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Cytological Studies on the Bushy Cells in the Pig *Ascaris* (*Ascaris suum*)

I. Ultrastructure and Secretory Function

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Introduction

As Chitwood and Chitwood (1950) have indicated in their review article, the bushy cells of the pig *Ascaris* (*Ascaris suum*) are originally not organs but giant cells. Since these cells are unusually large in size, however, some Japanese parasite morphologists (Dobashi, 1934; Nishituji, 1958; Fukuda, 1961) have described the bushy cells as organs or occasionally as cells, and there has been a confusion as to the true structural features of the cells. In a previous study (Ishikawa, 1960), the present author reported that the bushy cells are conceived to be an organ consisting of a cyst, a body, branches, minute branches, final branches and final swellings. According to the author's recent cytochemical studies on the bushy cells (Ishikawa and Yamada, 1972 a, b), the portion of the cells previously called cyst was found to exhibit positive Feulgen reaction and is, therefore, a giant nucleus containing DNA, whereas the rests of the cells previously named body, branches, minute branches, final branches and final swellings were confirmed to represent an abundant mass of cytoplasm.

In terms of the cytophysiological functions of the bushy cells as revealed by their morphology, Chitwood and Chitwood (1950) have suggested a secretory function, and Bolla *et al.* (1972) have recently recorded that the ultrastructures of the cells bespeak for the functions of both protein synthesis and secretion.

In the present study, the author has examined the detailed ultrastructural features of the bushy cells by means of electron microscopy and has obtained a series of findings which suggest that the cells are specifically differentiated secretory cells. The present report is hence concerned with the description and discussion of the ultrastructural aspects of the cells in particular relation to their secretory activity.

Materials and Methods

Forty five pig *Ascaris* (*Ascaris suum*) of both sexes were obtained from the slaughter house of Nagoya City, Japan. These worms were kept alive in physiological Ringer-solution maintained at 37 C until the time of their sacrifice. Bushy cells with neighboring tissues were removed from these living worms.

Preparation of tissues for light microscopy:

Immediately after the removal from the donor worms, the bushy cell-containing tissues were placed in either of the two fixatives; Bouin solution and 2% calcium acetate in 10% formalin. Fixation was performed at room temperature for periods ranging from 24 to 72 hr. The tissues were rinsed in water, dehydrated in graded ethanol series and embedded in paraffin. Sections were cut at 6 to 8 μ, deparaffinized, hydrated and subjected to staining with hematoxylin eosin.

Preparation of tissues for electron microscopy:

Immediately after the removal from the donor worms, the bushy cell-containing tissues were cut into tiny cubes with a side less than 1 mm 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 hr. The osmium tetroxide-fixed tissues were dehydrated in ethanol series and embedded in Epon 812, as prescribed by Luft (1961). The glutaraldehyde-fixed tissues were rinsed in cacodylate-buffer (pH 7.4) for 2 to 4 hr, postfixed at room temperature for 2 hr in cacodylate buffered 1% osmium tetroxide (pH 7.4), dehydrated and embedded in Epon 812. From these tissue blocks, thick sections with the thickness of about 1 μ were cut on a JUM No. 5 microtome, stained with toluidine blue and used for trimming tissues containing bushy cells. From the trimmed tissue blocks, ultrathin sections were cut on a Porter-Blum microtome (MT-1), mounted on copper grids and stained doubly with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963). The stained sections were examined in Hitachi HU-11 D or HS-4 electron microscope. Pictures were taken at original magnifications ranging from 2,000 to 10,000 times and photographically enlarged as desired.

Results

Light microscopy :

As is illustrated in Fig. 1, the bushy cells of the pig *Ascaris* are giant and range in dimension from 0.8 to 1.8 mm. The cells are stellate in shape and contain a huge oval nucleus which is centrally situated and cyst-like in appearance. The cytoplasm of the cells is unusually abundant and shows villous configurations in outline.

Electron microscopy :

1. Nucleus

The nucleus of the bushy cells is enclosed by a nuclear envelope which is irrergularly waved and at certain loci exhibits slender evaginations with terminal swellings extending into the cytoplasm (Fig. 3). A high power view of parts of the nucleus (Fig. 3 inset) reveals that the nuclear envelope is double in structure and consists of inner and outer leaflets. The nuclear envelope is provided with relatively numerous nuclear pores which often show a honeycomb appearance (Fig. 3 inset). The cytoplasmic surface of the outer leaflet of the nuclear envelope is studded with ribosomes (Fig. 3 inset). Within the nucleus varying concentrations of chromatin granules are scattered and embedded in amorphous substances of nuclear matrix (Fig. 2 and 3). In the nucleus prominent nucleoli are detected (Fig. 2). In addition, irregularly shaped dense bodies of unknown nature are embedded in the amorphous substances of nuclear matrix, and surrounded by clusters of chromatin granules (Fig. 2).

2. Cytoplasm

The cytoplasm of the bushy cells is bordered by a plasma membrane (Fig. 4). As in cells of higher animals, the plasma membrane of the bushy cells exhibits the structure of unit membrane, trilaminar structure consisting of two dense lines and a less dense intermediate layer (Fig. 4 inset). A well developed basal lamina with a uniform thickness is found to run in parallel with the plasma membrane (Fig. 4 inset). The plasma membrane of the cells is often seen to be invaginated into the cytoplasm and appears to represent the activities of pinocytosis or reversed pinocytosis (Fig. 4). In keeping with such findings, vesicles are occasionally found to exist in the cytoplasm subjacent to the plasma membrane (Fig. 4). At certain loci, above all at those where a slender process of the cytoplasm is inserted into other parts of the cytoplasm, the plasma membrane is found to form attachment plates which are desmosome-like in appearance (Fig. 5).

In the cytoplasm of the bushy cells, nearly all varieties of cell organelles and inclusions are visualized, such as mitochondria, Golgi apparatus, lysosomes, granular and agranular elements of endoplasmic reticulum, ribosomes, microfilaments, crystalloids, glycogen particles, lipid droplets and secretory granules

(Fig. 6).

Throughout the cytoplasm of the bushy cells mitochondria are disseminated here and there. The mitochodria are spherical, oval or rod-like, but occasionally filamentous in shape (Fig. 6). They are provided with tubular and lamellar forms of cristae and contain small numbers of tiny dense mitochondrial granules (Figs. 5 and 7). Not infrequently, mitochondria are closely associated with other cell organelles and inclusions such as the Golgi apparatus (Figs. 7 and 8), elements of endoplasmic reticulum (Figs. 7 and 11) and lipid droplets (Fig. 18) in the cytoplasm.

In the bushy cells, the Golgi apparatus occupies various territories of the cytoplasm; peripheral, central and juxtanuclear areas (Fig. 6). This cell organelle consists of arrays of flattened sacs and associated vesicles and small vacuoles (Fig. 8). These Golgi elements are found to contain substances of varying electron opacities (Fig. 8). As in the case with mitochondria, the Golgi apparatus is often in close vicinity to other cell organelles and inclusions; mitochondria (Figs. 7 and 8), elements of granular endoplasmic reticulum (Fig. 8 and 14) and secretory granules (Fig. 14).

Everywhere in the cytoplasm of the bushy cells, components of agranular and granular endoplasmic reticulum are observed (Fig. 7, 9, 10 and 11). Based upon their appearances, these components are grouped into at least three types; tubules, vesicles and cisternae. The cisternal components of the endoplasmic reticulum are, at some cytoplasmic loci, dilated (Fig. 9) with a flocculent substance of low electron opacity being enclosed in them, whereas at other loci they are flattened (Fig. 7). Another characteristics of particular note in the endoplasmic reticulum cisternae is that they occasionally undergo invaginations (Figs. 9 and 10). Such invaginations are singular and simple (Fig. 9) in some endoplasmic reticulum cisternae, but in others they are multiple and complex in configurations (Fig. 10). In certain inner areas of the cytoplasm, tubular and vesicular components of agranular endoplasmic reticulum are concentrated (Fig. 11). Such endoplasmic reticulum components are frequently seen to be in close association with mitochondria (Fig. 11). In line with this, dilated cisternae of granular endoplasmic reticulum are often closely associated with mitochondria (Fig. 9).

The cytoplasm of the bushy cells is more or less granular throughout, apparently due to the presence of free ribosomes (Figs. 8, 9 and 10). The ribosomes are at times clustered to form polysomes, which are localized preferentially around the membranous elements of endoplasmic reticulum cisternae (Fig. 10).

In the cytoplasm of the bushy cells, above all in its center and perinuclear areas, microfilaments are arranged in irregular networks and cross the cytoplasm (Fig. 12).

It is interesting to observe the possible sequence of secretory product formation, migration and discharge in the cytoplasm of bushy cells. Dense granules with an average diameter of 300 to 800 mμ are disseminated here and there in the cytoplasm (Fig. 6). These granules are membrane-limited, exhibit a thin less opaque halo immediately beneath the limiting membrane and contain dense materials which are homogeneous or vesicular in texture (Fig. 4). Parts of the dense materials within the granules are often found to show crystalline structures, in which periodic dense lines with an average interval of 75 Å are detected (Fig. 13). The membrane-limited granules must be of Golgian origin and secretory in nature; in the territory of the Golgi apparatus certain vesicles and vacuoles contain dense materials and exhibit structural features similar to those of the dense granules (Fig. 14). The dense secretory granules are occasionally accumulated in the peripheral cytoplasm and appear to migrate towards there (Fig. 15). However, the inclusions of the secretory granules accumulated in the peripheral cytoplasm are less electron opaque, as compared with those of the granules distributed in the rest of the cytoplasm and are often lucent in their center

or periphery (Fig. 15). Immediatly beneath the plasma membrane of the cells, vacuoles with lucent contents are found which are comparable in dimension to secretory granules (Fig. 15). In the peripheral cytoplasm, furthermore, images are observed which suggest that the lucent vacuoles are flattened and fused with one another (Figs. 4, 15 and 16).

In the bushy cells, the plasma membrane shows evidence of pinocytotic activity; the plasma membrane is often invaginated into the peripheral cytoplasm and possibly resultant vesicles of varying densities are seen right beneath the membrane (Fig. 4). In addition, some of these vesicles are coated (Fig. 4 inset).

In the cytoplasm of the bushy cells there occur lysosome-like dense bodies (Fig. 17), which appear at times in the vicinity of the Golgi apparatus. Most of these bodies are spherical or oval but some of them are irregular in shape. They are membane-limited and comparable in dimension to secretory granules. However, their inclusions are composed of vesicles and irregular membranous elements imbedded in homogeneous dense matrix (Figs. 17 and 18).

In certain loci of the cytoplasm of the bushy cells glycogen particles are found to occur which display spherical accumulations of different sizes (Fig. 19). Some of the glycogen particles tend to be distributed in close association with elements of agranular endoplasmic reticulum (Fig. 19).

In the cytoplasm of the bushy cells, above all in its central parts, lipid droplets of different sizes and shapes are demonstrated (Figs. 18 and 20). Some of the lipid droplets are so closely associated with mitochondria (Fig. 18) or elements of granular endoplasmic reticulum (Fig. 20) that the both structures are seen to be in direct contact with each other (Figs. 18 and 20).

In Fig. 21, a possible sequence of events taking place in the cytoplasm of the bushy cells is schematically illustrated with special reference to the secretory and pinocytotic activities of the cells and the relation of these activities to cell organelles and inclusions.

Discussion

Light microscopy:

As Chitwood and Chitwood (1950) described in their review article, the bushy cells are originally giant cells and not organs. The unusually large size of the cells, however, has led some Japanese parasite morphologists (Dobashi, 1934; Nishituji, 1958; Fukuda, 1961) to the uncertain description that the bushy cells are at one time cells and at other time organs. Thus, there has been a confusion as to the true nature of these cells. The present author (Ishikawa, 1960) has, likewise, reported previously that the bushy cells are believed to be an organ in which a cyst is noted. According to the author's recent cytochemical analyses on the bushy cells (Ishikawa and Yamada, 1972 a, b), however, the cyst was found to exhibit positive Feulgen reaction and therefore to be a nucleus. Thus, the concept that the bushy organ is in fact a giant cell has been established (Ishikawa and Yamada, 1972 a, b).

The results of the present light microscopic observations are taken to substantiate the validity of the previously established concept; the bushy cells have an abundant stellate cytoplasm provided with a well delineated cyst-like nucleus.

In an *Ascaris* worm, there occur four bushy cells which are situated within the upper one forth of the worm body and are placed in that part of celiac cavity between the lateral chords and intestine. If the bushy cells are observed light microscopically, these cells are found to be closely opposed to both the lateral chords and intestine. However, the canalicular structures connecting the nucleus of the cells and lateral chords as erroneously described previously (Ishikawa, 1960) are never detected, and this is taken to be a confirmation that the bushy cells are independent in structure and function from other tissues of the worm.

Electron microscopy;

1. Nucleus

In the bushy cells of the *Ascaris* the nuclear envelope is found to be irregularly waved and to exhibit characteristic evaginations. In the epithelial cells lining the uterus of *Ascaris lumbricoides*, Sato (1950) reported the presence of wave-shaped nuclear envelopes, which are similar in structural features to those observed in the present study. In the bushy cells examined here, the nuclear envelope is provided with relatively numerous pores showing a honeycomb appearance. In the same cells of the *Ascaris suum*, Bolla *et al.* (1972) have observed similar nuclear pores, and Pappas (1956) has described a honeycomb structure of the nuclear envelope in *Amaeba proteus*. In the present bushy cells the cytoplasmic surface of the outer leaflet of the nuclear envelope is studded with ribosomes, and such ultrastructural features of the nuclear envelope were noted previously by Bolla *et al.* (1972). All these ultrastructural properties of the nuclear envelope in the bushy cells appear to indicate the high activity of the boundary between the nucleus and cytoplasm. Such high activity of the nuclear envelope might possibly be due to the fact that the nucleus is to control the huge mass of the cytoplasm. In the nucleus of the present bushy cells, nucleoli are prominent, indicating that the activity of RNA synthesis within these nuclear components is high for regulating the protein synthesis in the huge mass of the cytoplasm. As mentioned above in the results, the nature of irregularly shaped dense bodies in the nucleus is unknown, however, these bodies may have something to do with chromatin granules, since they are surrounded by clusters of chromatin granules.

2. Cytoplasm

In keeping with the results of previous observations on the bushy cells (Fukuda, 1961; Bolla *et al.*, 1972), the plasma membrane of the bushy cells examined here exhibits the structure of unit membrane. Bolla *et al.* (1972) described previously that in the bushy cells infoldings of the cell membrane occur reminiscent of the formation of pinocytotic vesicles or as a result of the discharge of secretory products from the cell. In accordance with these results, images indicative of the activity of pinocytosis or reversed pinocytosis have been discerned in the plasma membrane and subjacent cytoplasm in the present bushy cells. Parallel with the plasma membrane of the bushy cells observed here, is found to run a well developed basal lamina, which certainly corresponds to an amorphous basement lamina reported previously (Bolla *et al.*, 1972).

In the bushy cells studied here mitochondria are almost identical in number, shape, internal structure and distribution with those observed previously in the same cells (Fukuda, 1961; Bolla *et al.*, 1972). The tubular and lamellar forms of cristae in the mitochondria of the present bushy cells are of particular note. Mitochondria with more or less similar ultrastructural features of cristae have been reported to occur in the parenchmal cells of liver fluke (*Fosciola hepatica*, L) (Björkman and Thorsell, 1962), and muscle cells of *Ascaris lumbricoides* var. *suum* (Rew and Satz, 1974). It seems likely that mitochondria with such characteristics are common to cells of parasites living physiologically in anaerobic conditions.

In the present bushy cells the elements of the Golgi apparatus are not very unusual in ultrastructural morphology and most of the ultrastructural features of this cell organelle are well consistent with those of the organelle described previously in the same cells (Bolla *et al.*, 1972). In view of the present result that the Golgi elements contain substances of varying electron opacities and are associated with other cell organelles and inclusions, it is obvious that this organelle is conceived to play an important role for the formation of lysosomes and secretory granules.

In the cytoplasm of the present bushy cells the ultrastructures of components of agranular and granular endoplasmic reticulum are mostly common to those reported by Bolla *et al.* (1972) in the same cells. Some of the cisternal components of the endoplas-

mic reticulum in the present bushy cells are dilated and contain a flocculent substance. This is taken to be a reflection of accumulation of various substances in the course of functional activities; viz., synthetic and transporting activities (Porter, 1961) of the endoplasmic reticulum. The invaginated cisternae of the endoplasmic reticulum observed in the present study correspond apparently to the K-bodies described by Fukuda (1961) in the same cells which represent either modifications of smooth endoplasmic reticulum or intensive folding of the plasma membrane cut in cross section, according to Bolla *et al.* (1972). The tubular and vesicular components of agranular endoplasmic reticulum are concentrated in certain inner areas of the cytoplasm of the present bushy cells. In light of the generally accepted functions of agranular endoplasmic reticulum (Fawcett, 1966), such components may possibly be engaged in a biosynthetic activity of steroids. This appears to be substantiated by the present finding that the endoplasmic reticulum components are often in close association with mitochondria which perhaps supply energy for the possible biosynthetic processes.

In the cytoplasm of the bushy cells studied, free ribosomes at times in the form of polysomes are present, and these are interpreted to be engaged in the synthetic activity of structural and functional proteins such as enzymes of different natures and proteinous moieties of secretory products.

In agreement with the results of previous observations on the bushy cells of *Ascaris*, microfilaments are detected in the present study. Although the exact functional significances of the filaments remain to be well known, the structures may exist as either cytoskeletal or contractile elements in the cytoplasm (Fawcett, 1966).

The present studies on the ultrastructure of the bushy cells in the pig *Ascaris* indicate that the activity of secretion is the function of primary importance in the cells. As the results of the present ultrastructural studies show, the products of secretion in the cells in the form of membrane-limited granules are originated from Golgi apparatus. The present results that the dense materials within the secretory granules are often crystalline in structure seem to indicate that a moiety of the secretory substances is protein in nature. According to the data of the previous cytochemical analyses of polysaccharides in the bushy cells (Ishikawa and Yamada, 1972 b) the secretory granules have been shown to contain proteins in addition to mucosaccharides with neutral and acidic groupings. Therefore, the results of the present ultrastructural studies are compatible with the data of previous cytochemical studies. It is interesting to presume the possible mode in which the products of secretion leave the cytoplasm in the bushy cells. In the bushy cells, the inclusions of the secretory granules accumulated in the peripheral cytoplasm are less electron opaque or lucent in their center or periphery, and vacuoles with lucent content are found immediately beneath the plasma membrane of the cells. These ultrastructural evidences are taken to imply that the products of secretion are released by mean of diacrine mode. In contrast, the evidence of pinocytotic activity in the plasma membrane and subjacent cytoplasm is to be interpreted in terms of resorption of materials from outside of the cells, and some of these material may possibly be available for the production of secretory substances.

In the cytoplasm of the bushy cells examined here the ultrastructural features of lysosomes or related bodies are presumed to suggest that they are produced in the Golgi apparatus and consist of different functional types of the cell organelles engaged in the processes of intracellular digestion (de Duve, 1965).

In cells of parasites such as *Hymenolepis diminuta* and *Lacistorhynchus tenuis*, glycogen is known to occur in two types of particles; (a) single granules (beta particles) 200 to 400 Å in diameter and (b) rossettes (alpha particles) consisting of varying numbers of 200 Å particles with an aggregate diameter of 600 to 2,000 Å, whereas mixtures of these

particles occur in particular cell types (Lumsden, 1965). From their ultrastructural features, the glycogen particles within the cytoplasm of the present bushy cells are conceived to be mixtures. The cytophysiological functions of glycogen particles in the bushy cells are not elucidated precisely, however, the close association of these inclusions with other organelles such as the elements of agranular endoplasmic reticulum tend to suggest the possible participation of the polysaccharide in some activities such as metabolism and synthesis.

In the bushy cells studied here, the functional significances of lipid droplets are likewise not well comprehended. The present finding that the lipid droplets are associated with other cell organelles such as mitochondria and elements of agranular endoplasmic reticulum bespeaks for the importance of the inclusion in the cytophysiology of the bushy cells (Fawcett, 1966).

From the results obtained in the present study, it is apparent that in the pig *Ascaris* the bushy cells are performing a secretory function, while the products of secretion being released in a diacrine mode. It remains, however, to be elucidated what function is to be ascribed to the products of secretion.

In various vertebrate species, the metabolic and functional activities of cells and tissues are controlled by at least two organ systems ; nervous and endocrine systems. It is well known that the endocrine system is phylogenetically lower than the nervous system. In the pig *Ascaris* which is apparently an invertebrate species phylogenetically lower than vertebrates, the presence of the nervous system was reported previously by the present author (Ishikawa, 1961). In view of all these facts, an endocrine system should be developed in the pig *Ascaris*. Such assertion is well correlated with the concept that the bushy cells are endocrine in nature. The possible endocrine functions of the bushy cells may be comparable to those of the interstitial cells in human testes which perform endocrine functions, even though they are distributed singly in interstitium. Furthermore, the topographical situation of the bushy cells in the pig *Ascaris* is taken to enable them to have a vast surface of contact with celiac fluid and thus to release easily their products of secretion into the fluid. This is also in keeping with the concept that the bushy cells are endocrine in nature.

Summary

In the pig *Ascaris* the so-called bushy organs are in fact giant cells. Numerous cytoplasmic processes are found to project from the surface of the cell cytoplasm, leading the cells to villous configurations. The nucleus of the bushy cells is likewise giant, irregular in outline and provided with a number of projections from the nuclear surface. Nuclear pores are outstanding in the nuclear envelope. In the cytoplasm of the bushy cells, usual cell organelles and inclusions are observed such as mitochondria, ribosomes, granular and agranular varieties of endoplasmic reticulum, Golgi apparatus, lysosomes, microfilaments, glycogen particles, lipid droplets, crystals, and secretory granules. Various transitional forms are noted between the elements of Golgi apparatus and secretory granules. As they approach the plasma membrane, secretory granules come to decline in electron opacity and their inclusions are thought to be released in a diacrine mode. On the plasma membrane and right beneath it pinocytotic invaginations and resultant vesicles (smooth and coated) are seen. All these ultrastructural features of the bushy cells in the pig *Ascaris* bespeak for that their major activity is secretory, whereas they are to some extent resorptive. In view of the apparent secretory activity of the bushy cells, their possible endocrine function has been discussed with reference to the presence of the nervous system and the topographical situation of the cells in the pig *Ascaris*.

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References

1) Björkman, N. and Thorsell, W. (1962) : The fine morphology of the mitochondria from parenchymal cells in the liver fluke (*Fasciola hepatica*, L). Exp. Cell Res., 27, 342-346.
2) Bolla, R. I., Weinstein, P. P. and Cain, G. D. (1972) : Fine structure of the coelomocyte of adult *Ascaris suum*. J. Parasit., 58, 1025-1036.
3) Chitwood, B. G. and Chitwood, M. B. (1950) : Nematology, Section 1, Monumental Printing Company, Baltimore, 53 pp.
4) de Duve, Ch. (1965) : The living cell, Freeman & Company, San Francisco & London, 72 pp.
5) Dobashi, S. (1934) : Studies on the isolation tissue and the phagocytic organ of *Ascaris*. Keio Igaku, 14, 217-242.
6) Fawcett, D. W. (1966) : The cell. Saunders Company, Philadelphia & London, 161, 233, 307 pp.
7) Fukuda, T. (1961) : Electronmicroscopical studies on stellate cells of *Ascaris lumbricoides*. Kumamoto Igaku, 35, 889-912.
8) Ishikawa, M. (1960) : Morphological and physiological studies on "bushy organ" of *Ascaris*. Jap. J. Parasit, 9, 631-635.
9) Ishikawa, M. (1961) : On the secretory phenomenon of nerve cell of *Ascaris*. Jap. J. Parasit., 10, 1-5.
10) Ishikawa, M. and Yamada, K. (1972 a) : Cytological and cytochemical studies on the bushy cells of the pig *Ascaris* (*Ascaris suis*). Jap. J. Parasit., 21 (Supl.), 22-23.
11) Ishikawa, M. and Yamada, K. (1972 b) : Polysaccharide cytochemistry of the bushy cells of the pig *Ascaris* (*Ascaris suis*). Histochemistry and Cytochemistry, 241-242.
12) Luft, J. H. (1961) : Improvements in epoxy resin embedding methods. J. Biophs. Biochem. Cytol., 9, 409-414.
13) Lumsden, R. D. (1965) : Macromolecular structure of glycogen in some *cyclophyllidean* and *Trypanophynch cestodes*. J. Parasit., 51, 501-515.
14) Morishita, K. (1975) : The argument of the 23th Katsurada-Prize. Yobo-Igaku J., 89, 27.
15) Nishituji, K. (1958) : Studies on the stellate cells. J. Kumamoto Med. Soc., 32, Suppl. 5, 761-800.
16) Pappas, G. D. (1956) : The fine structure of the nuclear envelope of *Amaeba proteus*. J. Biophys. Biochem. Cytol., 2, 431-435.
17) Porter, K. R. (1961) : The ground substance ; Observations from electron microscopy. In : The Cell. Brachet J. & Mirsky A. E. (Eds.), Vol. 2, Academic Press, New York, 621 pp.
18) Rew, R. S. and Saz, H. J. (1974) : Enzyme localization in the anaerobic mitochondria of *Ascaris lumbricoides*. J. Cell Biol., 63, 125-135.
19) Reynolds, F. S. (1963) : The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol., 17, 208-212.
20) Sato, M. (1950) : Electron microscopic studies on *Ascaris lumbricoides*. Yamaguchi Igaku, 8, 312-318.
21) Watson, M. L. (1958) : Staining of tissue sections for electron microscopy with heavy metals. J. Biophys. Biochem. Cytol., 4, 475-485.

ブタ回虫花房状細胞の細胞学的研究
I　超微構造と分泌機能

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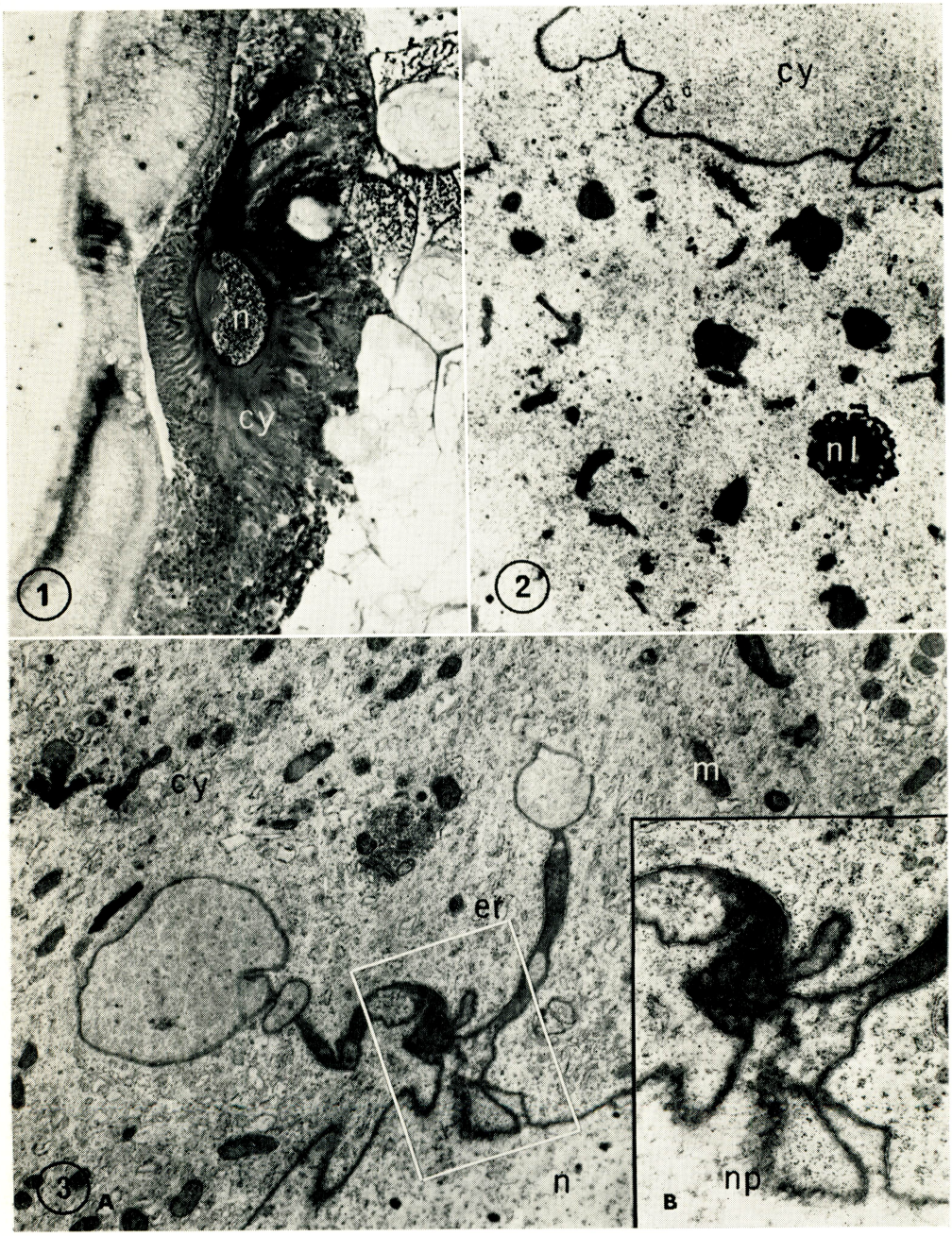
ブタ回虫のいわゆる花房状器官は1個の巨大細胞であつて，その細胞表面から細胞質突起が出て絨毛様の観を呈する．細胞の核は巨大で不規則な輪郭を示し，表面から多数の特異な突起を出す．また顕著な核膜孔が認められる．細胞質には糸粒体，リボ小体，粗面および滑面小胞体，Golgi 装置，リソゾーム，微細線維，グリコーゲン顆粒，脂質滴，結晶体，多数の分泌顆粒が存在する．分泌顆粒と Golgi 要素との間には種々の移行型が認められる．分泌顆粒は形質膜に近づくに従つて，電子密度が著明に減少するので，その内容は限界膜と形質膜を透過して胞体外に放出されるものと思われる（diacrine 様式の放出）．細胞膜には飲作用または逆飲作用の像がみられ，それに近接する細胞質には被覆あるいは滑面の小胞が存在する．これらの所見は，花房状細胞がおもに分泌機能を営み，わずかながら吸収機能をも備えていることを物語る．回虫は神経系を備えること，ならびに花房状細胞は体腔面に直接していることから，花房状細胞は内分泌活動を営む可能性がある．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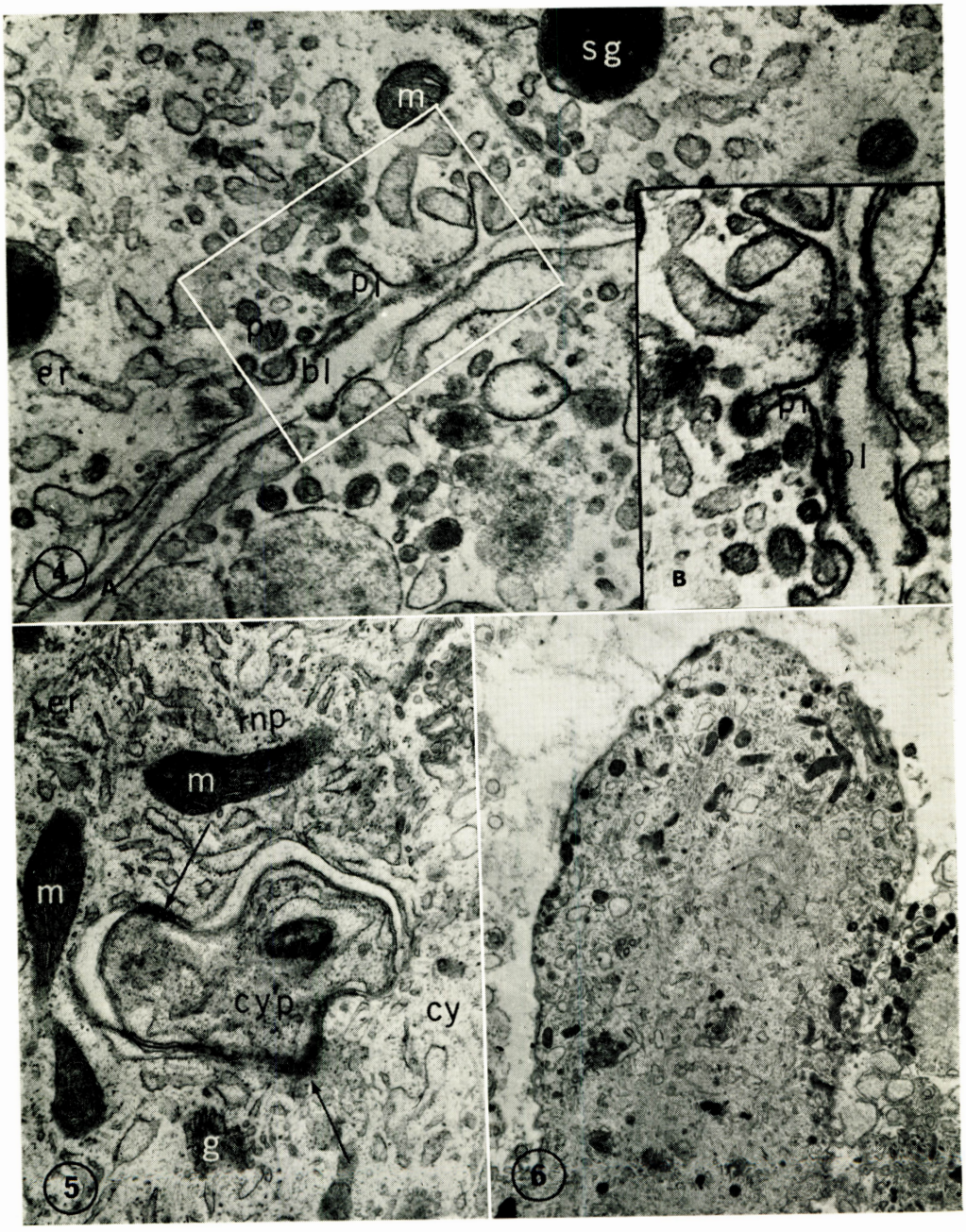
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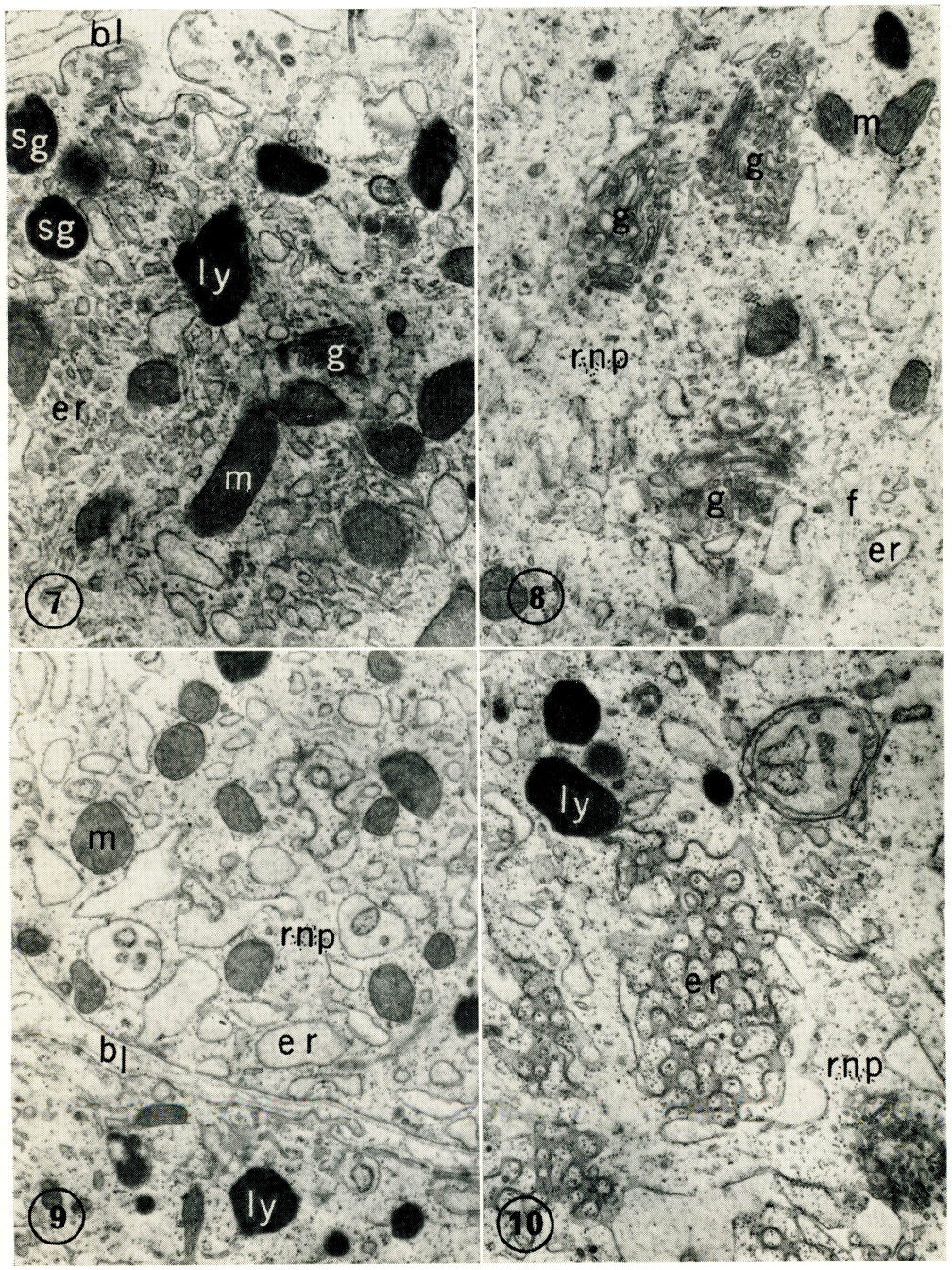
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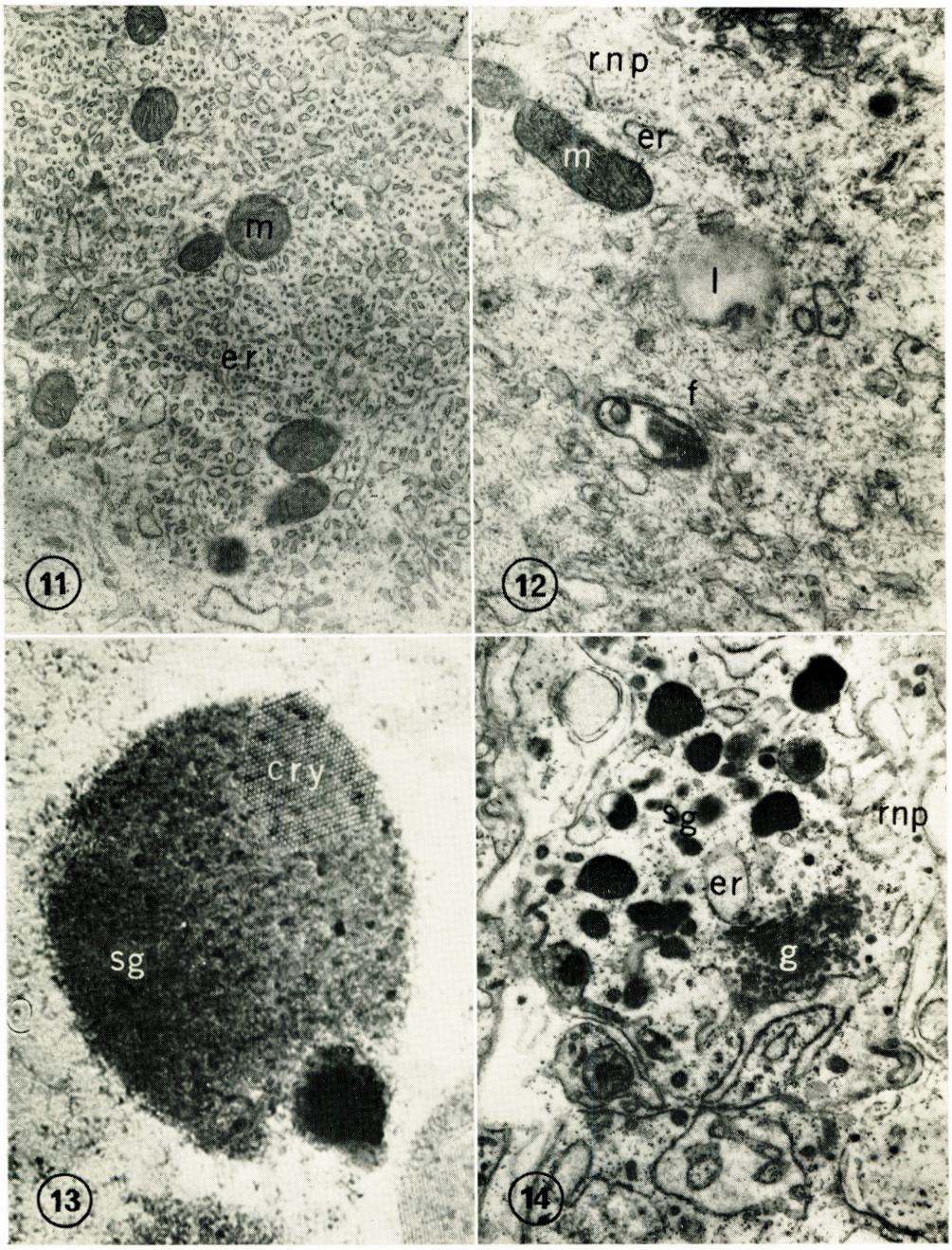
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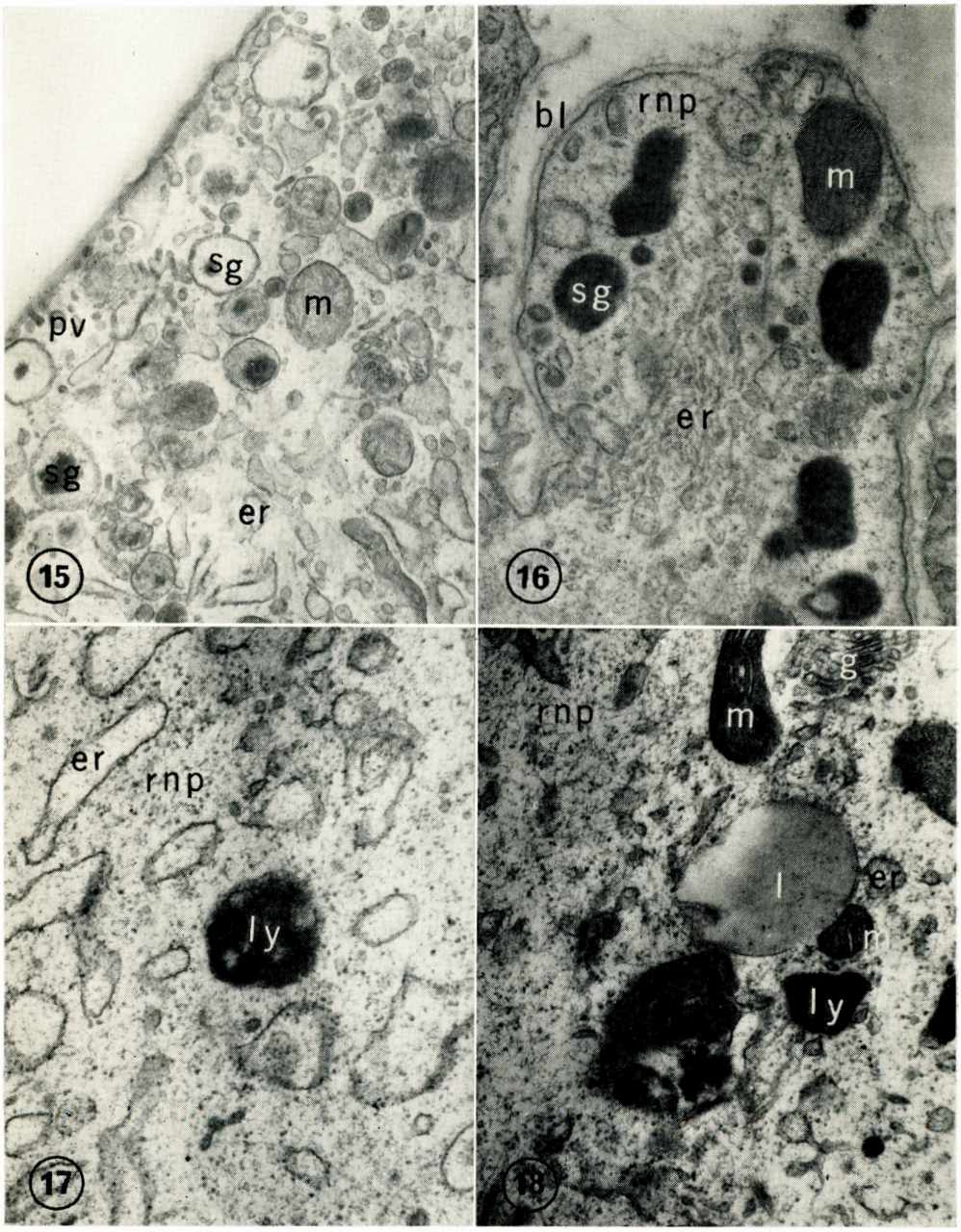
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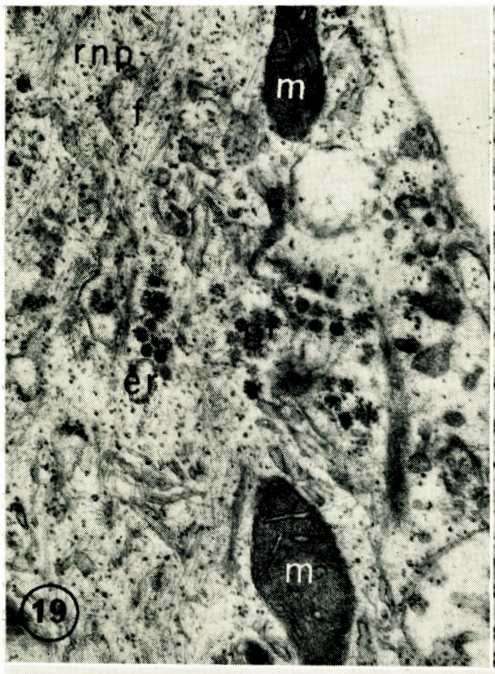
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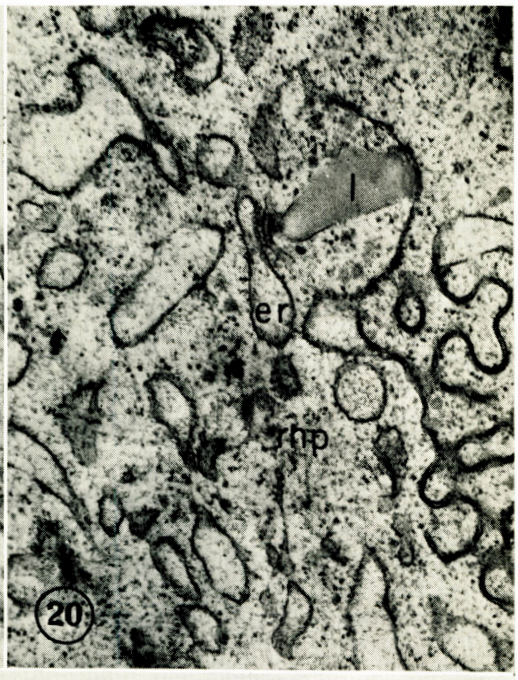
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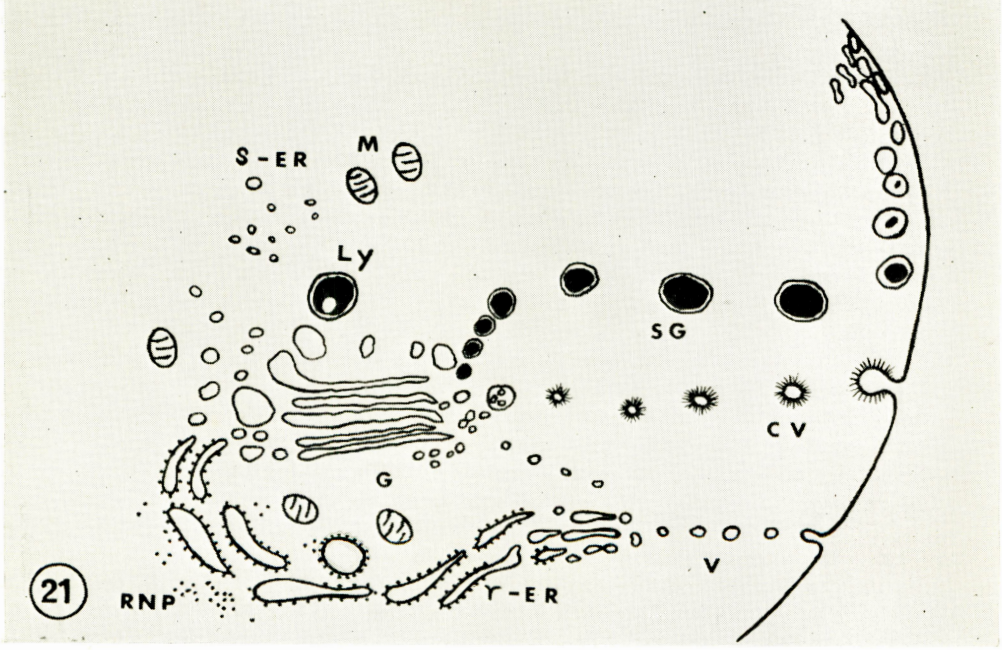
S-ER
M
Ly
SG
CV
G
V
21
RNP
r-ER

Legends for Figures

Fig. 1. A bushy cell in a pig *Ascaris*.
A huge oval nucleus (n) is embedded in an abundant cytoplasm (cy) with villous projections. Homatoxylin-eosin stained. × 56.

Figs. 2–3. Parts of the nucleus and cytoplasm of a bushy cell in a pig *Ascaris*.

2. Within the nucleus a nucleolus (nl) is seen and nuclear envelope exhibits an irregular configuration. Cytoplasm (cy). Uranyl acetate-lead citrate stained. × 3,900.

3(A). Note two slender evaginations with terminal swelling from the nucleus (n). Cytoplasm (cy), mitochondria (m), endoplasmic reticulum (er). Uranyl acetate-lead citrate stained. × 5,700.

3(B). High power view of the rectangled portion in Fig. 3(A). Numerous nuclear pores (np) are shown in the nuclear envelope. Uranyl acetate-lead citrate stained. × 11,000.

Figs. 4–20. Parts of the cytoplasm of a bushy cell in a pig *Ascaris*.

4(A). Pinocytotic invaginations (pi), vesicles (pv), secretory granules (sg), mitochondria (m), endoplasmic reticulum (er) and basal lamina (bl) are observed. Uranyl acetate-lead citrate stained. × 26,000.

4(B). High power view of the rectangled portion in Fig. 4(A). A pinocytotic invagination (coated) (pi) is obvious. Uranyl acetate-lead citrate stained. × 39,000.

5. A cytoplasmic projection (cyp) is invaginated into the surrounding parts of the cytoplasm (cy). Note a desmosome like structures (arrows). Golgi apparatus (g), mitochondria (m), endoplasmic reticulum (er), ribosomes (rnp). Uranyl acetate-lead citrate stained. × 18,000.

6. Low power view. Usual cell organelles and inclusions are detected. Uranyl acetate-lead citrate stained. × 3,800.

7. Mitochondria (m), Golgi apparatus (g), endoplasmic reticulum (er), secretory granules (sg), lysosomes (ly) and basal lamina (bl) are shown. Uranyl acetate-lead citrate stained. × 16,000.

8. Three areas of Golgi apparatus (g), endoplasmic reticulum cisternae (er), ribosomes (rnp), mitochondria (m) and microfilaments (f) are shown. Uranyl acetate-lead citrate stained. ×18,000.

9. Dilated cisternae of the granular endoplasmic reticulum (er), mitochondria (m), lysosomes (ly), ribosomes (rnp) and basal lamina (bl) are illustrated. Uranyl acetate-lead citrate stained. × 14,000.

10. Agranular endoplasmic reticulum elements (er) of multiple and complex configurations are shown. Lysosomes (ly), ribosomes (rnp). Uranyl acetate-lead citrate stained. × 13,000.

11. Accumulations of agranular endoplasmic reticulum elements (er) and mitochondria (m) are noted. Uranyl acetate-lead citrate stained. × 19,000.

12. Microfilaments (f) are distributed here and there. Mitochondria (m), ribosomes (rnp), endoplasmic reticulum (er), lipid droplets (l). Uranyl acetate-lead citrate stained. × 27,000.

13. A huge crystal with regular periodic substructures (cry) is noted. Secretory granules (sg). Uranyl acetate-lead citrate stained. × 57,600.

14. Golgi elements (g) with associated immature and mature secretory granules (sg) are shown. Ribosomes (rnp), endoplasmic reticulum (er). Uranyl acetate-lead citrate stained. × 16,000.

15. Note secretory granules (sg) with inclusions of declining electron opacity. Mitochondria (m), pinocytotic vesicles (pv), endoplasmic reticulum (er). Uranyl acetate-lead citrate stained. × 16,500.

16. Beneath the plasma membrane secretory granules (sg) with inclusions of varying electron density are localized. Ribosomes (rnp), mitochondria (m), endoplasmic reticulum (er), basal lamina (bl). Uranyl acetate-lead citrate stained. × 20,000.

17. A lysosome (ly) is surrounded by granular varieties of endoplasmic reticulum (er). Ribosomes (rnp). Uranyl acetate-lead citrate stained. × 33,000.

18. A lipid droplet (l) is in direct contact with a mitochondrion (m). Lysosomes (ly), ribosomes (rnp), Golgi apparatus (g), endoplasmic reticulum cisternae (er). Uranyl acetate-

lead citrate stained. × 26,000.

19. Glycogen particles (gl) are in close association with agranular varieties of endoplasmic reticulum (er). Mitochondria (m), microfilaments (f), ribosomes (rnp). Uranyl acetate-lead citrate stained. × 26,000.
20. A lipid droplet (l) is in association with granular varieties of endoplasmic reticulum (er). Ribosomes (rnp). Uranyl acetate-lead citrate stained. × 26,000.

Fig. 21. A schematic representation of a sequence of events taking place in the cytoplasm of the bushy cells during the course of secretory and resorptive activities. Vesicles (V), granular endoplasmic reticulum (r-ER), ribosomes (RNP), coated vesicles (CV), Golgi apparatus (G), mitochondria (M), agranular endoplasmic reticulum (s-ER), lysosomes (Ly), secretory granules (SG).