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Ultrastructural Changes at the Surfaces of Oocytes in a Sabellid Polychaete, *Pseudopotamilla occelata* Moore

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Structural changes at the surfaces of oocytes of *Pseudopotamilla occelata* were examined by electron microscopy. The oocytes, which grow up to the final stage in the same coelomic fluid, once released from the ovary at 5 μm stage, change in the structure of the vitelline envelopes. Microvilli were found to change greatly in structure, abundance and behaviour during oogenesis. Microvilli are short and bifurcated at the previtellogenic stages and grow in size, but the number increases only during previtellogenesis but decreases during vitellogenesis. Glycocalyx structures begin to form at the tips of microvilli at the early previtellogenic stages and become more abundant as oocytes grow and remain at the final stage of oogenesis. The tips of microvilli are separated from the stems at the late vitellogenic stages to form vesicles simultaneously with retraction of the microvilli. Vitelline envelope consists of outer, intermediate and inner layers at the previtellogenic stages. However, the inner layer becomes thickened and differentiated into two sublayers at 80 μm stage, while the outer and intermediate layers remain constant in the thickness. These structural changes were presumably the results of functional differentiation of the vitelline envelope throughout oogenesis even in the same milieu.

KEY WORDS: Electron microscopy, *Pseudopotamilla occelata*, Oocytes

In sabellid polychaetes immature oocytes grow in the coelomic sacs throughout the whole process, after they have been released into the sacs from the ovary. Since the process occurs at any time of the year, a mixture of oocytes of all stages is present in each coelomic sac. Once oocytes have grown to the final stages, they remain arrested without further growth until spawning. Even though oocytes of various stages grow in the same coelomic fluid, some physiological activities are varied during oogenesis (Lee, 1977: Lee and Whiteley, 1984). For example, formation of yolk granules becomes very active only in the oocytes larger than 80 μm in diameter. We assumed that the surface structures of oocytes including the vitelline envelopes are structurally and functionally different at the different stages, so that oocyte of each stage may be able to take up materials selectively from the outside and to carry out stage-specific process such as yolk formation. Permeability to ^{3}H-uridine was found to be variable during oogenesis of *Schizobranchia* (Lee, 1984). Electrophoretic analysis of the vitelline envelopes of the *Schizobranchia* oocytes showed that the protein components change during oogenesis (Lee, 1988). Furthermore coelomic fluid proteins were found to be selectively taken up predominantly into the oocyte ranging from 80 μm to 140 μm in diameter (Jang, 1988). Observations suggested that the oocyte surface may change in structure during oogenesis in accordance with physiological differentiation.

There have been several reports on the structural characteristics and changes of the oocyte surfaces in polychaetes (Franklin, 1966; Schroeder and Hermans, 1975; Huebner and Anderson,

1976; Heacox and Schroeder, 1981; Eckelbarger and Grassle, 1982; Eckelbarger, 1984) and *Urechis* (Gould-Somero and Holland, 1975). Even though most of these studies were confined to limited stages of oogenesis, it has been observed that microvilli are simple at the early stage but grow in size, becoming branched as oogenesis proceeds. We studied ultrastructural changes at the surfaces of oocytes of a sabellid polychaete, *Pseudopotamilla occellata*.

Materials and Methods

Pseudopotamilla occelata Moore, a tubiculous polychaete, was collected on the east coast in Korea and kept in sea water at 12°C after being brought to the laboratory. Oocytes were obtained directly from the female worms by opening the coelom of the segments with a razor blade and were washed a few times in Milipore-filtered sea water (MFSW) by gentle centrifugation at 200 x g. The oocytes were then fixed in 1% glutaraldehyde-90% MFSW and postfixed in 0.5% osmium tetraoxide at 4°C. For scanning electron microscopy the oocytes were treated with isoamyl acetate after dehydration with ethanol, dried at critical point with carbon dioxide and coated with gold.

For transmission electron microsocpy the dehydrated oocytes were treated with propylene oxide, embedded in an Epon 812 mixture, and polymerized at 60°C. Thin sections of 0.05 μm were made on a ultramicrotome (LKB 8800), and stained with uranyl acetate and lead citrate.

Results

Projections

Observations with a scanning electron microscope showed that the oocyte surfaces are covered with numerous projections which change in shape, size and number during oogenesis (Figs. 1 and 2). The projections are round, uniform and arranged regularly in oocytes 34 μm in diameter (34 μm stage), and become irregular in shape, size and distribution at 46 μm stage (Fig. 1A). The irregularity remains during the subsequent stages up to 115 μm stage (Fig. 2A). Then the shape and the arrangement of the projections abruptly change at 146 μm stage. They become polygonal with a regularity in size and almost a crystalline arrangement. This appearance persists up to the final 186 μm stage. Throughout most of the stages the projections appeared to be covered with fuzzy-looking structures. This fuzziness was increased at the stages later than 146 μm stage.

Microvilli

Microvilli, which are surrounded by plasma membranes and filled with cytoplasm, change greatly in size and shape as well as in number during oogenesis. The microvilli are 340 nm long and 70 nm wide at the 32 μm stage, but increase 4 fold in length with a slight increase in width during oogenesis (Fig. 3,4,5,6).

Most of the microvilli were observed to be branched at the stages earlier than the 115 μm stage but branching ceases some time prior to 160 μm stage. The length of the branch is variable among the microvilli, suggesting that branching is a possible way by which additional microvilli are made during previtellogenesis.

The tips of the microvilli are covered with glycocalyx structures arrayed in a fan shape, each being 60 nm long and 6-9 nm wide. The glycocalyx structures begin to appear before the 32 μm stage, the youngest stage examined. They develop into a fan shape by increaing in abundance to a maximum at 160 μm stage. The size of each of these structures remains constant throughout all stages. The fuzzy-looking apprearance of the projections observed previously by scanning electron microscope appeared to be due to the presence of these glycocalyx structures.

At the stages earlier than the 115 μm stage, the microvilli are continuous at the outermost surface with the bifurcated projections which bear the glycocalyx structures, but a few microvillar tips at 115 μm stage appear to be separated from the stems to form vesicles (Fig. 5A). By the 160 μm stage, these separated vesicles are abundant (Fig. 5B). The vesicles are completely surrounded by membranes, keeping the glycocalyx structures at the peripheral side. The vesicles seem to result from retraction of microvilli, leaving the tips behind, as oogenesis apporoaches toward the end,

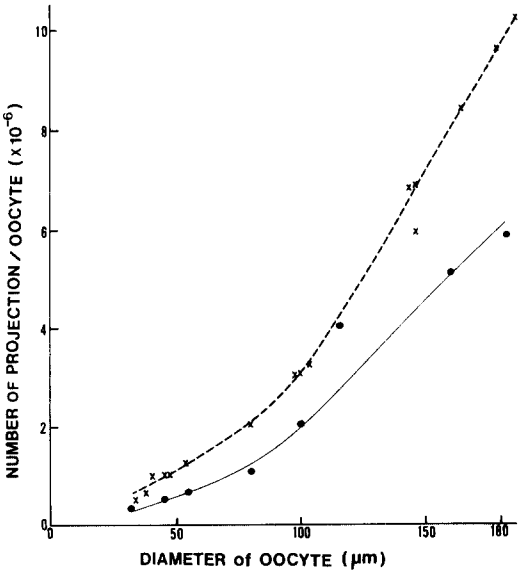

Fig. 7. Change in the number of projection during oogenesis. The projections, which include the tips of microvilli and the vesicle, were estimated from the scanning (x—x) and from the transmission electron micrographs.

since these two events were observed to occur simultaneously. At the final 186 μm stage the vesicles remain at the surface (Fig. 6). The projections, which had been observed with a scanning electron microscope (fig. 1 and 2), were identified to be the tips of microvilli at the stages earlier than 115 μm and vesicles plus microvillar tips at stages later than 160 μm with a transmission electron microscope. By this time, most of the surface of the oocyte exposed to the coelomic fluid, seen *en face* in Fig. 2C, is composed of these vesicles.

Changes in the Numbers of Projection and Microvilli

Number of projections, which occur either as the tips of microvilli or as vesicles isolated from the stems of microvilli, were estimated from scanning and transmission electron micrographs. In order to determine the total number of projections per oocyte from the scanning electron micrographs the number of projections per μm^2 of the oocyte surface was multiplied by the total surface area, assuming the oocyte to be a smooth sphere.

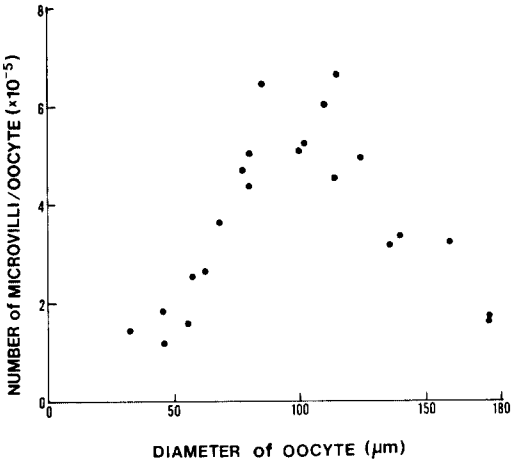

Fig. 8. Change in the number of microvilli. The number was estimated from the transmission electrn micrographs.

We found that the total number increases exponentially from 4.7 x 10^5 at 34 μm stage to 10.2 x 10^6 at the 186 μm stage, indicating that new projections are continuously made with the progress of oogenesis (Fig. 7). However, an increase in the surface area may be greater than the production of projections, since the density decreases with oogenesis from 147 projections per μm^2 at the 45 μm stage to 93 at the 186 μm stage.

The number of the projections was also estimated from the transmission electron micrographs. For this estimation the projection was counted from the electron micrograph equivalent to 1 μm long oocyte surface. The number was squared to get the number of projections in 1 μm^2 surface and then multiplied by the total surface area. The estimations showed that the number of projections increases exponentially from 3.2 x 10^5 at the 32 μm stage to 5.9 x 10^6 at the 186 μm stage (Fig. 7). These data obviously follow the trend obtained from the scanning electron micrographs except that the number of projections at each stage was underestimated in the transmission electron micrographs.

The number of microvilli was estimated for the oocytes of each stage from the transmission electron micrographs by similar calculation. The estimations showed that the number of the microvilli per oocyte increases from 1.2 x 10^5 at the 32

μm stage to a maximum of 5.0-6.7 x 10^5 at 80-115 μm stages when vitellogenesis takes place most actively and then decreases to 1.7 x 10^5 at the 176-186 μm stage (Fig. 8). Even though the estimation of microvilli might have been underestimated as well, presumably the overall profile in the change might be true. The projections may exceed the microvilli approximately 3-fold in number, because microvilli are branched at the tips. The ratio of projections to the microvilli is below 4 at the stages prior to the 100 μm stage, but increases remarkably thereafter to 35 at 186 μm stage, indicating that the microvilli increases in parallel with the change in the number of projections during previtellogenesis, but gradually disappear during the late vitellogenesis along with the increase in the number of vesicles.

Vitelline Envelope

The vitelline envelope, which surrounds oocytes, filling the space among the microvilli, becomes thickened and structurally changes during oogenesis (figs. 3,4,5 and 6). Because there are no accessory cells, this envelope is probably produced by the oocyte itself and, therefore, technically is a vitelline envelope. However, to conform with common usage. it will be referred to as chorion here. The thickness of the chorion is only 0.21 μm at 34 μm stage and increases about 5-fold during the rest of the oogenetic period. The inner layer of chorion is separated from the plasma membrane by a previtelline space. During previtellogenesis up to the 55 μm stage, the chorion consists of outer, intermediate and inner layers, among which the former two layers are constant in thickness throughout oogenesis. However, the inner layer, which is only one layer up to the 55 μm stage, greatly increases in thickness and becomes distinctly bipartite at the stages later than 80 μm stage. The filaments of I_2 are sometimes seen as extended fibers between the microvilli, as in Fig. 5A and B, and sometimes appear to surround to the microvilli where these filaments are cut in cross section (Fig. 6). These patterns indicate that the filaments of layer I_2 are arrayed concentrically around the stem of the microvilli. Filaments aligned parallel to the cell surface were observed in the inner layer, in which the structures are more densely packed in the upper layer than in the lower layer.

Discussion

The studies demonstrate that the surfaces of the oocytes including microvilli with glycocalyx structures and chorion change greatly in structure during oogenesis. Microvilli, which change most prominently at the oocyte surfaces of *Pseudopotamilla*, have been usually observed in the oocytes of various species of polychaete (Franklin 1966; Huebner and Anderson 1976; Eckelbarger and Grassle 1982; Eckelbarger 1984). The structures were also observed in the oocytes of many other non-polychaete species including *Urechis*, ascidian, fish, frog, and mite, indicating that microvilli are general structures occurring at the surfaces of oocytes (Gould-Somero and Holland, 1975; Reger, 1977; Hosokawa, 1985; Pinto *et al.*, 1985). However, structural changes of the microvilli throughout the whole period of oogenesis have been studied only in *Urechis* (Gouls-Somero and Holland, 1975). The microvilli were usually interpreted to be a route for transporting substances from the outside into oocytes in most of polychaete species examined, regardless of whether the species shows endocytotic activity or not during oogenesis (Dhainaut, 1967, 1970, 1976; King *et al.*, 1969; Potswald, 1972; Eckelbarger, 1976, 1980; Eckelbarger and Grassle, 1982). IN *Pseudopotamilla*, microvilli were found to be quantitatively changed during oogenesis, reaching a miximum at the most active vitellogenic stages, suggesting that microvilli may be correlated with the uptake of yolk precursor proteins.

Glycocalyx structures invested at the tips or at the peripheral sides of the vesicles did not show any change in shape and size but in abundance. These structures were observed in the oocytes of *Sabellaria* (Franklin, 1966), *Capitella* (Eckelbarger and Grassle, 1982) and *Urechis* (Gould-Somero and Holland, 1975). Change in density of the glycocalyx structures, which were found to be indigestable with trypsin in *Schizobranchia* (Lee, unpublished data) and identified to be a polysaccharide in mite (Reger, 1977), seems to be significant in the function of the microvilli.

A semi-quantitative estimation of the projections and microvilli showed that the vesicles including the tips of microvilli almost linearly increase along with the progress of oogenesis, even though the number of microvilli do not follow in accordance with the vesicles. Microvilli actually declines toward the final stage of oogenesis. This suggests that microvilli continue to be formed and withdrawn at the late stage of oogenesis, leaving the vesicles at the surface.

Structural changes at the surface of oocytes during oogenesis was difficult to correlate to a function yet, but the changes appear to be significant in exploring the role of the vitelline envelope.

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안점의 꽃갯지렁이 *Pseudopotamilla occelata* Moore의 난모세포 표면의 미세구조적 변화

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안점의 꽃갯지렁이(*Pseudopotamilla occelata*) 난모세포의 표면의 구조적 변화를 전자현미경으로 연구하였다. 일단 난소로부터 방출되어나온 직경 5 μm정도의 난모세포가 동일한 체강액에서 직경 185 μm까지 성장하는 난자형성중 난모세포의 난황막은 구조적으로 변하였다. 미융모(microvilli)는 구조, 수량 및 행동에 변화를 보였다. 전난황형성기에 미융모는 길이가 짧고 두갈래로 갈라져 있었다. 난자형성이 진행됨에따라 미융모의 길이는 계속 증가하는 반면 수는 전난황형성기까지만 증가하고 난황형성기에는 점차 감소하는 경향을 보였다. 미융모 끝에 Glycocalyx 구조가 전난황형성초기에서부터 형성되는 것이 보이는데, 이 구조는 난모세포의 성장과 함께 수적으로 점차 증가하다가 마지막 단계에서는 일정하게 유지되었다.

난자형성 말기에 미융모의 수축과 동시에 미융모의 끝이 줄기로부터 분리되면서 조그만 소낭이 형성되어 난황막 표면에 남게되었다. 난황막은 처음에는 외층(outer layer), 중간층(intermediate layer) 및 내층(inner layer)의 3층으로 되어있었으나, 외층과 중간층은 난자형성 전기간을 통하여 구조적으로 거의 일정하지만, 내층은 그 두께가 두터워지고 80 μm의 난모세포에서부터는 두개의 층으로 다시 나뉘어지는 것을 볼 수 있었다. 이와같은 구조적인 변화는 아마도 난모세포의 표면이 비록 동일한 환경에서 일지라도 단계에따라 기능적으로 다르게 분화한 결과로 생각되었다.

Figure Legends

Fig. 1. Scanning electron micrographs of the surfaces of previtellogenic oocytes of 34 μm (A), 46 μm (B), 60 μm (C), and 80 μm in diameter (D). Projections are irregular in size, shape and distribution. Scale bar 1 μm.

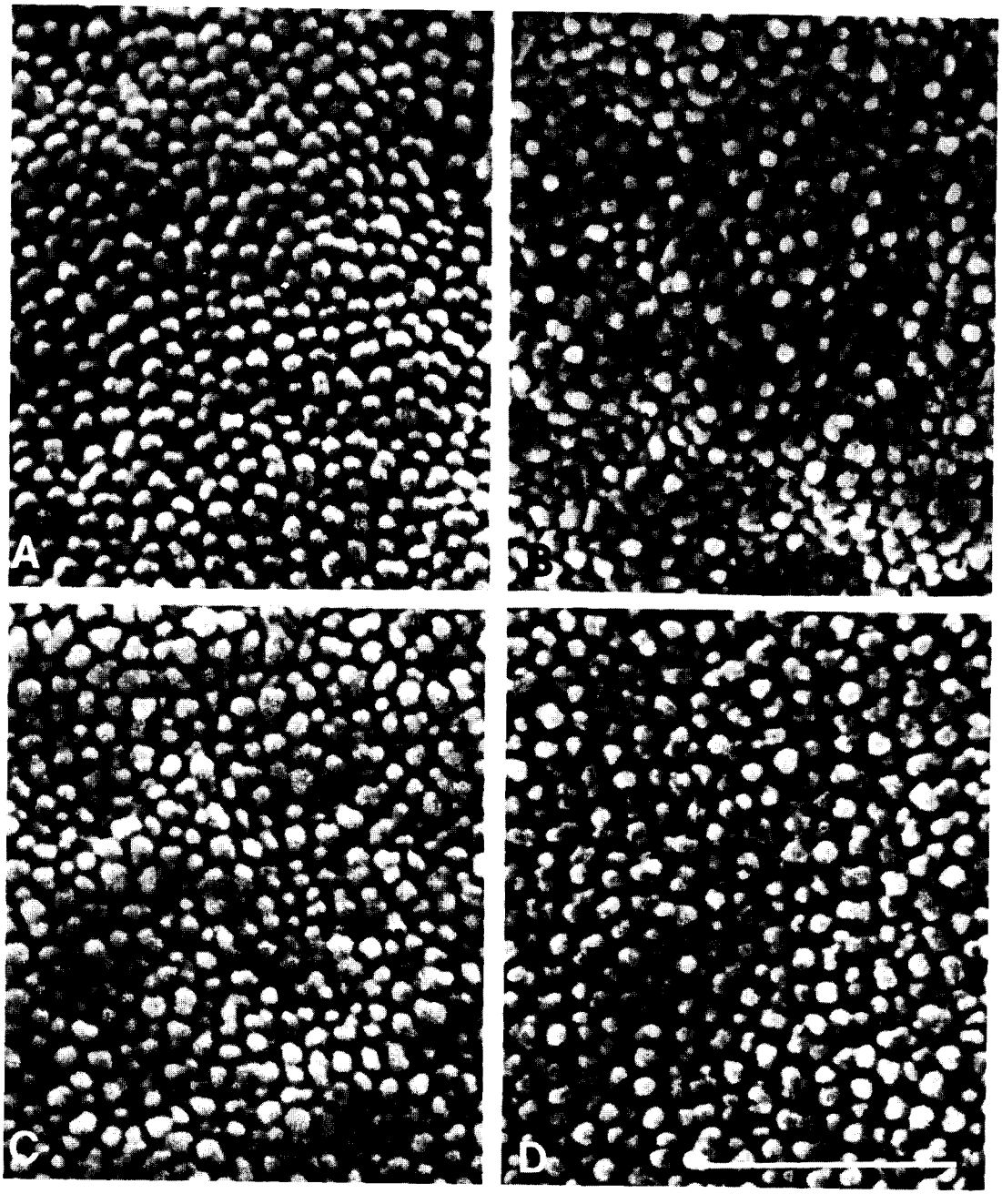
Fig. 2. Scanning electron micrographs of the surfaces of oocytes during vitellogenesis with diameters of 115 μm (A), 146 μm (B), 164 μm (C) and 186 μm (D). Projections are irregular in A, but become consistent and regular in size and shape in B, C, and D. Scale bar 1 μm.

Fig. 3. Transmission electron micrographs of the surfaces of oocytes of 32 μm (A) and 55 μm in diameter (B). Microvilli (Mv) are surrounded with plasma membrane and bifurcated into branches of different lengths. At the tips of microvilli glycocalyx structures begin in form (arrowhead). Chorion (C) consists of outer (O), intemediate (M) and inner layers (I) and perivitelline space(PVS). Scale bar 1 μm.

Fig. 4. Transmission electron micrographs of the surfaces of oocytes of 80 μm (A) and 100 μm in diameter (B). The glycocalyx structures increases in abundance but remain constant in size (arrowhead). The inner layer of chorion becomes bipartited into two sublayers (I_1 and I_2) at the 100 μm stage. Scale bar 0.5 μm.

Fig. 5. Transmission electron micrographs of the surfaces of oocytes of 115 μm (A) and 160 μm in diameter (B). During this vitellogenic period the glycocalyx structures become abundant. The two inner sublayers increase greatly in the thickness. Isolated tips of microvilli remain as vesicles (V) with disappearance of some microvillar stems. Filaments of I_2 surrounding the micriovilli are indicated by arrowhead. Scale bar 0.5 μm.

Fig. 6. Transmission electron micrograph of 186 μm in diameter, the final stage of oogenesis. Fragments of microvilli are observed with the vesicles and glycocalyx structures. Scale bar 0.5 μm.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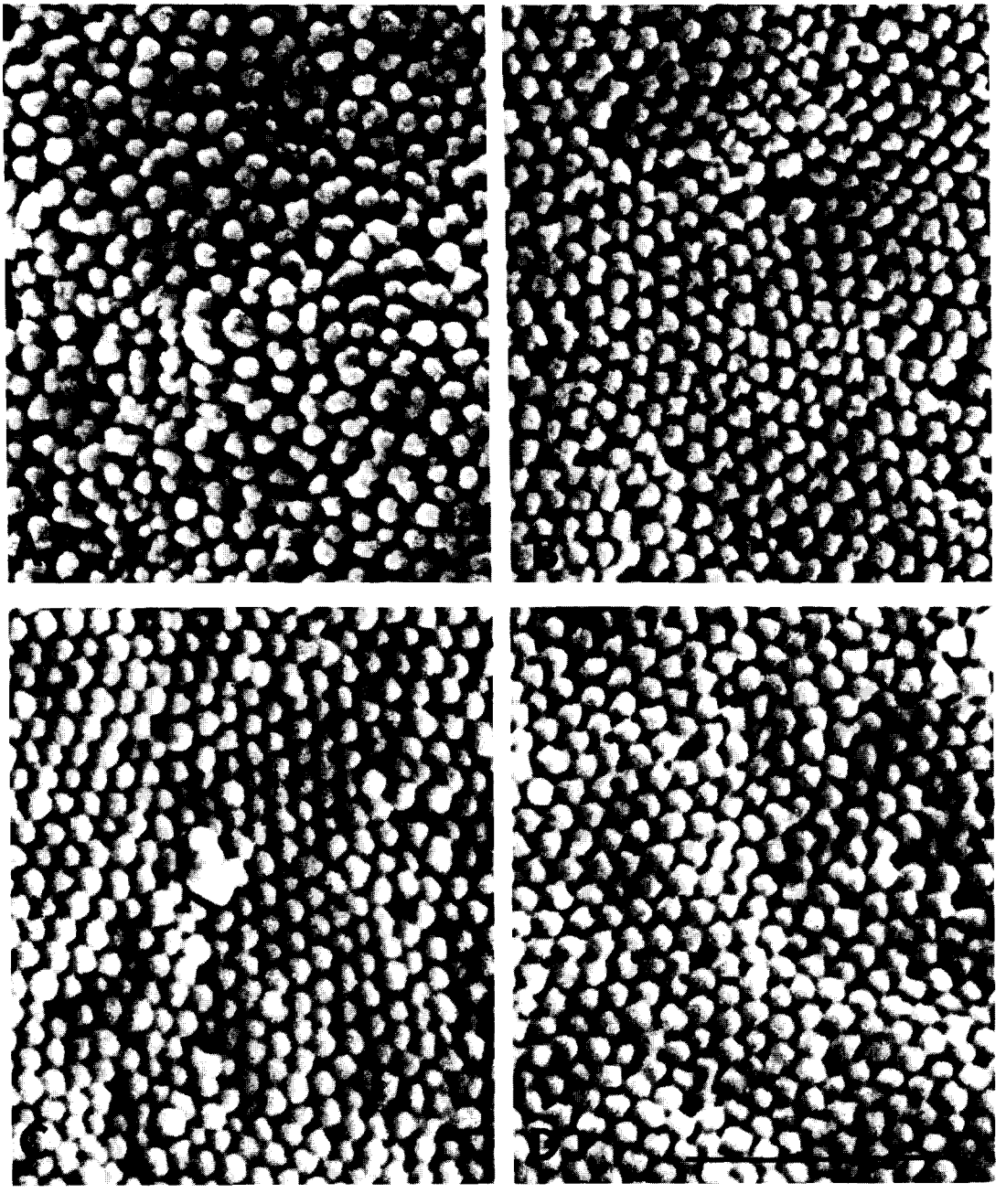

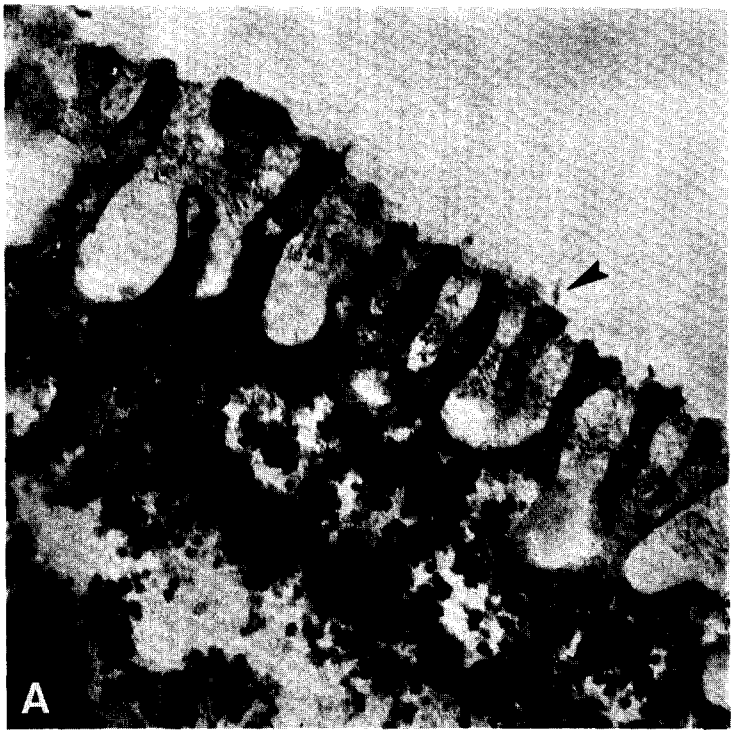
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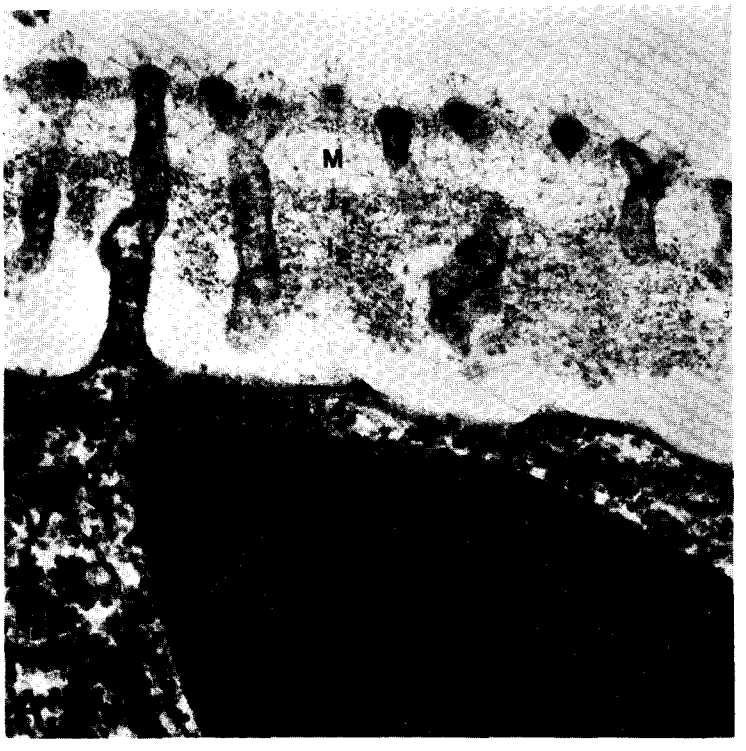
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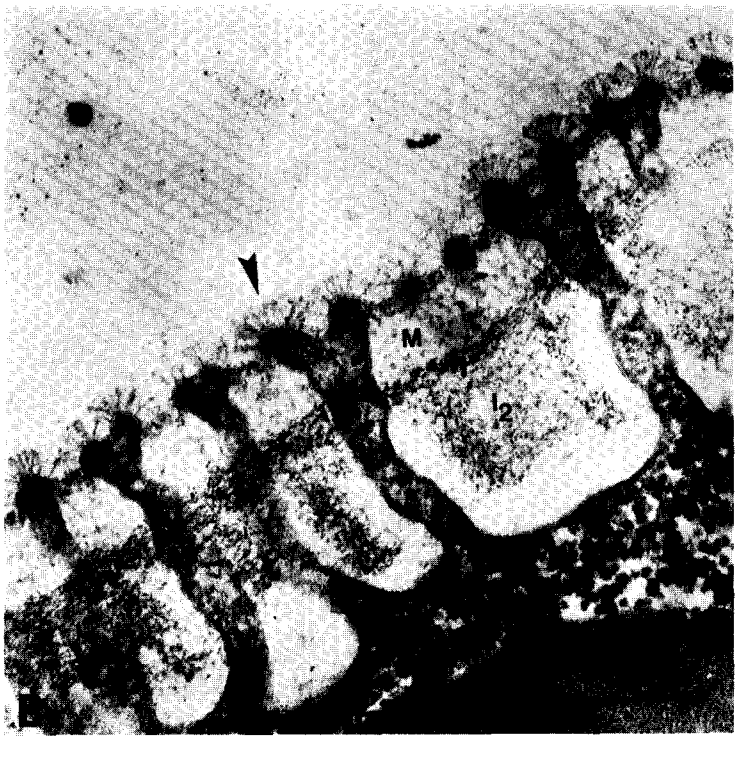
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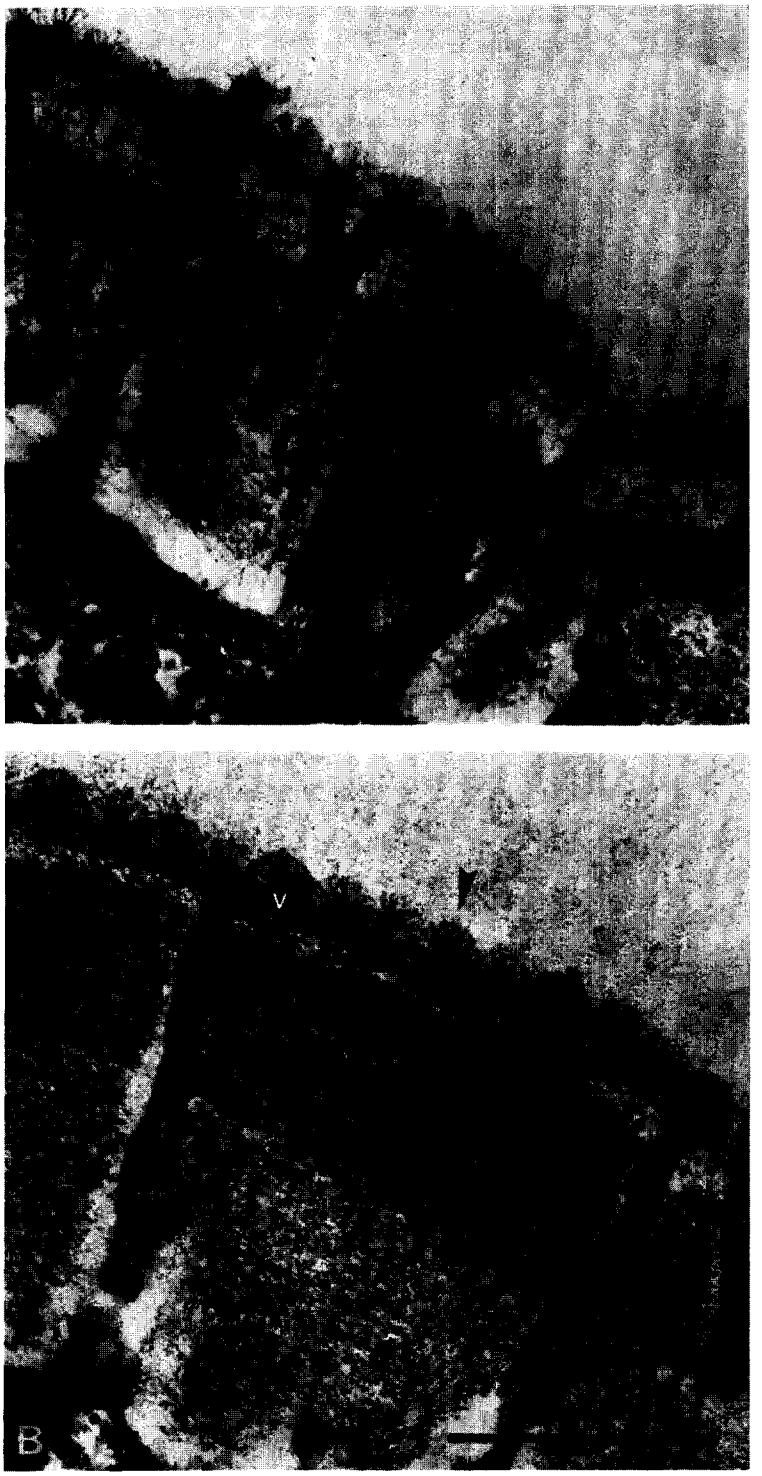
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B