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# Observations on the Ultrastructure of the Excretory Canal of the Cestode, *Spirometra erinacei*

(cestode/excretory canal/SEM)

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**The excretory system of the plerocercoid and the adult worm of *Spirometra erinacei* were studied using transmission and scanning electron microscopes. Attention was concentrated on flame cells, primary excretory ducts, secondary excretory ducts and collecting excretory canals. Special attention was paid to the basic structure of the duct wall with reference to functional morphology. The duct wall was composed of a superficial cytoplasmic layer and a fibrous basal membrane. Bleb-like protrusions were particularly conspicuous in the plerocercoid and in the neck of adult worm, while particles on the duct wall were observed mostly in the mature proglottid of the adult worm. The significance of these structures are discussed herein. Considering all the characteristics of the fine structure we suggest that the superficial layer of the duct wall is simultaneously functional in the exocytosis-endocytosis cycle and the apocrine secretion.**

Recently many workers have given more and more attention to the structure and function of the tegument of cestodes, yet have shown little interest in the excretory system of cestodes. Though there are studies on the excretory system (1—5), the functional morphology of the duct wall has rarely been documented.

Parshad and Guraya (6) reported the histochemical localization of lipids and the presence of enzyme activity in association with excretion of the duct wall. It is still under debate whether the particles on the surface of the duct wall are C-type virus or microvilli (7, 8).

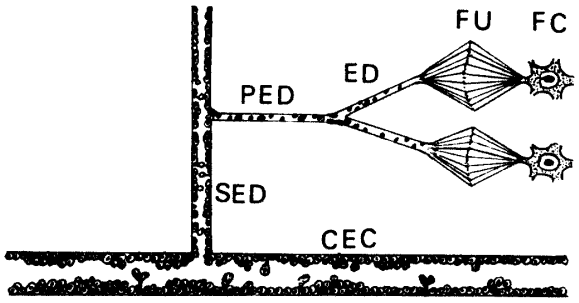


Fig. 1. Simplified excretory system of adult *Spirometra erinacei*. FC, flame cell ; FU, funnel of flame cell ; ED, efferent duct ; PED, primary excretory duct ; SED, secondary excretory duct ; CEC, collecting excretory canal.

The present paper is concerned with the morphological findings of the excretory system of plerocercoid and adult worm of the cestode, *Spirometra erinacei*. The functional morphology of the duct wall and the nature of granular substances in the lumen are given particular emphasis. Since there is some disparity in the nomenclature of components of the excretory system of cestodes in general, a diagram of the excretory system, applying the nomenclature used in this paper is included (Fig. 1).

## MATERIALS AND METHODS

Plerocercoids of *Spirometra erinacei* were collected from naturally infected snakes, *Elaphe quadrivirgata*. Adult worms were obtained experimentally by feeding plerocercoids to dogs. Fresh materials were immersed in physiological saline, rinsed with 0.1 M phosphate buffer (pH 7.4) and dissected into small pieces. Then the materials were fixed in 5% glutaraldehyde for 6 hr, postfixed in 1% $OsO_4$ in 0.1 M phosphate buffer (pH 7.4) for 4 hr, at 4°C, dehydrated in an ethanol series and finally embedded in Epon 812. Ultrathin sections were cut in a Porter-Blum ultramicrotome, were double-stained with uranyl acetate and lead citrate, and examined under a Hitachi HU-12A transmission electron microscope operated at an accelerating voltage of 70 kV.

Some of the materials which had already been fixed successively in 5% glutaraldehyde and 1% $OsO_4$ were dehydrated, and soaked in amylacetate and freeze-fractured. These specimens were then dried by the critical point drying method, immediately coated with Pt-Pd alloy, and examined under a MSM-V scanning electron microscope operating at 10 kV.

## RESULTS

Fundamental elements of the excretory system of plerocercoids and adult worms are flame cells scattered in the parenchymal layer, efferent ducts, primary excretory ducts, secondary excretory ducts and collecting excretory canals (Figs. 3—6). All these ducts observed had the same basic morphology. The fine structure of the duct wall of plerocercoids resembles closely the structure of the duct wall in the neck of adult worms (Figs. 7—9). The superficial layer of the excretory canal is 1—2 $\mu$ in thickness. There are many vacuoles and vesicles with a diameter of 0.5—0.7 $m\mu$ and mitochondria in this layer. This superficial layer is separated from the parenchymal layer by a basal membrane which is about 0.2—0.4 $\mu$ in thickness. The basal membrane has many infoldings toward the superficial layer and seems to be related with the metabolic function of the superficial layer.

Nuclei of the cells in the superficial layer invaginate into the parenchmal layer, and an abundant storage of glycogen granules and mitochondria are found in the cytoplasmic bridge. Lipid droplets and glycogen granules are also scattered in the parenchymal layer. Beneath the basal membrane, we observed some cross sections of the secondary excretory canal with a single layer of globular particles on the wall (Fig. 5). As for the basic morphology of the

duct wall, there are no distinct differences between plerocercoids and adult worms. It is remarkable that there are bulging protrusions on the wall of the canal of plerocercoids and the neck region of adult worms (Figs. 4 and 8). These protrusions seem to be in process of release from the wall surface. The superficial cytoplasmic layer is well developed in both the life stages, and contains many vesicles and vacuoles in the basal region. The basal membrane is also well developed and is composed of a fibrous structure. Thick muscle bundles are arranged among glycogen granules and lipid droplets in the parenchymal layer.

The excretory canal in each immature, mature and gravid proglottid of adult worm has structural features similar to those of plerocercoids and the neck region of adult worms (Figs. 10—12). The superficial cytoplasmic layer is well developed and contains many vacuoles and mitochondria (Fig. 13). This may be characteristic of the excretory duct of mature proglottids. The mitochondria in this layer are scarce in the cristae, round in shape, and variable in size. There are abundant glycogen granules, large lipid droplets and ambiguous whirl structures in the parenchymal layer.

Numerous particles with a distinct core are observed on the superficial cytoplasmic layer of the excretory duct. These particles are not distributed equally, but are disposed in a stratum in some parts and swarmed in a lump in the other parts. The cross section the structure of the particles is quite different from that of microvilli, and there are no tubular structures such as microvilli on the surface of the cytoplasmic layer (Figs. 14—16).

Most of these particles are 0.7—1.0 $m\mu$ in diameter, some having a core, that is a dense circular center with diameters of 0.3—0.4 $m\mu$. These particles are found not only in the collecting canal, but also in the primary and the secondary ducts. No particles are found in the efferent duct, which is directly connected with the flame cell. The particles seem to have occurred from the cytoplasm beneath the cytoplasmic membrane (Fig. 16). Morphological evidence of the reverse pinocytotic activity was also observed on the luminal surface of the duct (Fig. 14). A higher manginfication showed Y-shaped or bleb-like protrusions budding from the cytoplasmic membrane among numerous particles (Fig. 15).

The particles on the surface of the superficial cytoplasmic layer of the collecting excretory canal are especially dense in mature proglottids. They are distributed more densely on the wall of the collecting excretory canal and the secondary excretory duct than on the wall of the primary excretory duct. It is interesting that in the superficial cytoplasmic layer circular or elliptic vesicles and vacuoles surrounded by the limiting membrane are evidently in process of forming a core in each of them. The nearer they are to the surface of the superficial cytoplasmic layer, the more distinct are the cores.

It is true that many of the particles have a similar structure and size to C-type viruses. The process of particles formation resembles the budding of the C-type virus. However, some particles display multiple budding in the

form of letter "Y" and numerous bleb-like protrusions, which are evidently cytoplasmic extensions, and particles were observed on the surface of the duct wall. We also observed invaginations of the plasma membrane and vesicles in the cytoplasm, which is generally considered to be the occurrence of pinocytosis (Fig. 14). These particles may be waste materials produced by the exocytosis.

We observed under SEM how the excretory canal ran and ramified revealing the outlets of the secondary excretory canal opening into the collecting excretory canal (Figs. 17—21). Lipid droplets and glycogen were observed as granules around the collecting excretory canal (Fig. 17). The collecting excretory canal is filled with amorphous contents or debris which may be excretion (Fig. 20). On the inner surface of the collecting excretory canal of the adult worm there are fine granular substances or debris, but no thickly growing microvilli (Fig. 21).

## DISCUSSION

As the fundamental elements of the excretory system of *Spirometra erinacei*, flame cells, funnel of flame cells, efferent ducts, primary excretory canals, secondary excretory canals, and collecting excretory canals are all evident. The relationship among these elements is shown as a diagram in Fig. 1. These fundamental structures of the excretory system are common with those of other kinds of cestodes (1, 3). The complex network of the excretory system is

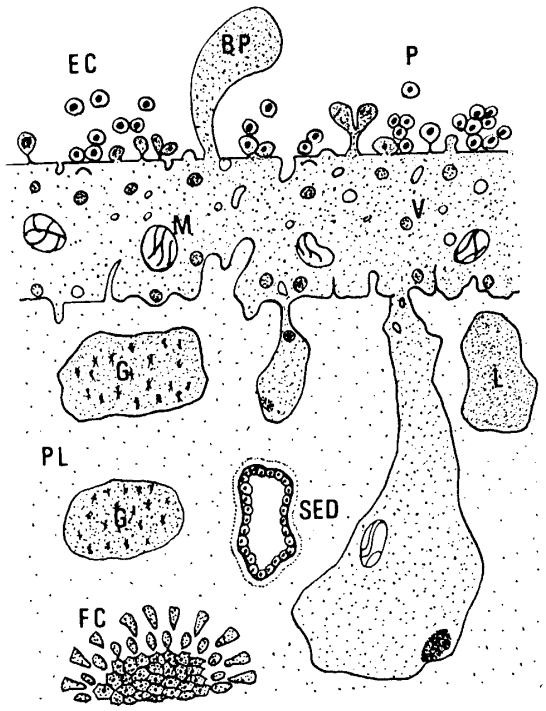


Fig. 2. Diagrammatic representation showing fine structure of the duct wall of excretory canal. EC, excretory canal ; BP, bulging projection ; P, particles ; M, mitochondria ; V, vesicle ; G, glycogen granules ; L, lipid droplets ; PL, parenchymal layer ; SED, secondary excretory canal ; FC, flame cell.

situated in the parenchymal layer of the plerocercoid and the adult worm. Morphological evidence of the ramification of the excretory duct was observed under the scanning electron microscopy. The network of the system including ramification of the duct is of such a structure as to be convenient for excretory, osmoregulatory and hydrostatic functions.

The basal morphology of the excretory duct is shown in Fig. 2. The ultrastructure of the excretory duct is usually composed of a cytoplasmic superficial layer, a fibrous basal membrane, and a cytoplasmic bridge in the parenchymal layer. Such a structure is common in the excretory ducts of plerocercoids and adult worms. Numerous vesicles in the cytoplasmic layer and invaginations of the basal membrane are characteristic features which are evident throughout the larval and adult stages. In adults, these features can be seen more evidently in mature proglottids than in immature proglottids. As is generally known, it is characteristic of pinocytosis or reverse pinocytosis to form vesicles in the cytoplasmic layer. This applies to the tegument of cestodes. Concerning basal invaginations they are characteristically seen in the cells involved in water transport, e. g., kidney tubule cells. Thus the superficial cytoplasmic layer of the excretory duct is related both to excretion and resorption of materials.

Smyth (9) described that although some of the end products of metabolism were presumably excreted, the basic physiology of excretion in cestodes had not been closely examined. The fine structure substantiating these excretory and other functions has not been sufficiently documented. We observed that the excretory duct has a luminal plasma membrane showing various stages of micropinocytosis. Besides pinocytotic vesicles, we found bleb-like protrusions and numerous particles budding from the cytoplasmic membrane. Baron (4) reported the extension of the wall, the bleb-like process and rod-shaped bodies on the bladder wall of *Cysticercus bovis*, and suggested that these structures were involved in the secretory function. Our observation also confirmed that the more direct form of excretion such as bleb-like protrusions is seen mainly in the part of the neck. Bennett (10) observed such pinocytosis in the luminal surface of flame cells, efferent ducts, and primary ducts of juvenile *Fasciola hepatica* during migration in the host. He also found that the excretory lipid is released by apocrine secretion with droplets or parts of droplets nipped off by the apical plasma membrane and released into the lumen.

The results of the present study suggest that the superficial cytoplasmic layer has an excretive and resorptive function, and probable mechanisms of these functions are exocytosis-endocytosis and apocrine secretion. Howells (1) found that the epithelium lining the excretory canal of *Moniezia expansa* has a structure suggesting that it is metabolically active. He also postulated that the alkaline phosphatase activity of the central canal might be associated with the active transport of materials into and from the canals.

Swiderski *et al.* (5) also studied the excretory system of three cyclophyllidean cestodes and reported that characteristics of the ultrastructure of the epithelium of canal walls provided some confirmation of the high metabolic activity. Parshad and Guraya (6) proved that the ingredients accumulated in the lipid

around excretory canals were triglyceride, phospholipid and lipoprotein. He thought that these ingredients participated in the formation of structural material, which was related with the secretory process. The existence of phosphatase on the wall of excretory canals is generally known, and it is thought to be connected with selective resorption and excretory function or transport sugar (Threadgold (11)). Our conclusion is that the excretory system with its mechanism for secretion and reabsorption in cestodes is able to adapt to conditions of the environment, and is involved in the preservation and regulation of body fluid.

Concerning structures on the surface of the duct wall there are various interpretations. Many investigators have observed microvillar structures on the duct wall of the excretory canal of various cestodes such as *Catenotaenia pusilla*, *Hymenolepis diminuta*, *Inermicapsifer madagascariensis*, *Moniezia expansa*, *Cysticercus bovis*, *Echinococcus granulosus*, *Cysticercus longicollis*, and *Cysticercus* of *Taenia crassiceps* (1, 3, 4, 5, 12, 13).

Other investigators observed that *Spirometra*, *Diphyllobothrium* and *Ligula* bore particles which morphologically resembled to the C-type virus, even the details (7, 8). The true nature of this structure like microvilli or viruses and the function of the surface structure of anucleate epithelium lining the canals is still debatable. Mueller and Strano (7) found a large number of virus-like particles of about 850 Å in diameter on the surface of the collecting duct of plerocercoids of *Sparganum proliferum*, Spargana of *Spirometra mansoni* and *Spirometra mansonoides*. They thought that these particles were C-type virus particles and suggested that they might play a role in the transformation of a non-proliferating sparganum to proliferating larva, since virus particles of similar morphology had been associated with tumor production. Daly *et al.* (14) also found the same particles in a non-proliferating sparganum of human origin. Dougherty *et al.* (8) described that similar particles extruded from the wall of excretory ducts of *Spirometra*, *Diphyllobothrium* and *Ligula*. He said that in spite of their similarity to the C-type virus, the viral nature of these particles was in doubt because of the apparent lack of nucleic acid.

We also observed similar particles on the wall of excretory canals of every segment of plerocercoid, and of scolex, neck, immature proglottids of adult worms. Perfect particles with a distinct core in each center are found mainly in excretory canals of mature proglottids, and gravid proglottids. It seems that in the excretory canals of plerocercoids and the scolex and the neck of adult worms particles without a perfect electron dense core are predominant. In mature proglottids particles are not distributed evenly on the wall of excretory canals, but they are thick in some parts and thin in the other.

Thus we ascertained that the structure of these particles is evidently different from that of so-called microvilli in intestines of mammals and from that of microtriches on the tegument of cestodes. In cross sections of the particles, there are no microtubules, septa, numerous electron dense core, such as are seen in the microthrix. Under SEM we did not observe thickly grown microvilli, but rather granular substances and debris on the wall of excretory

canals. The former are apparently not microvilli but particles. These particles have a close resemblance to C-type viruses in structure, but considering the existence of a superficial cytoplasmic layer with abundant vesicles and vacuoles it is most probable that the particles are vesicles produced by the excretory and secretory function of this layer.

Recently investigators have reported particulate inclusions in malaria and *Entamoeba histolytica* (15, 16, 17). The particles have been given various names such as cristalline protein inclusions, crystalloids, rickettsiae, viruses and virus-like particles. One supposed that these particles had to be viruses, because of their appearance of crystalline aggregations, regularity in size, great density and the tendency to be arranged in ageometric configuration. The viruses seen in protozoa are evidently different from the C-type virus particles seen in *Spirometra*. We are now attempting to clarify the relationship between parasite and virus in order to determine the true character of the particles seen on the wall of excretory canals of cestodes.

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## EXPLANATION OF FIGURES

Fig. 3. Section through the collecting excretory canal (EC) of plerocercoid showing particles (P), superficial cytoplasmic layer with invagination, basal membrane (BM) and glycogen granules (G). (×12,000)

Fig. 4. Numerous particles (P) lining the duct wall of plerocercoid. Note the relationship of the infoldings of basal membrane (arrows), vesicles (V) and particles (P). (× 36,000)

Fig. 5. Cross section of primary excretory ducts (PED) of plerocercoid showing several particles on the wall. (× 10,000)

Fig. 6. Longitudinal section of the funnel (FU) and flame cell (FC) of plerocercoid. (× 8,000)

Fig. 7. Cross section on neck region of adult worm showing collecting excretory canal (CEC), particles (P) and basal membrane (BM). (×6,000)

Figs. 8. and 9. Bulging projections (BP) arising from duct wall in neck region of adult worm. Note particle with core and amorphous substance in the canal (arrows). (× 20,000)

Fig. 10. Cross section of excretory canal in mature proglottids of adult worm showing numerous particles, abundant glycogen granules (G) and mitochondria (M). (× 10,000)

Fig. 11. Horizontal section of excretory canal in mature proglottids showing the ramification of canals. (× 3,000)

Fig. 12. Cross section of excretory canal in mature proglottids showing budding form of particle, lipid droplets (L) and glycogen granules. (× 1,000)

Fig. 13. Cross section of excretory canal in gravid proglottids. Note numerous vesicles and vacuoles (V) in the superficial cytoplasmic layer, whirl structures (W) and flame cell (FC). (× 25,000)

Fig. 14. Pinocytosis in the superficial cytoplasmic layer of the duct wall of mature proglottids showing numerous particles, vesicles and vacuoles (V). (× 33,000)

Fig. 15. High magnification of the superficial cytoplasmic layer. Note budding and branching forms of particles (arrows) arising from walls. (× 40,000)

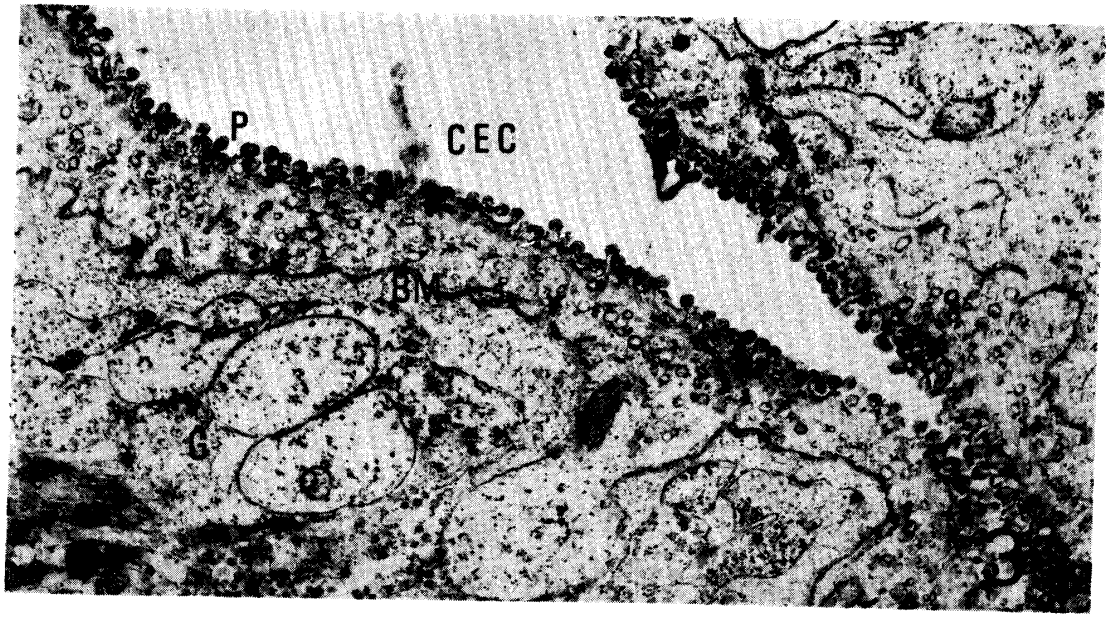
Fig. 16. Particles in the process of release from the surface of lacunae (arrow) near the secondary excretory duct. (× 40,000)

Fig. 17. Scanning electron micrograph of longitudinal section of the collecting excretory canal in mature proglottids. (× 5,000)

Figs. 18 and 19. Scanning electron micrographs of cross sections of the collecting excretory canal in gravid proglottids. (× 2,500, × 5,000)

Fig. 20. Scanning electron micrograph of cross section of the collecting excretory canal in plerocercoid. Note bulging projections (BP), concretions (C) in the canal, and pores in the duct wall. (× 10,000)

Fig. 21. The inner surface of the collecting excretory canal in mature proglottids showing numerous particles and concretions. Note absence of microvilli-like structures. (× 10,000)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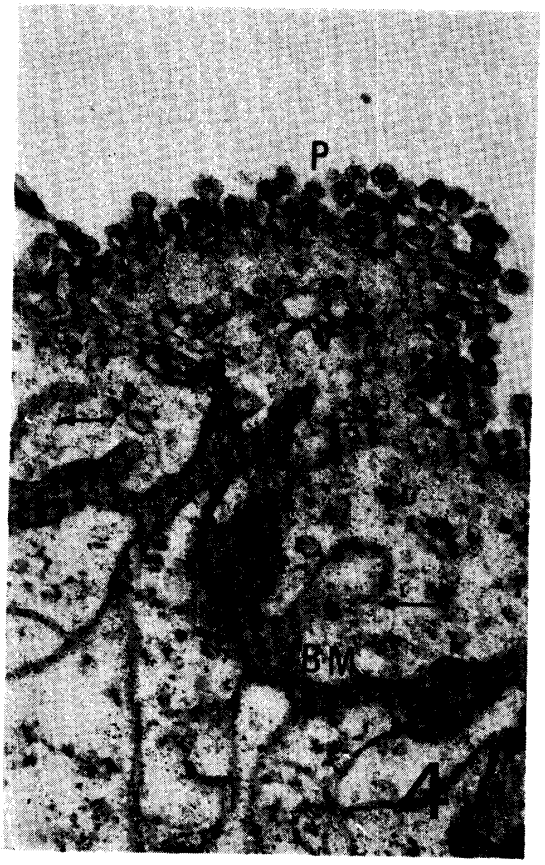
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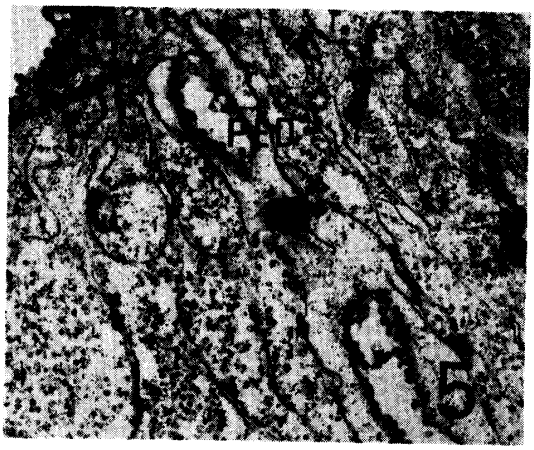
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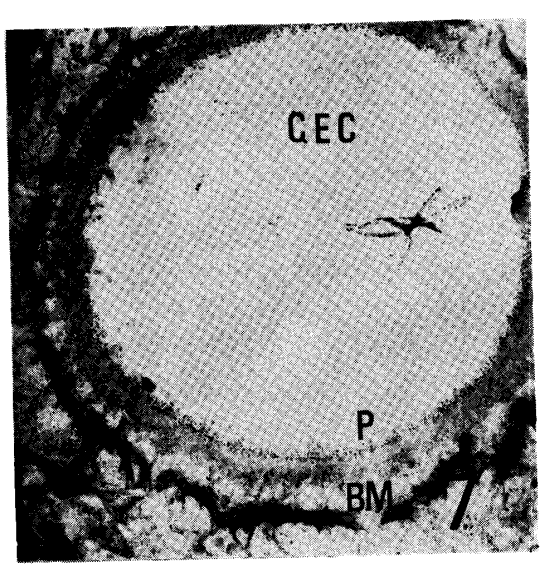
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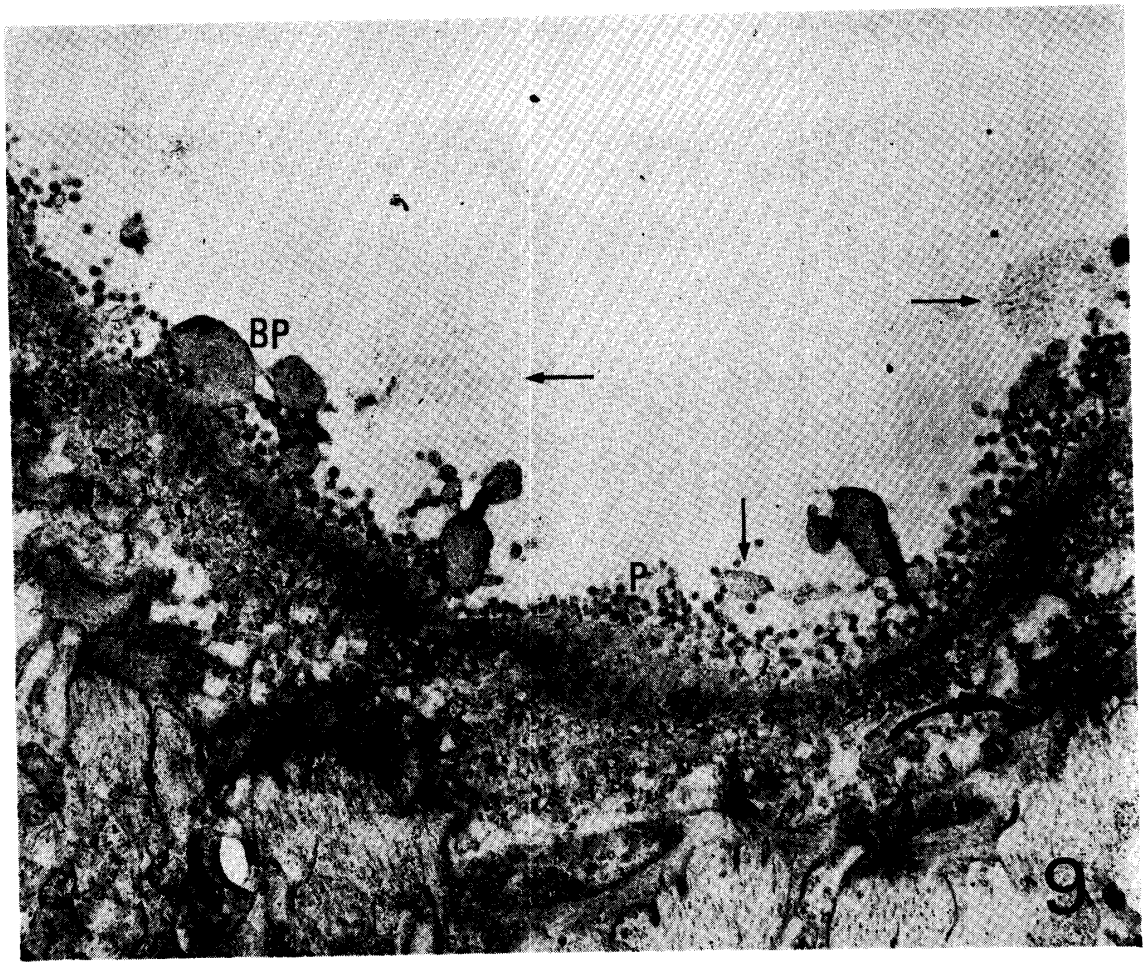
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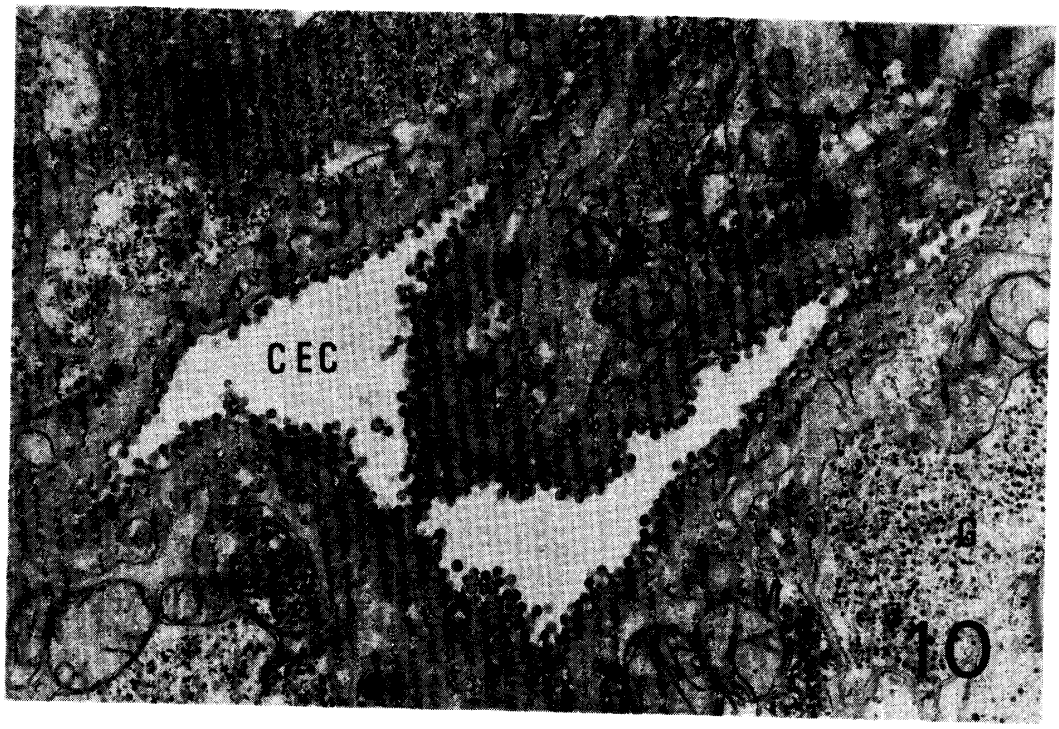
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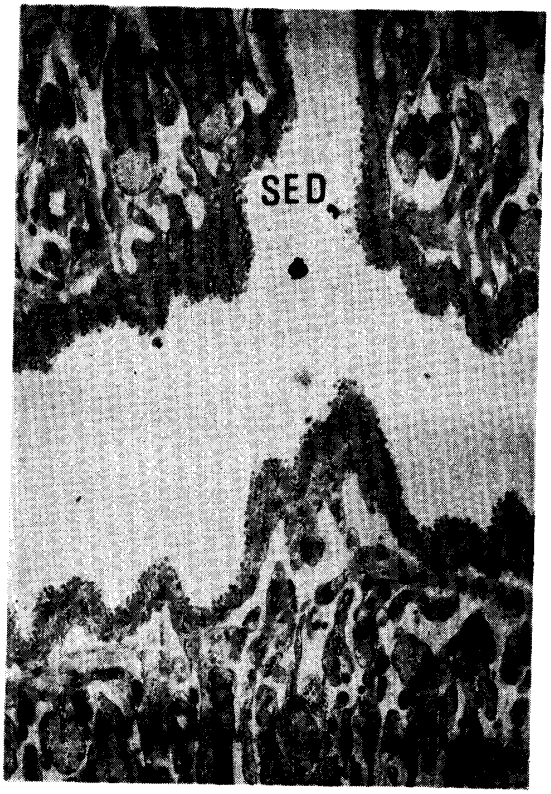
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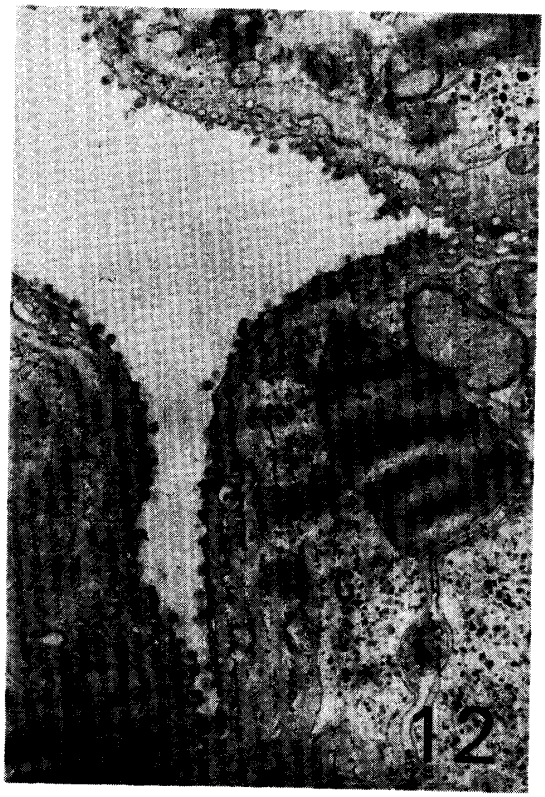
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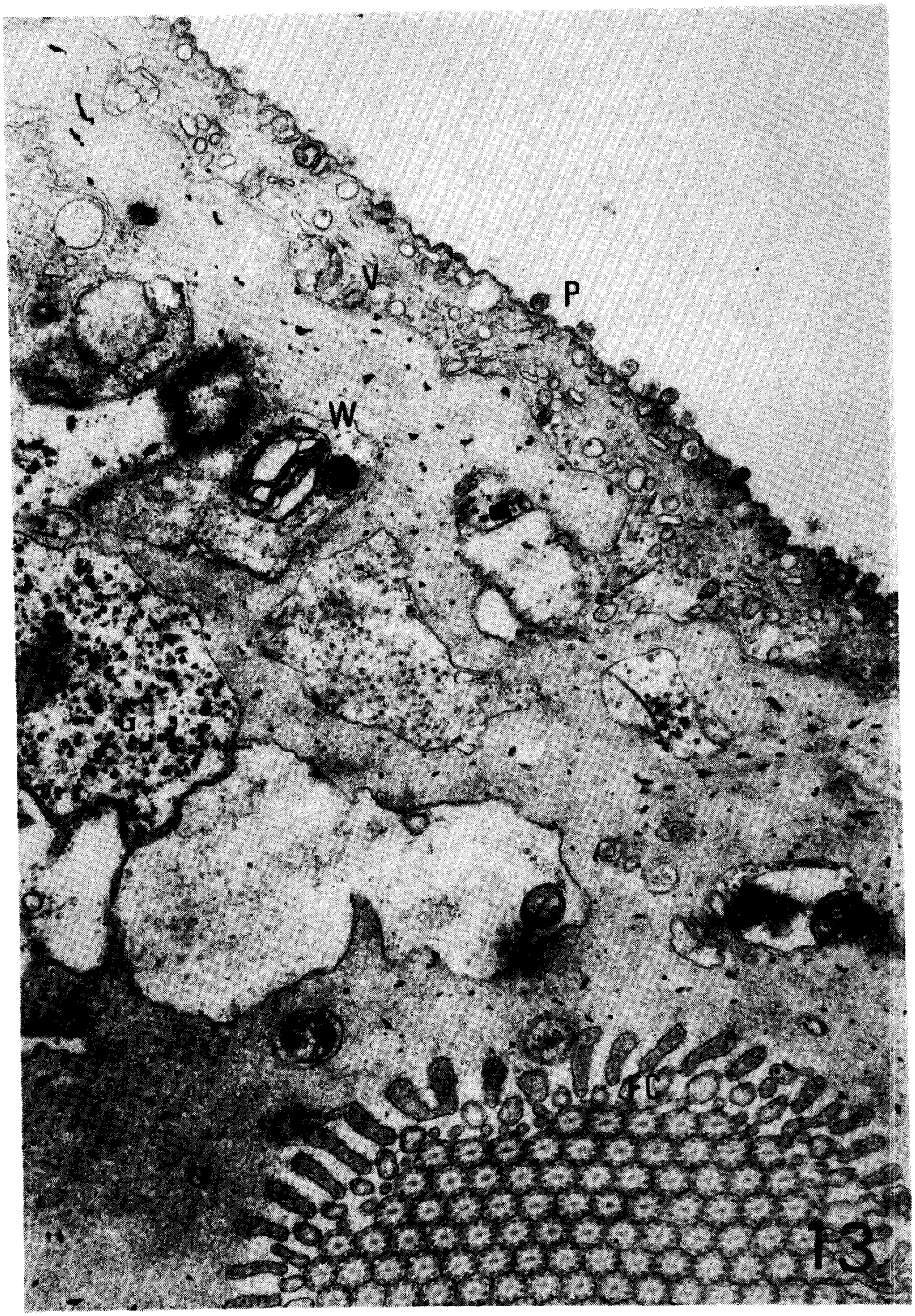
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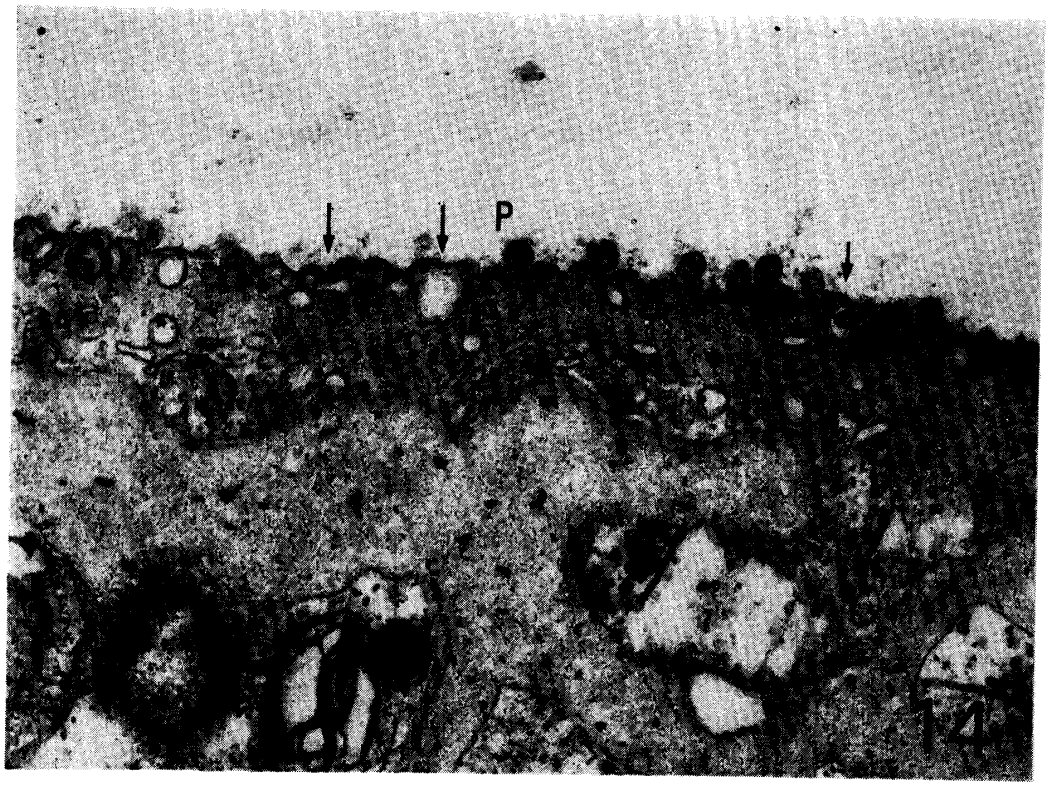
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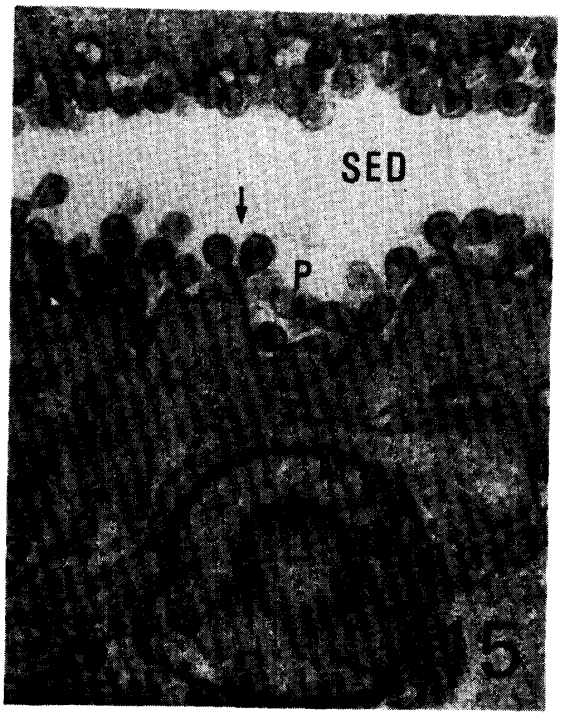
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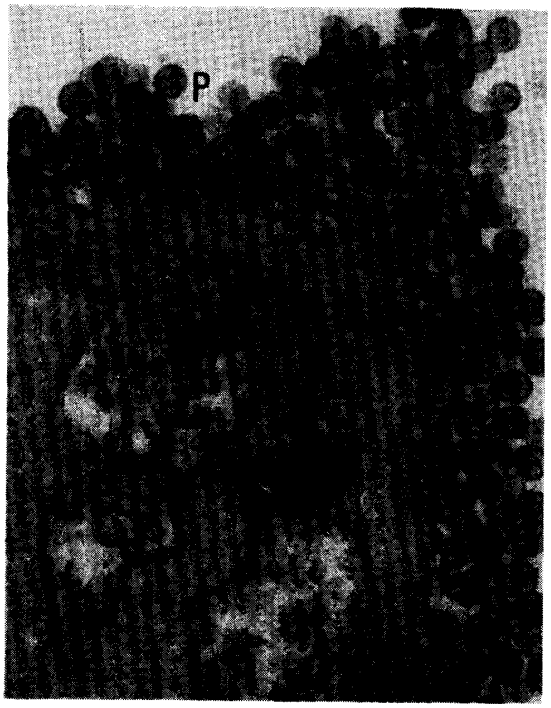
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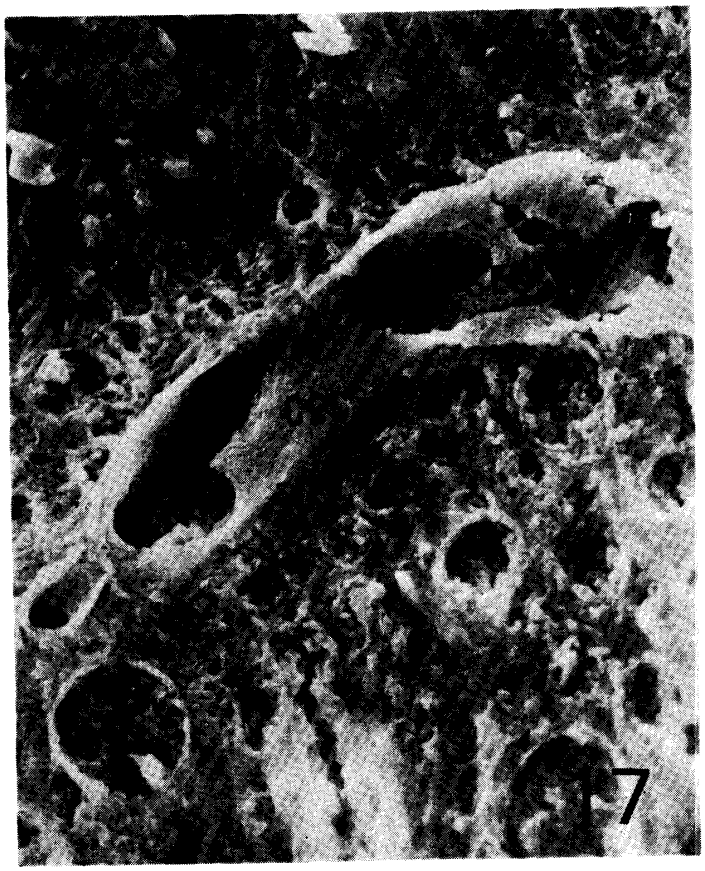
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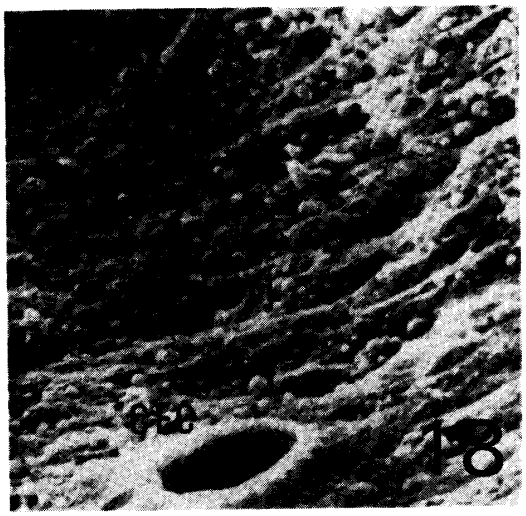
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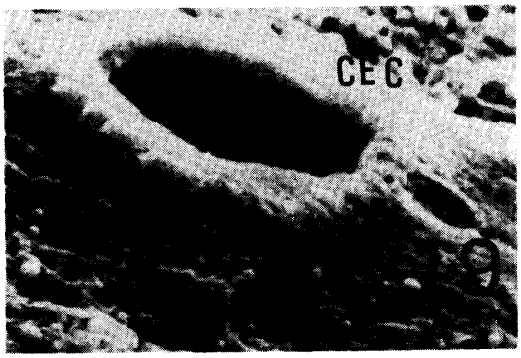
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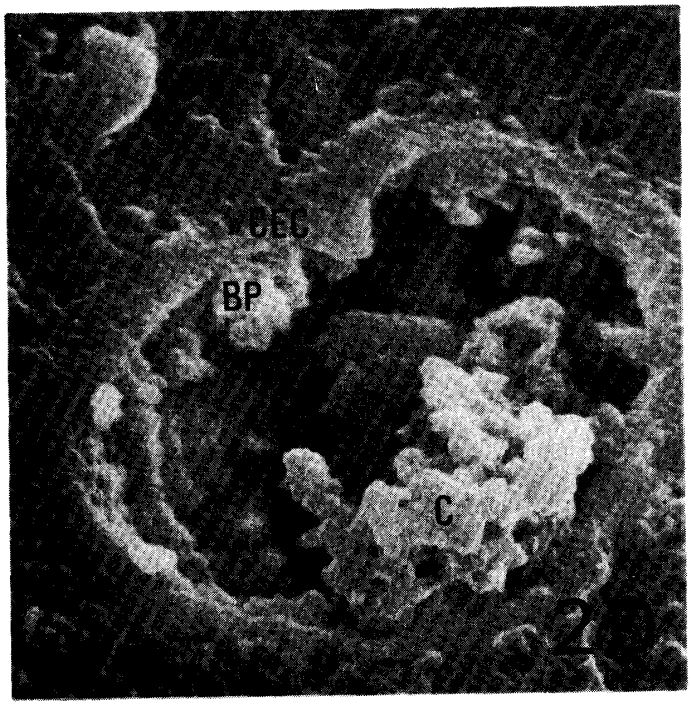
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BP
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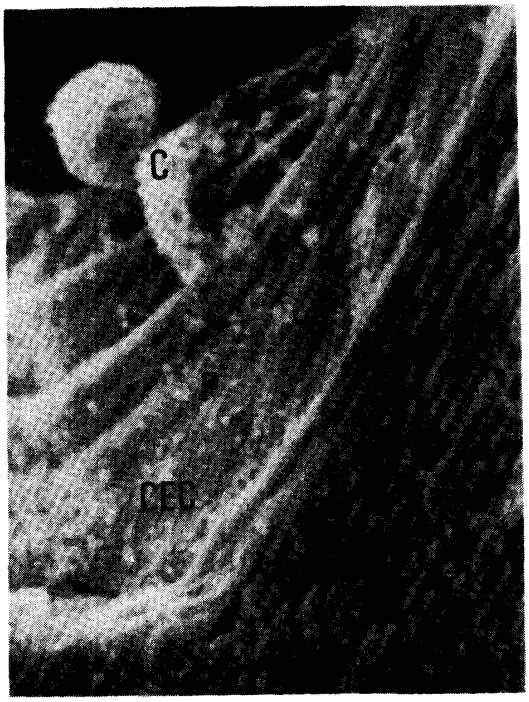
2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