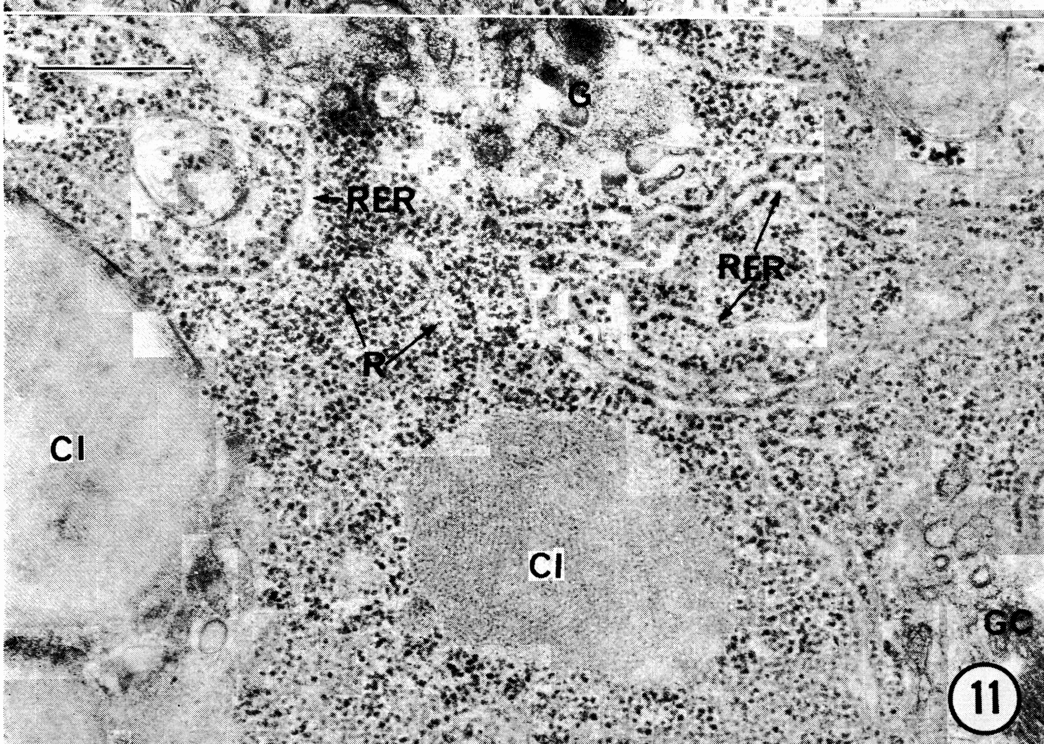
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Fig. 1 Part of the body wall of newly excysted juvenile. Subtegumental cell is surrounded by the parenchymal cells. Chromatin is dispersed in the nuclear matrix of the subtegumental cell. Note mitochondria and many granules in the subtegumental cell. (Scale : 2 μm)

Fig. 2 Part of the subtegumental cell of newly excysted juvenile. There are many ribosomes and sparse rough endoplasmic reticulum in the cytoplasm. (Scale : 0.5 μm)

Fig. 3 Part of the parenchymal cell of newly excysted juvenile. Note numerous glycogen granules. Mitochondria and ribosomes are also visible near the nucleus. (Scale : 0.5 μm)

Fig. 4 Subtegumental cell of five-day-old juvenile from rat. Note heterochromatin throughout the nucleoplasm in the nucleus. Many ribosomes and a few mitochondria are visible in the cytoplasm. (Scale : 1 μm)

Fig. 5 Part of the parenchymal cell of five-day-old juvenile from dog. In the cytoplasm at the periphery of the nucleus, few ribosomes are observed. (Scale : 1 μm)

Fig. 6 Parenchymal cell of 10-day-old juvenile from rat. The heterochromatin is observed in the nucleus. Note numerous glycogen granules and few mitochondria in the cytoplasm. (Scale : 1 μm)

Fig. 7 Part of the subtegumental cell of 10-day-old juvenile from dog. Heterochromatin of the nucleus occurred adjacent to the nuclear envelope. Note nuclear pore and nucleoplasmic continuity with the cytoplasm (arrowhead). Numerous ribosomes, developed rough endoplasmic reticulum and some mitochondria are in the cytoplasm. (Scale : 0.5 μm)

Fig. 8 Parenchymal cell of 10-day-old juvenile from dog. Note heterochromatin throughout the nucleoplasm in the nucleus. Few ribosomes, sparse rough endoplasmic reticulum and a few mitochondria with few short cristae are observed in the cytoplasm at the periphery of the nucleus. A delicate branching of the endoplasmic reticulum, that formed a lace-like network is also seen. (Scale : 1 μm)

Fig. 9 Subtegumental and parenchymal cells of 15-day-old juvenile from rat. Note few ribosomes, sparse rough endoplasmic reticulum in the subtegumental cell and numerous glycogen granules in the parenchymal cell. (Scale : 2 μm)

Fig. 10 Part of the subtegumental cell of 100-day-old adult from dog. Numerous ribosomes and well-developed rough endoplasmic reticulum and numerous mitochondria with well-developed cristae are visible. (Scale : 2 μm)

Fig. 11 Part of the subtegumental cell of 100-day-old adult from dog. Note numerous ribosomes and well-developed rough endoplasmic reticulum. Crystalline inclusions are also seen. (Scale : 0.5 μm)

CI	Crystalline inclusion	N	Nucleus
G	Granule	NP	Nuclear pore
GC	Golgi complex	NU	Nucleolus
GG	Glycogen granule	PC	Parenchymal cell
L	Lipid	R	Ribosome
M	Mitochondrion	RER	Rough endoplasmic reticulum
		SC	Subtegumental cell

mal cells contained numerous mitochondria with very few cristae. There were lots of glycogen granules in the muscles and parenchymal cells.

Discussion

The nucleus is known to be the source of informational macromolecules that control synthetic activities of the cytoplasm. The DNA of heterochromatin is present as nucleohistone, and is thought to be metabolically inactive (Fawcett, 1981). In the excysted metacercariae and all of the juveniles recovered from rats, the nuclei of the subtegumental and parenchymal cells were rich in masses of heterochromatin except for the subtegumental cells of the metacercariae. Rough endoplasmic reticulum was sparse in the cytoplasm of the subtegumental and parenchymal cells of the metacercariae and the juveniles from rats. Consequently, it seems that protein synthesis is not active in these cells. Similar observations were reported for the rough endoplasmic reticulum in the tegumental cells of *Fasciola hepatica* by Bennett and Threadgold (1975). It has also been known that rat serum, antiproteases and immunoglobulins inhibit the worm's acid hemoglobin protease, which hydrolyzes hemoglobin (Hamajima *et al.*, 1979, 1982 a ; Hamajima and Yamagami, 1980, 1981 a ; Iwata *et al.*, 1982 ; Hamajima *et al.*, 1982 b, 1983). From these results, it is possible that the worms are not able to digest rat blood or provide the essential nutriments for the synthesis of DNA, RNA, protein and glycogen in their cells (Hamajima and Yamagami, 1981 b). It would appear, therefore, that the majority of the worms stayed as juveniles in the body of rats.

The chromatin of the nuclei was euchromatic in the subtegumental cells of the worms recovered from dogs, except in 10-day-old worms. Being euchromatic is thought to be more active metabolically than heterochromatic. Thus, it seems likely that the nuclei of this form are active in transcription. In contrast, heterochromatin was found in the nuclei of the parenchymal cells and subtegumental cells of the 10-day-old worms. It was also observed that some nucleoplasm passes through the nuclear pores directly into the cytoplasmic matrices in the subtegumental cells of 10-day-old worms. These observations suggest that RNA moleculues move from the nucleus to the cytoplasm for the arrangement of components of the ribosomes on which polypeptide chains are built and for the messengers from DNA in the nucleus for the aerobic transformation of the juveniles into adults.

The arrangements of the ribosomes and rough endoplasmic reticulum in the subtegumental cells of 15-day-old worms from dogs was similar to those of adults. In *P. westermani* adults, the cytoplasm of the subtegumental cells contained a large number of ribosomes and extensive rough endoplasmic reticulum. These results are similar to those reported for *Schistosoma* by Hockley (1973) and *F. hepatica* by Threadgold (1967). Thus, it may be probable that some enzymes are synthesized via the ribosomes and rough endoplasmic reticulum and is utilized for metabolism. Moreover, in the worms from dogs, the cristae of mitochondria in the subtegumental cells were more developed than those in the parenchymal cells. The number of mitochondria increased in the subtegumental and parenchymal cells of 15-, 25- and 100-day-old worms. Mitochondria contained well-developed cristae in the subtegumental cells. These observations are different from those reported by Hockley (1973) for the number of mitochondria in the tegument of the adult *Schistosoma*. In contrast to the mitochondria of the subtegumental cells in the juveniles and adults those of the parenchymal cells in these stages bear the sparse and poorly developed cristae. This is in agreement with the observations in *F. hepatica* (Threadgold and Gallagher, 1966). These observations indicate that the mitochondria of the parenchymal cells are correlated with the anaerobic metabolism in the liver fluke and lung fluke worms (Björkman and Thorsell, 1964 ; Hamajima, 1975 ; Hamajima *et al.*, 1979 ; Hamajima *et al.*, 1982 c).

In contrast to rat serum, the dog serum did not markedly inhibit hemoglobin protease of the worms(Hamajima *et al.*, 1979 ; Hamajima *et al.*, 1983). And also some lung flukes ingested dog blood cells easily (Hata *et al.*, 1982 ; Hata *et al.*, 1983). For these reasons, it seems to be a good possibility of ingestion of nutrition in the worms of dogs. Thus, it is concluded, therefore, that dogs are a suitable host for these worms.

Summary

The present study was carried out by electron microscopy to elucidate the ultrastructure of the body wall of *Paragonimus westermani* (triploid type) during the development within rats as an unfavorable host and dogs as a suitable host. In newly excysted metacercariae, the chromatin was dispersed throughout the nuclei of all the subtegumental cells except for those of the parenchyma. However, in the juveniles recovered from rats, heterochromatin was found in the nuclei of both the subtegumental and parenchymal cells. The morphology of the cytoplasmic organelles and inclusions in the body wall of the worms from rats was essentially similar to those in newly excysted metacercariae. In the worms from dogs, chromatin of nuclei of the subtegumental cells was euchromatic in the majority of cases except that of 10-day-old worms. In contrast, nuclei of the parenchymal cells contained mainly heterochromatin. The cytoplasms in the subtegumental cells of 15-, 25- and 100-day-old worms contained large numbers of free ribosomes, polyribosomes and well-developed rough endoplasmic reticulum and mitochondria with well-developed cristae. The mitochondria with few short cristae were numerous in the parenchymal cells. Furthermore, abundant granules of glycogen were contained in the muscles and parenchymal cells of these worms. Therefore, the appearance of these cytoplasmic organelles in 15-day-old worms was similar to that of adult worms.

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終宿主からえたウェステルマン肺吸虫の体壁における核，細胞小器官および後形質の形態

浜島房則　福田孝一　尾熊文実
（防衛医科大学校寄生虫学教室）

本研究では，ウェステルマン肺吸虫（3n 型）メタセルカリア，ラットおよびイヌからえた幼若虫および成虫の体壁における核，細胞小器官および後形質の微細構造を電子顕微鏡で観察した．その結果，メタセルカリアの体壁における上皮細胞の核には多くの真正染色質がみられたが，ラットからえた虫体では異質染色質が多くみとめられた．しかし，ラット感染虫体では，メタセルカリアの細胞小器官および後形質からの著しい体壁微細構造の変化はみられなかつた．以上のような核の性質，細胞小器官およびグリコーゲン顆粒の微細構造の状態からみて，ラットは本吸虫の非好適宿主であると考えられる．一方，イヌからえた虫体の上皮細胞の核では，ほとんどのものに多くの真正染色質がみられたが，柔細胞のそれは，多少の例外を除いて，異質染色質であつた．感染後15，25および 100 日令虫体では上皮細胞に多くのリボゾーム，ポリゾーム，よく発達した粗面小胞体およびミトコンドリアがみられたが，柔細胞にはクリステの発達の悪いミトコンドリアが多数みられた．さらに，これらの虫体の筋肉および柔細胞にはグリコーゲン顆粒が多くみとめられた．感染後15日令虫体の細胞小器官の発達は成虫にみられるそれらとほぼ同様となり，本虫体はイヌでは感染後15日令で細胞小器官の発育を完成した．以上の体壁微細構造の観察から，イヌは本吸虫の好適宿主であると考えられる．