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Scanning Electron Microscopic Observation of the Reproductive System in *Diphyllobothrium latum*

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Introduction

Scanning electron microscopy (SEM) has been extensively employed in studying the surface structure of cestodes from the morphological or taxonomic point of view (Andersen, 1975a, 1975b, 1975c; Berger and Mettrick, 1971; Bortoletti, 1979; Boyce, 1976; Bylund, 1975; Gabrion and Euzet-Sicard, 1979; Hess and Guggenheim, 1977; Hilliard, 1972; Jilek and Crites, 1979; Jones *et al.*, 1979; Jones, 1979; Swilenov *et al.*, 1976; Ubelaker *et al.*, 1973; Verheyen *et al.*, 1978; Yamane *et al.*, 1975, 1976).

These observations have given us comprehensive informations on the surface structure of cestodes. The three-dimensional topographic analysis was especially useful for reconstructing the structure. Most of the observations by SEM have been made on the surface structure of the cestodes, however, the internal organs have scarcely been observed (Coil, 1977; Yamane *et al.*, 1982a, 1982b).

The present contribution deals with the internal structure of the reproductive system of *Diphyllobothrium latum*. Observations will be confined to the cirrus, cirrus-sac, seminal vesicle, and testis as the male reproductive system, and the yolk gland, uterus, vagina, oviduct and ovary as the female reproductive system.

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Materials and Methods

Several mature proglottids of *Diphyllobothrium latum* were obtained from a man who evacuated a strobila spontaneously. For SEM preparation, small pieces of proglottids were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 hr at 4 C. Following buffer rinse, the specimens were post-fixed in 1% OsO_4 in 0.1 M phosphate buffer (pH 7.2) for 4 hr at 4 C. Fixed specimens were dehydrated in a graded series of ethanol, soaked in amylacetate, dried by the critical point drying method, sputter-coated with gold-palladium alloy in GIKO IB-5 ion coater, and observed with a Hitachi HFS-2ST scanning electron microscope.

Some fixed specimens were embedded in styren after dehydration to get cross fractures of the reproductive system in proglottids. After polymerization for 48 hr at 60 C, the specimens were cracked under a stereoscope. The cracked specimens were soaked in propylene oxide to dissolve the polymerized styren, and then soaked in

amylacetate for the critical point drying method (Tanaka *et al.*, 1974).

The ion-etching was carried out on some dried specimens for 5–15 minutes using ion coater at an accelerating voltage of 300 V and an ion current of 3 mA to get clearly the cellular structure in the cracked specimens (Tanaka *et al.*, 1976). The ion-etched specimens were dealt with in the same procedure as mentioned above.

Several proglottids were fixed in 70% alcohol, dehydrated in ethanol series and embedded in paraffin wax. Cross and sagital sections were cut and stained with Hematoxylin-Eosin for the light microscopic observations to compare with the results observed by SEM.

Results

The male reproductive system

The cirro-vaginal aperture was situated in the middle of a small elevation in the first half of the ventral surface of each proglottid. The cylindrical cirrus, 60 μm in diameter, usually protruded from the genital pore. The cirrus became slightly slender at the top, opening a small ejaculatory pore. Many hemispherical genital papillae, 20 μm in diameter, surrounded the genital pore (Fig. 1, arrowheads).

Microtriches grew densely all over the surface of the cirrus. These microtriches were rather stout, as compared with those of the tegument, and showed a characteristic form with spined tips and bulb-like roots (Fig. 2, arrows).

Microtriches covered the tegument and also the genital papillae which spermatozoa with mucus substances often attached (Fig. 3). In a sagital fracture of the cirro-vaginal aperture, genital papillae were arranged around the root of the cirrus at the deep inside of the genital atrium. Uterine loops in which mature eggs were packed opened to a uterine pore (Fig. 4, arrow).

A sagital fracture showed that the cirrus consisted of duct in the center (Fig. 5). The spherical cirrus-sac, 400 μm in length and 220 μm in width, was located somewhat obliquely to the ventral surface of the proglottid in the sagital fracture. The round seminal vesicle, 360 μm in diameter, was situated dorsally to the cirrus-sac (Fig. 6).

The contracted cirrus was invaginated in the cirrus-sac, showing a muscular, porous, sponge-like structure with a convoluted ejaculatory duct in the center (Fig. 7). The seminal vesicle formed a round sac with the thick muscular wall, 30 μm thick, and packed numerous mature spermatozoa (Figs. 8, 9). The spermatozoa showing thread-like, cylindrical body and some round granules, 0.3–0.5 μm in diameter, were often bulging from the surface cytoplasmic layer of the spermatozoa. The longitudinal groove was observed along the body of a spermatozoon (Fig. 10).

Testes of 70 μm in diameter and vas deferens were observed in the parenchymal layer, being surrounded with numerous particles. Many spermatozoa with long thread-like flagella were packed in vas deferens (Figs. 11, 12).

The spermatogenesis was observed in a fracture of the testes. Spermatogonia had large nuclei and numerous particles in the cytoplasm (Fig. 13). The spermatogonia completed the cell division to form the primary spermatocytes. The primary spermatocyte was also composed of a large nucleus and less cytoplasm. The conglomerated nuclei were arranged in a common cytoplasmic mass (Fig. 14). The early spermatocytes showed rosette formation, and were surrounded by the residual cytoplasmic capsule with the thread-like projections (Fig. 15).

The female reproductive system

The follicles of yolk glands, which contained round cells of 15 μm in diameter

were arranged directly beneath the tegument (Fig. 16). Vitelline cells in the yolk gland had a round nucleus and numerous granules, 0.5 μm in diameter, in the cytoplasm (Fig. 17). The vagina, 50 μm in diameter, ran in the ventral side of the uterine loops, being surrounded with a thick wall. Microvillar structure was seen on the surface of the vaginal wall (Fig. 18).

The oviduct (450 μm in diameter) which had a peculiar thick muscular wall was observed near the proximal part of uterine loops (Fig. 19). The thin microvilli and thick cilia-like processes of 0.2–0.4 μm in diameter grew densely on the inner wall of the uterus (Fig. 20). The uterine loops were supported by porous, interstitial tissue. Numerous granules, mucus substance and immature eggs were observed in the uterus (Fig. 21).

A fracture of the ovary showed primordial germ cells which had numerous particles and follicles (Fig. 22). The oogonia were distributed around the primordial germ cells, and showed a mulberry-like appearance with complicated microplicae and small crypts on the surface (Fig. 23). The oocytes, the more advanced stage of oogonia, developed to a cluster of cells in the ovary (Fig. 24).

Discussion

The general reproductive system of diphyllobothriid cestodes was precisely described by such authors as Andersen, 1971, Rausch and Hilliard, 1970, and Smyth, 1969. The cirrovaginal aperture was situated in the middle of a small elevation in the anterior half of the ventral surface of the proglottid. A cirrus which protruded from the genital atrium bore densely growth of microtriches all over the surface. These microtriches were morphologically different from microtriches on the tegumental surface. Microtriches of a cirrus showed stout and spine-like figures, and may serve for the insertion and the fixation of cirrus on the copulation rather than for the nutrients absorption.

An extruded cirrus of *D. latum* was surrounded with the genital papillae. The shape and arrangement of these papillae differed from those of the other diphyllobothriid cestodes. Andersen (1975a) described that the genital papillae had an elliptical shape in *D. dendriticum* and a circular shape in *D. ditremum*, while in *D. latum* the shape was either round or elliptic. The genital papillae of *Diplogonoporus grandis*, and *Spirometra erinacei* somewhat differed from those of *D. latum* (Yamane *et al.*, 1975). The difference in their contour and arrangement might have a taxonomic significance. The function of the genital papillae has been poorly understood. Andersen (1975b) observed sensory endings, probably tangoreceptors, in the area between the genital papillae and sometimes in the genital papillae themselves in *D. ditremum* and *D. dendriticum*, and suggested that the "papillae" together with the sensory endings constituted the structural basis for an adaptation in the copulation process. In the present study any sensory endings could not be found out around the genital papillae, but it is assumed that these genital papillae serve the cirrus to hold itself after the insertion into the vagina in the copulation process.

The position of cirrus-sac and the seminal vesicle had a taxonomic significance in diphyllobothriid cestodes (Andersen, 1975a). In the present study an oval cirrus-sac was situated horizontally in the sagital fracture of the proglottid, and the seminal vesicle was connected with the cirrus-sac at the caudal part.

Spermatogenesis in cestodes was studied by transmission electron microscopy (Featherston, 1971; Rosalio, 1964; Rybicka, 1966). The common structure of a spermatozoon of cestodes was composed of a cy-

lindrical body, an elongated nucleus and a flagellum. Featherston (1971) reported small cytoplasmic enlargements were seen developing on the spermatozoa body at irregular intervals along its length. The cytoplasmic enlargement did not show any characteristic cellular structures. The presence of cytoplasmic enlargements was supposed to be related to the elongation process of the spermatozoa tails. The present study revealed round bulgings on the spermatozoa bodies and tails in the seminal vesicle, and it seemed that these bulgings coincided with the 'cytoplasmic enlargement' which was shown in the transmission electron microscopic study by Featherston (1971) and 'the migrating nuclei' by Bonsdorff and Telkkä (1965).

The presence of a double axial filament in a spermatozoon of *D. latum* (Bonsdorff and Telkkä, 1965) has been thought to be characteristic of pseudophyllidean cestodes. The present SEM observations revealed that the body of the spermatozoa of *D. latum* was not completely cylindrical, but showed a longitudinal groove along the body. These grooves seemed to be coincident with the presence of a double axial filament.

Rybicka (1966) described that the general pattern of spermatogenesis was similar in all cestodes, resembled that of Trematoda. The spermatogonia arose in the germ cells, occurred the divisions, resulted in a cluster of spermatogonia, and subsequently formed the spermatocytes. The displacement of the nuclei was followed by the maturation division and the spermatozoa formation. In the present study the process of spermatogenesis was partly observed. The spermatogonia with relatively large nuclei formed the spermatocytes by the maturation division. The cluster of nuclei of the spermatocytes showed a rosette formation. Many thread-like projections, which seemed to be identical with the origin of the cylindrical bodies of spermatozoa, developed in a residual cytoplasmic mass which wrapped the cluster of the nuclei.

Different egg-forming systems (oogenotop) in cestodes have been studied and grouped into four types (Löser, 1965a, 1965b, 1965c; Rybicka, 1966; Smyth, 1956; Smyth and Clegg, 1959). Vitelline cells contributed yolk and eggshell material to the embryo. Oocyte was released from the ovary through a single oviduct which had a thick muscular wall. Vitelline cells which contained both shell and yolk precursors passed through a common vitelline duct and joined with the zygote at the ootype where the eggshells were formed with the granules of shell precursor.

In the present study yolk glands of *D. latum* consisted of well-developed lobate follicles and each vitelline follicle was composed of numerous, round vitelline cells. Each vitelline cell was packed with many miliary granules. Swiderski and Mokhtar (1974), Mokhtar and Swiderski (1976) observed the developed membrane system, aboundant lipid droplets, protein vesicles and glycogen granules in the cytoplasm of the mature vitelline cells. Many round granules in the vitelline cells granules (Fig. 14) corresponded to the protein vesicles in their transmission electron micrographs.

These granules in the vitelline cells surrounded eggs in the proximal part of uterus, suggesting the close relationship with the eggshell formation. Bogomopova and Chavpova (1961) observed the formation of the eggshell took place in the initial part of the uterus in *D. latum*. Lethbridge (1976) showed the random arrangement of the globule-like units and their fusion between two or three neighbouring globules on the surface of the eggshell by SEM. According to the transmission electron microscopic studies, the vitelline cells of cestodes contained a large amount of nuclear and cytoplasmic glycogen which

were speculative to serve for the energy reservoir to the developing embryo. On the architecture of eggshell, the pattern and arrangement of the pits on the eggshell have suggested the significance for the taxonomy and the metabolic activity on the hatching (Hilliard, 1972; Coil, 1975).

Rybicka (1966) described that the oogonia appeared in the primordium of the ovary and multiplied to the oocytes. The oocytes grew rapidly in the ovary. In the present study oocytes with microplicae and small crypts on the surface were observed between the primordium and the interstitial tissue in the ovary, and the conglomeration of the matured oocytes grouped around the pore in the center of the ovary.

Summary

The reproductive system of *Diphyllobothrium latum* was studied by scanning electron microscopy. Observations were made on cirrus, cirrus-sac, seminal vesicles, testis, and spermatozoa as the male reproductive system. The female reproductive system—vagina, uterus, oviduct, yolk gland, and ovary—was also observed. The processes of spermatogenesis and oogenesis were also studied in some degree.

The application of the styren resin cracking method and the ion-etching method enabled stereoscopical observations of the internal fine structure of the reproductive organs. From the standpoint of functional morphology the results were discussed by reference to other observations obtained previously with the light and the transmission electron microscopy.

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広節裂頭条虫（*Diphyllobothrium latum*）の生殖器系に関する走査電子顕微鏡的観察

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広節裂頭条虫成虫にみられる生殖器系の各器官の立体的微細構造を走査電子顕微鏡によって観察した．雄性生殖器として陰茎，陰茎嚢，貯精嚢，精巣，精子について，雌性生殖器として，腟，子宮，輸卵管，卵黄腺，卵巣，卵子などについてその立体的微細構造を明らかにするとともに，精子及び卵子形成過程の一端を明らかにすることを試みた．

さらに機能形態学の観点から陰茎表面の小棘，生殖口周辺乳頭，精子形成過程，精子体部膨満部，卵黄細胞と細胞質内微細顆粒，卵殻形成，卵子形成過程などについて考察した．

また，表面構造のみならず，内部構造の走査電子顕微鏡による立体的微細構造の観察には，イオン・クリーニングの効果をふくめて，イオン・エッチング法が有効であることを明らかにした．

Explanation of Figures

Fig. 1 The cirrus (C) and the genital papillae (GP, arrowheads) on the tegument of the proglottids. (×500).

Fig. 2 Spines on the surface of the cirrus, showing bulb-form roots (arrows). (×20,000).

Fig. 3 The surface of genital papillae (GP), showing the dense growth of microtriches and spermatozoa (arrow). (×3,500).

Fig. 4 A cracking fracture of the cirro-genital aperture and genital papillae (GP), showing the cirrus (C), a part of uterine loop (U) and a uterine pore (arrow). (×400).

Fig. 5 A cracking fracture of the cirrus, showing a convoluted ejaculatory duct (arrow). (×500).

Fig. 6 A cracking fracture of a cirrus-sac (CS) and a seminal vesicle (SV) being adjacent to distal uterine loop (U). (×200).

Fig. 7 Inner structure of the cirrus-sac, showing a convoluted ejaculatory duct (ED, arrowhead) in the invaginated cirrus. (×800).

Fig. 8 A cracking fracture of the seminal vesicle (SV) in which numerous spermatozoa (S) were reserved.

Fig. 9 Inner structure of the seminal vesicle, showing numerous spermatozoa (S) attached to the inside of the wall (W). (×1,200).

Fig. 10 Spermatozoa (S) in the seminal vesicle which showed round bulgings (arrows) on the cytoplasmic layer of the tails. Note the longitudinal groove along the tail (arrowheads). (×12,000).

Fig. 11 A cracking fracture of the testis (T) and the vas deferens (VD) which were surrounded with numerous particles (P). (×850).

Fig. 12 Conglomeration of spermatozoa (S) in a cracking fracture of vas deferens (VD). (×2,000).

Fig. 13 A cracking fracture of the primary spermatogonia (SG) which had a large nucleus (arrows) and many particles (P) in the cytoplasm. (×3,000).

Fig. 14 The developed spermatogonia in the more advanced stages (SG), showing the conglomerated nuclei (N) which were arranged around the cytoplasmic mass (CP). (×7,000).

Fig. 15 Early spermatocytes (SC) in spermatocytogenesis showing rosette formation and the development of thread-like projections (arrowheads) around the residual cytoplasm. (×5,000).

Fig. 16 A cracking fracture of the yolk glands (YG) with the vitelline cells (V, arrowheads). (×4,000).

Fig. 17 The vitelline cell (V) in the yolk gland, showing a nucleus (N) and vitelline granules. (×7,200).

Fig. 18 A cracking fracture of the vagina (VA), showing the microvilli (arrows) on the muscular wall. (×1,600).

Fig. 19 A cracking fracture of the oviduct (OV) with a thick muscular wall. (×450).

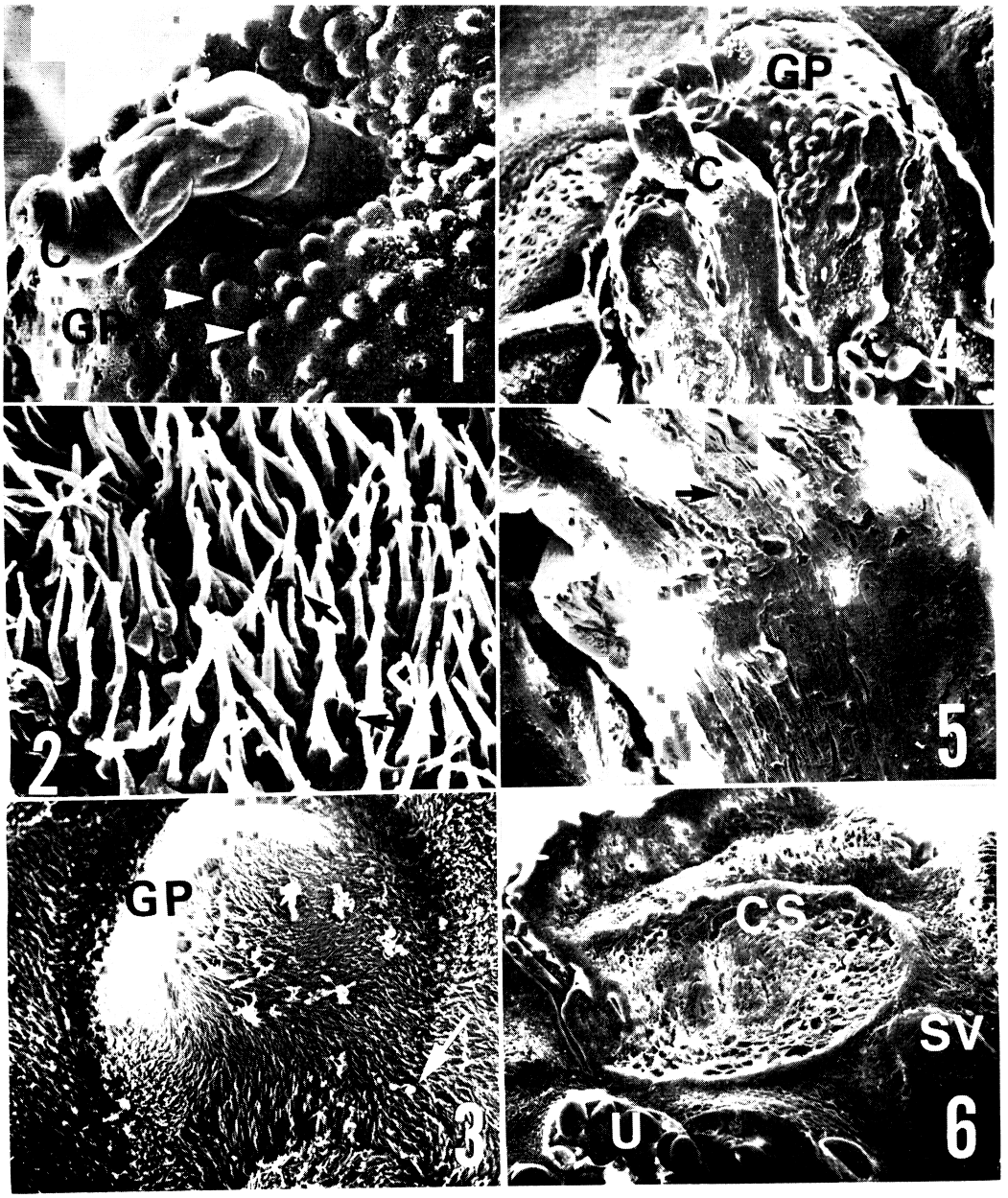
Fig. 20 Inner surface of the uterus, showing the dense growth of thin microvilli (arrows) and thick cilia-like processes (arrowheads). (×41,000).

Fig. 21 A cracking fracture of the uterine loop, showing an egg (E) and vitelline granules (arrows). (×500).

Fig. 22 A cracking fracture of the ovary showing primordia germ cell (PG) and many follicles (arrows). (×3,000).

Fig. 23 A higher magnification of the oogonium (OG) showing complicated microplicae and crypts (arrowheads) on the surface. (×27,000).

Fig. 24 Conglomeration of some developed oocytes in the ovary. (×5,000).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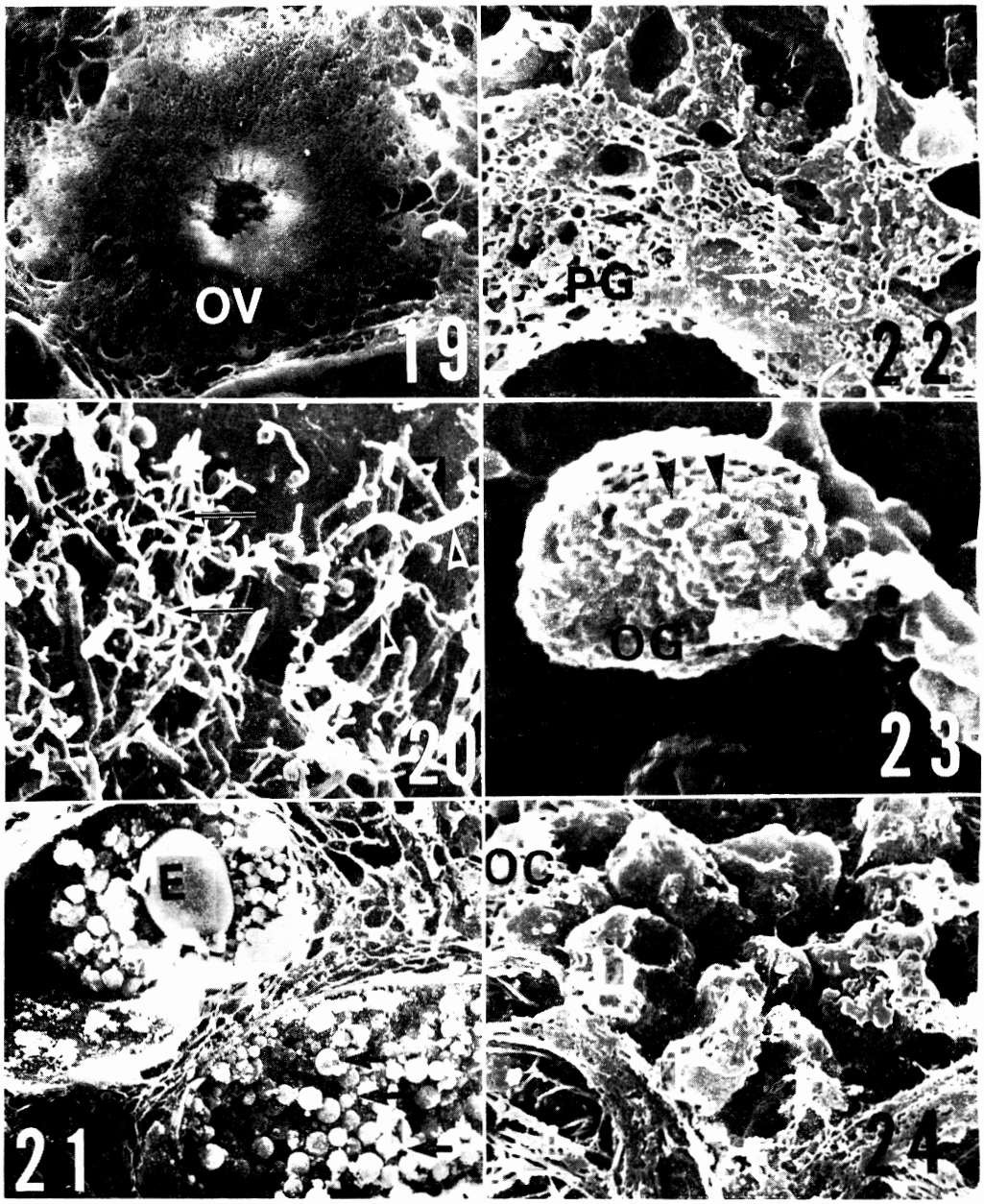
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