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Cytological Studies on the Lateral Canal Cell in the Pig Ascaris (*Ascaris lumbricoides suum*)
I. Ultrastructure of the Lateral Canal Cell

MICHIO ISHIKAWA

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Key words: *Ascaris lumbricoides suum*, lateral canal cell, ultrastructure, excretory function

Introduction

Since Chitwood and Chitwood (1950) previously regarded the lateral canal cell of pig ascaris as a system or an organ, there has been dispute as to the structural features of the cell. In the previous study (Ishikawa, 1974), it was shown that the intracellular canals, formerly called as the excretory organ, was a specialized canal structure in the cytoplasm. The canal structure exhibited respective characteristics in its different portions; perinuclear region, lateral canal in lateral line and excretory pore. The present study deals with the ultrastructural features of perinuclear region of the canal cell.

Materials and Methods

Thirteen females of adult *Ascaris lumbricoides suum* were obtained from pigs sacrificed in a slaughter house of Nagoya City, Japan. These worms were kept alive in Ringer's solution maintained at 37°C. Perinuclear portions of the lateral canal cell with their neighboring tissues were removed from these living worms.

I. Preparation of tissue specimens for light microsopy:

Perinuclear portions of the lateral canal cell were fixed in Bouin solution or 2 % calcium acetate in 10 % formalin (Leppi, 1968) for 24 to 72 hours at room temperature. The tissue specimens were then rinsed in water, dehydrated in graded ethanol series, and embedded in paraffin. Sections were cut at a thickness of 4 to 8 μm, deparaffinized, hydrated, and stained with hematoxylin and esosin.

II. Preparation of tissue specimens for electron microscopy:

Perinuclear portions of the lateral canal cell were cut into tiny cubes and fixed in chilled (4°C) phosphate-buffered (pH 7.2) 2 % osmium tetroxide or in chilled (4°C) cacodylate-buffered (pH 7.2) 2.5 % glutaraldehyde for 1.5 to 4 hours. The osmium tetroxide-fixed tissues were dehydrated in graded ethanol series and embedded in Epon 812. (Luft, 1961). The glutaraldehyde-fixed tissues were rinsed in cacodylate-buffer (pH 7.4) for 2 to 4 hours, postfixed for 2 hours in cacodylate buffered 1 % osmium tetroxide (pH 7.4) at room temperature, dehydrated in graded ethanol series, and embedded in Epon 812 (Luft, 1961). Thick sections for orientation with a thickness of about 1 μm were cut on a JUM No. 5 microtome and stained with 1 % toluidine blue. Ultrathin sections were cut on a Porter-Blum microtome (MT-1), mounted on copper grids, and doubly stained with uranyl acetate (Watson, 1958) and lead citrate (Raynolds 1963). The stained sections were examined in a Hitachi HU-11D or HS-4 electron microscope.

Results

Light microscopy:

A pair of lateral canals in the lateral lines were found from the esophageal region towards nearly the middle of the worm body. In the second quarter of the body the canal lacked a continuous lumen. The lateral

Department of Anatomy, Nagoya City University Medical School, Nagoya, Japan.

canals were connected to the transverse canal which opened into the ventral excretion pore (Fig. 1). The nucleus of the lateral canal cell was situated in that swelling part of the cytoplasm where the lateral canal was connected to the transverse canal. The nucleus was huge in size and irregularly shaped (Fig. 2). In the perinuclear area of the cytoplasm, there occured numerous canaliculi (Fig. 2). Such images bespeak the fact that both the lateral canals undergo repeated branchings, forming ramifications and terminal canaliculi (Fig. 2).

Electron Microscopy :

I. Nucleus

The nucleus was huge in size, irregular in shape and enclosed by a nuclear envelope (Fig. 3). Small nuclear pores were observed in the nuclear envelope (Fig. 4). Within the nucleus, varying concentrations of chromatin granules were scattered and embedded in amorphous substances of the nuclear matrix (Fig. 3). In addition, a large nucleolus was found within the nucleus (Fig. 3).

II. Cytoplasm

The cytoplasm limited by a plasma membrane was faced to the lateral line tissues. On the other hand, the cytoplasm enclosed the lumen of the lateral canals.

(A) Perinuclear Regions of the cytoplasm

Nearly all types of cell organelles and inclusions were found.

Mitochondria were scattered, (Fig. 4) and were spherical or oval in shape and provided with small numbers of cristae. Usually, these organelles were closely associated with other organelles and inclusions such as Golgi complexes, elements of endoplasmic reticulum, lipid droplets and glycogen particles (Fig. 4). A small number of Golgi elements occupied the perinuclear territory of the cytoplasm (Fig. 5). They consisted of arrays of flattened sacs associated with vesicles and small vacuolus. These Golgi elements contained substances of varying electron densities (Fig. 5). Some of the Golgi elements were often in close vicinity to mitochondria, elements of granular endoplasmic reticulum, secretory granules and lysosomes (Fig. 5).

A number of elements of both granular and agranular endoplasmic reticulum were detected in the cytoplasm (Fig. 6). These elements were grouped into at least three forms ; tubules, vesicles and cisternae. The cisternal elements were at certain cytoplasmic loci, dilated and intracisternal granules were often noted (Fig. 6). Frequently, tubular and vesicular components of agranular endoplasmic reticulum were accumulated, and condensing secretory granules limited by a membrane were scattered here and there (Fig. 7). These granules were often in close association with mitochondria.

The perinuclear cytoplasm was more or less finely granular due to the presence of free ribosomes (Fig. 6). They were at times clustered to form polysomes which were localized preferentially close to the membranous elements of endoplasmic reticulum (Fig. 6).

Lysosome-like dense bodies were also found (Fig. 7). They were spherical, oval, or irregular in shape and comparable in dimension to secretory granules. They were membrane-limited, electron opaque and appear at times in the immediate vicinity of Golgi elements.

Varying numbers of glycogen particles were discerned which display spherical accumulations of different sizes (Fig. 4). Some of these particles tended to be distributed in close association with mitochondria (Fig. 8). Furthermore, lipid droplets of different sizes and shapes were detected (Fig. 4). Like glycogen particles, some of the droplets were closely associated with mitochondria (Fig. 4).

(B) Marginal regions of the cytoplasm

In the marginal regions of the cytoplasm abutting upon the lumen, a number of both the secretory granules and lysosome-like dense bodies were accumulated (Fig. 9). In the marginal cytoplasm, these granules and dense bodies tended to be less electron opaque, as compared with those distributed in the rest of the cytoplasm and were often lucent in their center or periphery (Fig. 9). Microtubules were abundant, which were approximately 250 Å in diameter, variable in length and straight or slightly curved (Fig. 9). They tended to be accumulated right beneath the plasma membrane abutting the lumen (Fig. 9). The

cytoplasmic matrix contains fine filaments (Fig. 9). These were approximately 100Å in thickness, variable in length and accumulated immediately beneath the plasma membrane.

The marginal cytoplasm of the lateral canal cell lined the lumen of the terminal canaliculi. Such lumen was therefore bordered by the plasma membrane of the cell (Fig. 9). In this part of the cytoplasm, a number of granules of varying structural features were observed, which appeared to be either secretory or lysosomal in nature (Fig. 10). Here, the plasma membrane exhibited infoldings, and cytoplasmic bulgings of different sizes were found here and there (Figs. 9 and 10). In the canal lumen, cytoplasmic debris of varying shapes and sizes were found (Figs. 9 and 10).

In the marginal cytoplasm bordering the lumen of the ramifying canal, membrane-limited electron lucid vesicles and vacuoles of varying figures were found (Figs. 11 and 12). Not infrequently, huge irregularly shaped protrusions loaded with numerous vesicles and vacuoles were noted in the lumen (Fig. 12). These appeared to be secretory or excretory in nature ; they appeared to leave the cytoplasm and to be discharged into the lumen.

The lateral line tissues were deeply invaginated into the cytoplasm of the lateral canal cell and formed complex foldings (Fig. 13). These foldings were reminiscent of functionally modified adsorption. In the cytoplasmic region abutting upon the lateral line tissues, varying numbers of vesicles of different dimentions were visualized (Fig. 14).

Discussion

The results obtained in a previous light microscopic study on the lateral lines of *A. lumbricoides suum* (Ishikawa, 1961) are in keeping with the ultrastructural images revealed in the present study.

The electron microscopical images of the nucleus within the lateral canal cell of *A. lumbricoides suum* observed in the present study were similar in structure to those in *A. lumbricoides* (Dankwarth, 1971). Dankwarth (1971) described that the nucleus of the lateral canal cell of *A. lumbricoides* was approximately 65 μm in diameter and contained about 50 nucleoli. Lee *et al.* (1973) reported that the nucleus of *Anisakis* larva (Nematoda : Anisakidae) was enclosed by a nuclear envelope with a thin perinuclear cisterna. In this nucleus, nuclear pores were detected at numerous sites and dense areas of heterochromatin accumulations were interspersed between less dense nucleoplasm.

In the present study, mitochondria in the cytoplasm were provided with only a small number of cristae. Dankwarth (1971) reported similar mitochondria in the lateral canal cell of *A. lumbricoides*. Lee *et al.* (1973) reported that mitochondria were in close association with granular endoplasmic reticulum elements in the cytoplasm of the excretory gland of *Anisakis* larva. In the present report, mitochondria were in close association with other cell organelles and inclusions such as Golgi apparatus, endoplasmic reticulum, glycogen particles, and lipid droplets. These images bespeak that mitochondria supply energy to or exchange it with other cell organelles and inclusions.

The present study indicated that there occured relatively few elements of Golgi complexes in the cytoplasm on the lateral canal cell in the pig ascaris. However, numerous elements of Golgi apparatus had been found in the excretory system of *A. lumbricoides* (Dankwarth, 1971) and of adult *Nippostrongylus brasiliensis* (Lee, 1971). According to the present results, the Golgi elements contained substances of varying electron opacities and were associated with other cell organelles and inclusions such as lysosomes and secretory granules. It is, therefore, obvious that the Golgi complexes play an important role for the formation of both lysosomes and secretory granules.

In the present study, the cytoplasm abutting the lumen of the fine canals contained varying numbers of secretory granules, lysosomes, numerous microtubules and filaments. In the cells observed here, secretory granules were thought to arise originally from elements of the granular endoplasmic reticulum and then

from those of the Golgi apparatus. The marginal cytoplasm of the lateral canal cell was provided with microtubules, membrane-limited electron lucid vesicles, and vacuoles of varying sizes. In addition, huge irregular shaped protrusions loaded with numerous vesicles and vacuoles were observed in the lumen of the ramifying canals. From these images, the cytoplasm abutting upon the ramifying canals were presumed to perform secretory or excretory functions. It remained, however, to be determined whether the canals are truly secretory or excretory in function.

It is generally accepted that the lateral canal cell is performing excretory functions (Chitwood and Chitwood, 1950). In *Anisakis simplex*, however, Müller (1927) found light microscopically and chemically that the lateral canal cell showed secretory functions. In *Anisakis* larva (Nematoda Anisakidae), Lee *et al.* (1973) reported, on the basis of electron microscopic images, that the excretory glands of the worms were not only excretory but secretory in function. In *Ascaris lumbricoides*, likewise, Koizumi (1954) demonstrated light microscopically and chemically the excretion of toxic substances through the excretion pores of the lateral canal.

In the present study, fine filaments were found in the cytoplasm of the lateral canal cell of the pig ascaris. In keeping with this, Lee *et al.* (1973) detected filaments in the cytoplasm of the excretory gland cells of Anisakis larva. They described further that these filaments seemed to serve as an element for cytoskeletal functions. Fawcett (1966) reported that intracellular filaments exist as either cytoskeletal or contractile elements in the cytoplasm.

In the lateral canal cell observed here, microtubules were accumulated in the cytoplasm surrounding the canals. However the true nature of these microtubular functions remained to be elucidated. In this connection, Rhodin (1974) recorded that the function of microtubules within cilia and flagella are related to motility. In the cell studied here, therefore, the functional activity of microtubules was presumed to be concerned with motility.

Summary

The ultrastructures of nucleus and perinuclear and marginal regions of the cytoplasm in the lateral canal cell have been described in the pig ascaris.

Usually a lateral canal cell was involved in one worm. This cell was usually huge in size, H-shaped and uninucleate. The nucleus was large and irregular in shape, and enclosed by an envelope. In the nuclear envelope were observed small nuclear pores. Within the nucleus chromatin granules, nuclear matrix and nucleoli were found.

In the cytoplasm of the cell studied, a series of morphoplasm were visualized, such as mitochondria, Golgi complexes, elements of granular and agranular endoplasmic reticulum, free ribosomes, lysosome-like dense bodies, microtubules, filaments, secretory granules, glycogen particles, and lipid droplets.

The cytoplasm contained a specialized inclusion, canal structures. In the perinuclear cytoplasm these canals were ramifying and appeared to be concerned with secretion or excretion.

In the marginal cytoplasm abutting upon the ramifying and terminal canals, some microtubules and vesicles and vacuoles of varying sizes were demonstrated. These vesicles and vacuoles appeared to leave the cytoplasm and to be discharged into the canal lumen.

The lateral line tissues were deeply invaginated into the cytoplasm of the lateral canal cell and formed complex infoldings, which were reminiscent of functionally modified absorption.

Acknowledgements

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ブタ蛔虫側管細胞の細胞学的研究
I. 側管細胞の超微構造

石川道雄
（名古屋市立大学医学部第2解剖学教室）

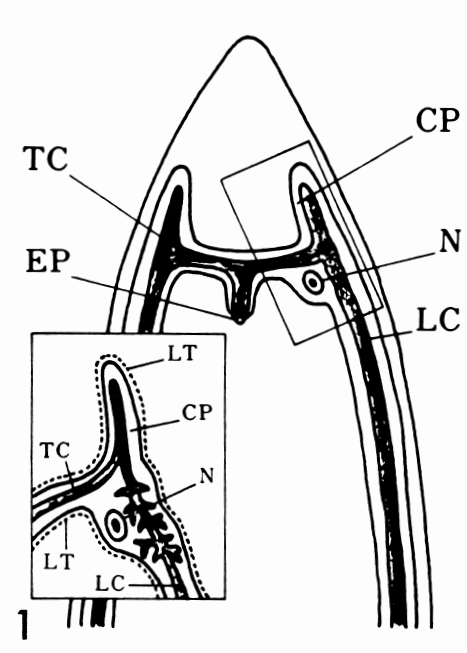
ブタ蛔虫の側管細胞の核，核周辺ならびに周縁部の超微構造を観察した．側管細胞はH字型を呈する巨大細胞で，その核は巨大であり，辺縁は不規則で，核膜孔を備える核膜に包まれ，内部にクロマチン顆粒，核基質および大きい核小体が存在する．

細胞質には糸粒体，Golgi 複合体，小胞体，リボ小体，水解小体，微細管，微細線維，分泌顆粒，グリコーゲン顆粒，脂質滴などが認められる．

核周辺の細胞質には分化した分岐する管構造が存在する．これらは排泄ないし分泌に関与するものと考えられる．

分岐管，終末管に面する細胞質辺縁部には微細管，微細線維，小胞および空胞が存在し，これらの小胞と空胞は管腔内に遊離するものと考えられる．

側線組織は側管細胞の細胞質に深く浸入し，吸収機能を思わせる複雑なヒダを形成する．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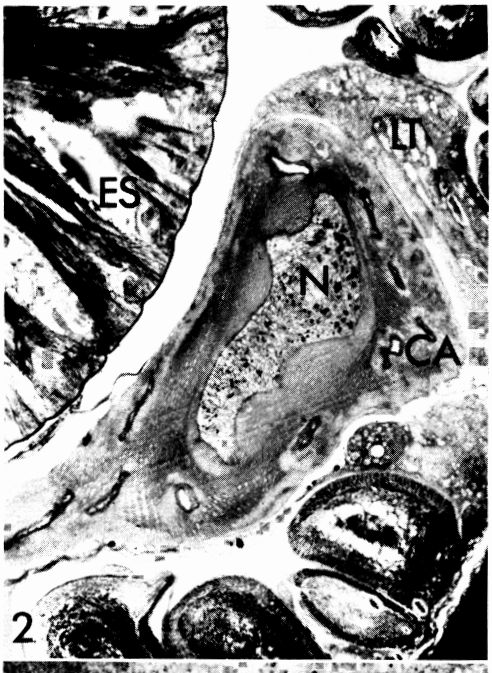
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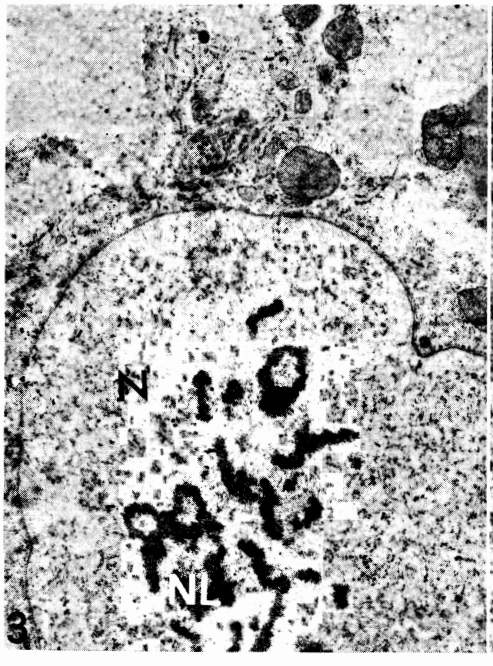
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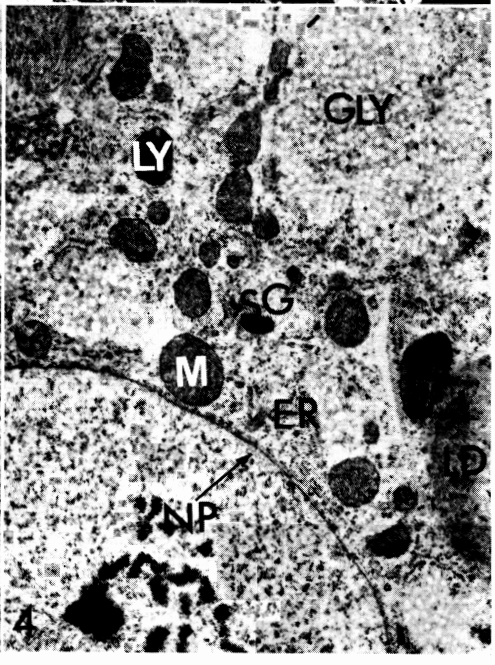
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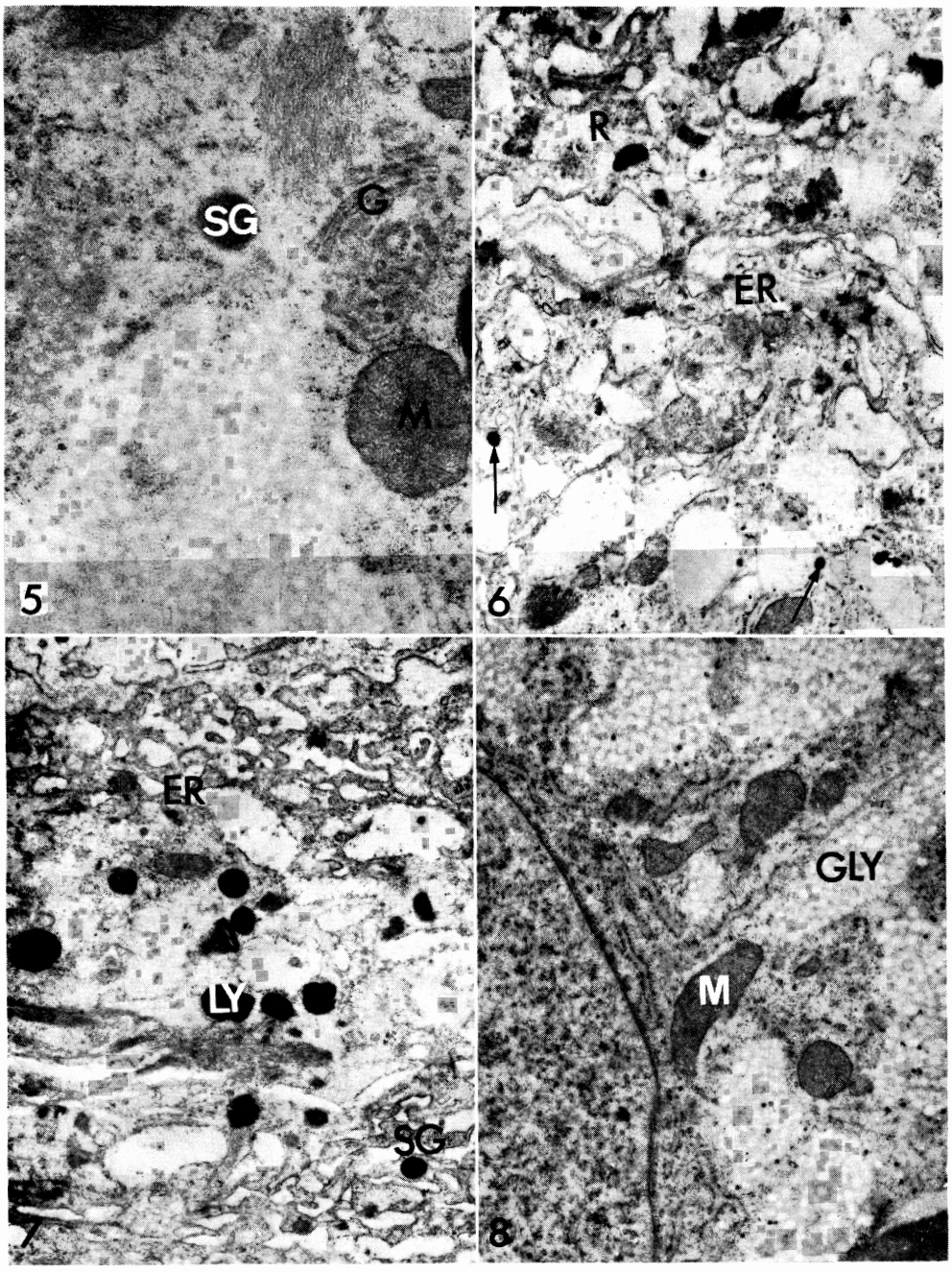
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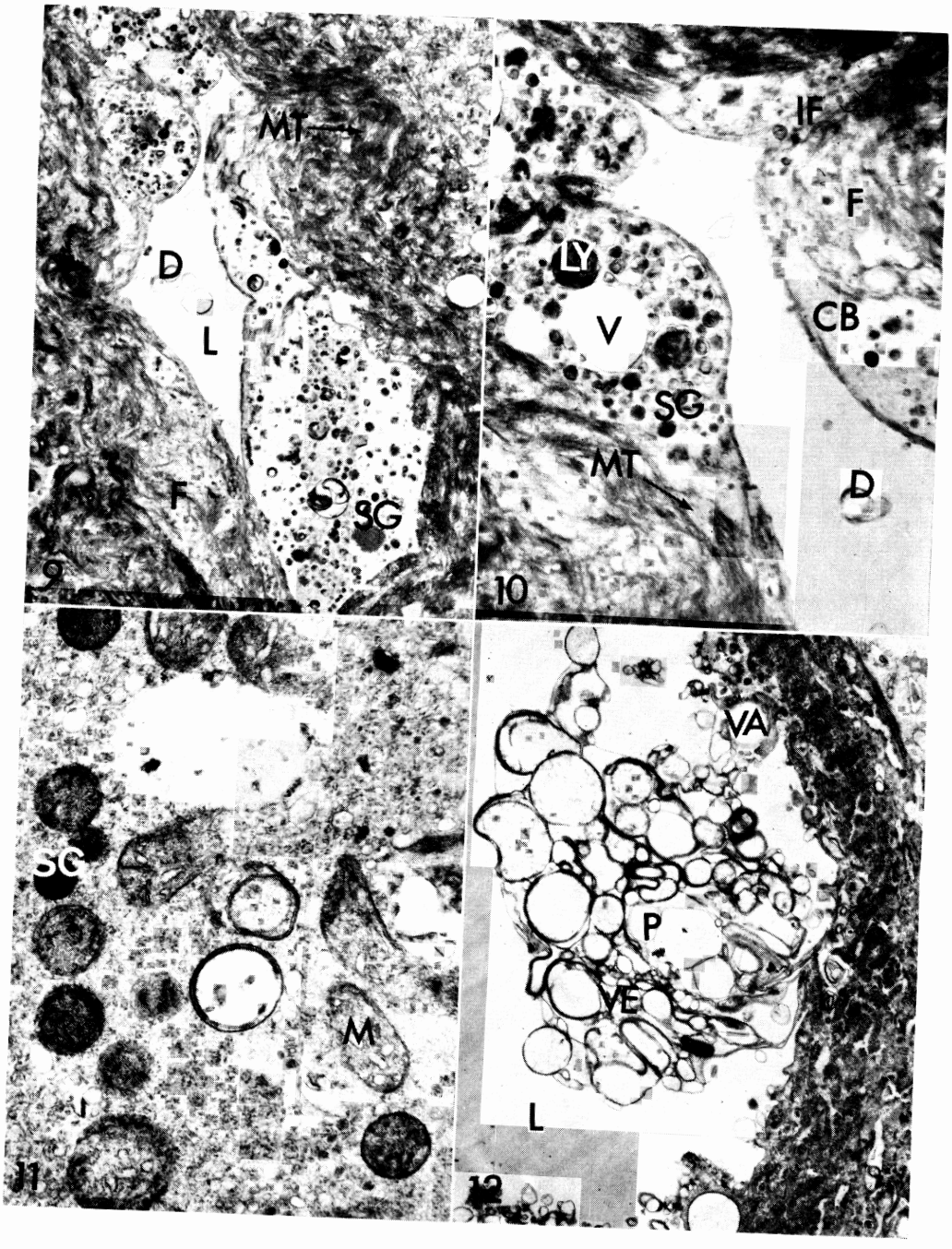
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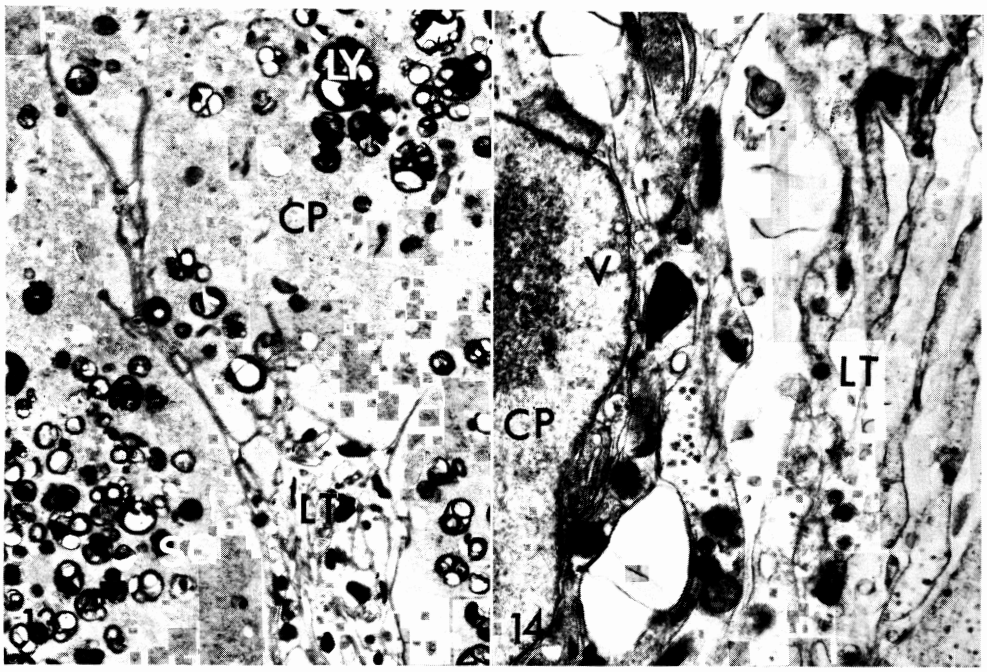

Figs. 1–14 are of infected animals

Fig. 1 A shematic illustration of the lateral canal cell in a pig ascaris (ventral view). Nucleus (N), lateral canal (LC), lateral line tissues (LT), excretory pore (EP), transverse canal (TC), cytoplasm (CP). Modification of Chitwood and Chitwood (1950).

Fig. 2 Part of the cytoplasm of the lateral canal cell. A huge nucleus (N), and canaliculi (CA) embedded in the cytoplasm are noted. Lateral line tissues (LT), esophagus (ES). (×100)

Fig. 3 Part of the cytoplasm of the lateral canal cell. The nucleus (N) is irregular in shape. Within the nucleus chromatin granules and a nucleolus (NL) are seen. (×3,600)

Fig. 4 Perinuclear cytoplasm of the lateral canal cell. Nuclear pore (NP. arrow), mitochondria (M), endoplasmic reticulum (ER), lysosome (LY), lipid droplet (LD), glycogen particle (GLY), secretory granule (SG). (×14,000)

Fig. 5 Perinuclear cytoplasm of the lateral canal cell. Mitochondria (M), Golgi complex (G), secretory granule (SG). (×22,000)

Fig. 6 Perinuclear cytoplasm of the lateral canal cell. The cisternae of the endoplasmic reticulum (ER) are dilated. Intracisternal granules (arrows) are noted. Ribosomes (R). (×14,000)

Fig. 7 Perinuclear cytoplasm of the lateral canal cell. The secretory granules (SG) are noted. Agranular endoplasmic reticulum (ER), lysosomes (LY). (×14,000)

Fig. 8 Perinuclear cytoplasm of the lateral canal cell. Glycogen particles (GLY) tend to be distributed in close association with mitochondria (M). (×26,000)

Fig. 9 Marginal cytoplasm abutting upon the terminal canaliculi in the lateral canal cell. Microtubules (MT, arrow), filaments (F), secretory granules (SG), lumen (L), debris (D). (×3,600)

Fig. 10 Cross section of a fine canal of the lateral canal cell. The plasma membrane covering the cytoplasm exhibits infoldings (IF), and cytoplasmic buldings (CB) are found. Lucent vacuoles (V), debris (D), microtubules (MT, arrow), secretory granules (SG), lysosomes (LY), filaments (F). (×14,000)

Fig. 11 Marginal cytoplasm of the lateral canal cell. Mitochondria (M), and secretory granules (SG) are noted. (×14,200)

Fig. 12 Cross section of ramifying canaliculi of the lateral canal cell. Protrusion (P), vacuoles (VA), vesicles (VE), lumen (L). (×6,400)

Fig. 13 Part of the cytoplasm of the lateral canal cell. Lateral line tissues are deeply invaginated into the cytoplasm and form complex foldings. Secretory granules (SG), lysosome-like bodies (LY), cytoplasm (CP), lateral line tissues (LT). (X26,000)

Fig. 14 Parts of the cytoplasm of the lateral canal cell and the lateral line tissues (LT). In the cytoplasmic region (CP), abutting upon the lateral line tissues, vesicles (V) are visualized. (×26,000)