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Fine Structure of Pericanalicular Cytoplasm of Taurocholic Acid-treated Rat Liver as Revealed by Deep Etching with Rapid Freezing

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Taurocholic acid 투여 흰쥐 담세관주위세포질의 미세구조에 관한 급속동결 deep etching법에 의한 연구

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요 약

본 연구에서는 급속동결 deep etching법을 이용하여 흰쥐에서 담세관주위세포질에 있는 미세사와 용해소체 및 소포등 세포내소기관들이 어떻게 담즙분비에 관여하는지를 알아 보고자 하였다. Taurocholic acid를 투여한 군은 정상군에 있어서와 같이 미세사가 담세관 주위를 둘러싸면서 담세관 원형질막과 다른 세포내소기관에 부착되어 있었다. 이들 담세관 주위의 미세사들은 미세융모속에서 다발을 이루고 있었으며 담세관 둘레에서는 관주위직을 형성하면서 연접복합체에 결합되어 있었다. 미세사는 또한 담세관 원형질막과 소포사이에 걸쳐있는 것도 관찰되었다.

이상의 소견으로 미루어 미세사는 담세관에서의 담즙의 분비를 돕는 소기관임을 알 수 있는데 특히 담세관에 융합할 수 있도록 소포의 전위를 유도하는 기구로서 작용할 것으로 추정된다.

Key words : Pericanalicular cytoplasm, Golgi apparatus, Vesicle, Microfilament, Liver, Rat, Deep etching, Rapid freezing

INTRODUCTION

Although transport pathways of bile acid are unclear at present, a vesicular transport theory has been postulated (Albert *et al*., 1980; Richard *et al*., 1980).

Recent studies have demonstrated that the vesicular traffic from the Golgi apparatus to the pericanalicular region is enhanced by bile acids (Hayakawa *et al*., 1990; Crawford *et al*., 1991), and that bile salts may stimulate the movement of vesicles towards the bile canaliculi (Dubin *et al*., 1980; Boyer and Meier, 1990; Reynier *et al*., 1992; LeSage *et al*., 1993). The vesicles are in two types; coated and uncoated. The coated

vesicles are known to engaged in receptor mediated endocytosis (Pearse and Bretscher, 1981; Adams and Pollard, 1986), while uncoated vesicles observed in the pericanalicular cytoplasm are known to mediate exocytotic transport into the bile canaliculi (Oda *et al.*, 1974: Jones *et al.*, 1979; Gobhard, 1984). It has been suggested, however, there is no direct evidence for the endocytosis (LaRusso, 1984).

In the field of hepatology, the cytoskeleton has been focused in researches for recent years (French *et al.*, 1982; French and Davirs, 1975; Okanoue *et al.*, 1985). Microfilaments take place in various forms of movement in nonmuscle cells (Huxley, 1973). The pericanalicular cytoplasm has an elaborate network of microfilaments in the form of micrvillous core filaments and the pericanalicular web (Ishikawa, 1987; Naramoto *et al.*, 1990). The microfilaments preserve the structure of bile canaliculi (Naramoto *et al.*, 1990) and play a main role in the contraction of bile canaliculi (Oda *et al.*, 1974; Phillips *et al.*, 1975; Oshio and Phillips, 1981). Thus, those in the pericanalicular cytoplasm of hepatocytes are expected to participate in the processes of bile formation, such as transport of secretory vesicles, exocytosis, contraction of the canalicular walls, or perhaps reabsorption through the canalicular membranes.

The present study was attempted to investigate the organelles such as microfilaments, vesicles and bile canaliculi in the pericanalicular cytoplasm participating in the bile secretion.

MATERIALS AND METHODS

Albino rats (Sprague Dawley, male, 250~280 g) were used in the present study. The animals were divided into two groups: normal and experimental. The experimental group was that 10% dehydrocholic acid (DA, 0.05 mg/g body weight) was injected at 40 min prior to the sampling. The liver tissues were taken under sodium pentobarbital (Nembutal, 0.015 mg/g body weight) anesthesia at 3 hr after the last feeding. And then liver tissues were taken immediately after laparotomy.

For thin sections, the liver tissues were cut into small pieces and immediately immersed in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.3) for 2 hr at room temperature. The tissue pieces were postfixed with 1% osmium tetroxide in the same buffer for 2 hr at 4°C. The fixed tissues were dehydrated in a series of graded ethanol and embedded in Epon mixture. The fixed tissues were dehydrated in a series of graded ethanol and embedded in Epon mixture. The thin sections were cut on an ultramicrotome (LKB 2088), and stained with uranyl actate and lead citrate, and were observed by using transmission electron microscope (Hitachi H-600).

Rapid freezing method:

For freezing at the temperature of liquid helium, freezing device (RF-10, Eiko engineering Co) was used. Liver tissues directly mounted on the specimen stage were put on the top of the plunger. The tissues were frozen by dropping the plunger on to the cooper block cooled with liquid helium. The frozen tissues were lifted again together with the cooper block. The cooper block was replaced with a new one in every experiment.

Deep-Etching replication method:

Frozen tissues were put into narrow grooves on the specimen stage in a liquid nitrogen pool, and then put into the freeze fracture apparatus (FD-2S Eiko Engineering Co) with a carrier. The frozen tissues were immediately fractured perpendicularly to the setting position in vacuum

at 2×10^{-7} mmHg. For deep etching, fractured tissues were left in a high vacuum for a further 20~30 min, while the specimen temperature was raised to about −90°C. The etched surface of the tissue was shadowed by evaporation of platinum and carbon successively while the specimen was rotated at 0.5 rps. The shadowed tissues were immersed in a detergent containing NaClO. The replicas were separated from the tissues and washed three times with distilled water.

RESULTS

1. Thin sections

Both in normal and experimental groups, the bile canaliculi appeared as spaces between the adjacent hepatocytes and were provided with microvilli. The zonula occludens, zonula adherens and macula adherens were orderly arranged to form a junctional complex at both sides of bile canaliculi. The pericanalicular cytoplasm showed densely packed microfilaments. The microfilaments around the bile canaliculi extend into the microvilli to form a core of filaments, which were in contact with the inner leaflet of luminal plasma membrane of bile canaliculi. The organelles such as ER, Golgi apparatus, lysosomes and vesicles were observed in the vicinity of bile canaliculi. The cis Golgi cisterns or ER adjacent to the cis Golgi face were almost devoid of visible contents or contained only a few materials of low electron density. The cis Golgi cisterns usually faced toward the bile canaliculi. In the taurocholic acid-treated rats, the cis Golgi cisterns showed linear saccular fashions or buds (Fig. 1). The vesicles appeared to increase near the bile canaliculi and some of them fused to them. The vesicles were usually round, oval or short tubular in shape. They were coated and lerger uncoated ones.

2. Deep etching replicas

The deep-etching with rapid-freezing enables us to examine three-dimensional ultrastructures of pericanalicular cytoplasms. The pericanalicular filaments formed an undercoat of the canalicular membrane so as to encase the bile canaliculi. The bile canaliculi were observed between hepatocytes with microvilli protruding into the canalicular lumen. The microfilaments were especially rich in the canalicular microvilli and the pericanalicular web. Microfilaments in the microvilli form a core bundle originating from the tip of the microvilli (Fig. 2). They were observed as filaments running vertically along the long axis of microvilli. At the base of the microvilli, they extended vertically to the pericanalicular web but never went across the web. They appeared to turn occasionally to a horizontal direction, suggesting a continuation of some of the core filaments to the pericanalicular web. The microfilaments in meshwork patterns were directly attached to the cytoplasmic sides of cell membranes (Figs. 2, 3, 4, 5, 6). Around the junctional complex, a large number of fiaments ran perpendicular to the lateral plasma membrane, converging mainly to the zonula adherens and zonula occludens, whereas smaller number ran parallel with the membrane.

In the normal group, the ER and Golgi apparatus were located in the vicinity of bile canaliculi. In the experimental groups, however, the membranous organelles including vesicles were increased near the bile canaliculi (Figs. 1, 2, 3, 5, 6). The vesicles were empty and showed smooth surfaces and were similar to those seen in thin sections. A small number of microfilaments running perpendicular to the canalicular wall were found to link internally to the canali-

cular membrane and externally to the vesicles (Fig. 6). The pericanalicular microfilaments were also attached to the vesicular structurtes (Figs. 2, 3, 5, 6).

DISCUSSION

The pericanalicular microfilaments form mainly meshwork structures (Ishikawa, 1987; Naramoto *et al.*, 1990). The microfilaments are a dynamic structure, and elongation and shortening occur at their plus end under conditions that enhance assemling or disassembling actin monomers (Tilney *et al.*, 1981). Indeed, the circumferential bundle of the contractile intestinal brush border is known to contain bidirectionally arranged actin filaments (Hirokawa *et al.*, 1983). In addition, hepatocytes contain myosin near by the canalicular membrane as viewed with the immunofluorescent microscopy (Yasuura *et al.*, 1989), suggesting that myosin may be located between microfilaments of the pericanalicular web. In the hepatocytes, therefore, microfilament contractions probably induce the bile canalicular peristalsis and vesicle transport.

In the present study, the experimental group showed that the vesicles appeared to increase near the bile canaliculi and some of them fused to them. The vesicles were in two types; coated and uncoated. Recent morphological evidence has suggested the association of microfilaments with coated pits and vesicles (Salisbury *et al.*, 1980; Ishii *et al.*, 1991). However, the presence of the coat on the vesicles in this area indicates that they are engaged in endocytosis rather than exocytosis (Pearse and Brestcher, 1981; Naramoto, 1990), because coated vesicles shed their coat within seconds after being formed from coated pits (Pearse and Bretscher, 1981). But uncoated vesicles observed in the pericana-licular cytoplasm are known to mediate exocytotic transport into the bile canaliculi (Oda *et al.*, 1974: Jones *et al.*, 1979; Gobhard, 1984). The vesicles in the present study seemed to participate in the intracellular transport of bile acids is consistents with that suggested by previous observations (Shin, 1976, 1986, 1988). The vesi-cles were larger than coated ones, and were almost devoid of visible contents in the thin sections. It seems useful to consider that the transport process of bile constituents is as water secretion, since most of the bile constituents would be excreted as a water soluble glucuronide (Cook *et al.*, 1952; Schmid, 1956; Schmid *et al.*, 1958) or secreted in the conjugated form, which is water soluble. Studies by Lake *et al.* (1985) using fluid phase markers suggest that water may be released from vesicles into bile. The finding of vesicles were in agreement with the chemical property of bile. In the deep etch-ing replica, the vesicles were empty and showed smooth surfaces. This implies that the vesicles would be compatible with those seen in the thin sections. The transport of the vesicles is dependent on the integraty of microtubules (Kawahara and French, 1990). Thus the functional cooperation of microfilaments and microtubules might be necessary for the bile secretion (Stein *et al.*, 1974; Gregory *et al.*, 1978; Dubin *et al.*, 1980). The microfilaments observed in the present study were directly attached to other cytoskeleton and appeared to be installed to link to both the canalicular membrane and vesicles.

This may indicate that these specialized microfilaments participate in the translocation of vesicles in the pericanalicular cytoplasm, and support the view that vesicles from the Golgi apparatus and possibly vesicles of the SER are involved in the intracellular transport of bile acids before canalicular secretion.

ABSTRACT

To elucidate how microfilaments and vesicles participate in bile formation, the pericanalicular cytoplasms were observed in the liver of rats treated with taurocholic acid by deep etching with rapid freezing, and copmpared them with the findings on convensional thin sections.

The microfilaments were identified around the bile canaliculi in the forms of core filaments of microvilli, filaments of pericanalicular web running in parallel to the border of bile canaliculi, and filaments on the junctional complex. In taurocholic acid-treated rats, microfilaments could be visualized around the bile canaliculi and along their borders. The microfilaments appeared to be installed to link to both the canalicular membrane and vesicles. Such specialized microfilaments are considered to participate in the translocation of vesicles in the pericanalicular cytoplasm.

From the evidence, it is assumed that the microfilament induces the vesicles to transport and fuse to bile canaliculi into which bile acids is secreted by exocytosis.

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FIGURE LEGENDS

Fig. 1. Electron micrograph on the thin section shows portions of the hepatocyte from a rat after 20 min of taurocholic acid treatment. The microfilaments are localized around the bile canaliculi (BC). A vesicle (vacant arrow) still attached to the cis Golgi cistern (G) which faces toward the bile canaliculi. The vesicles (vacant arrowhead) are seen in the vicinity of bile canaliculi. A vesicle (vacant arrow) is fused to a bile canaliculus. M: mitochondria. Bar=1 μm

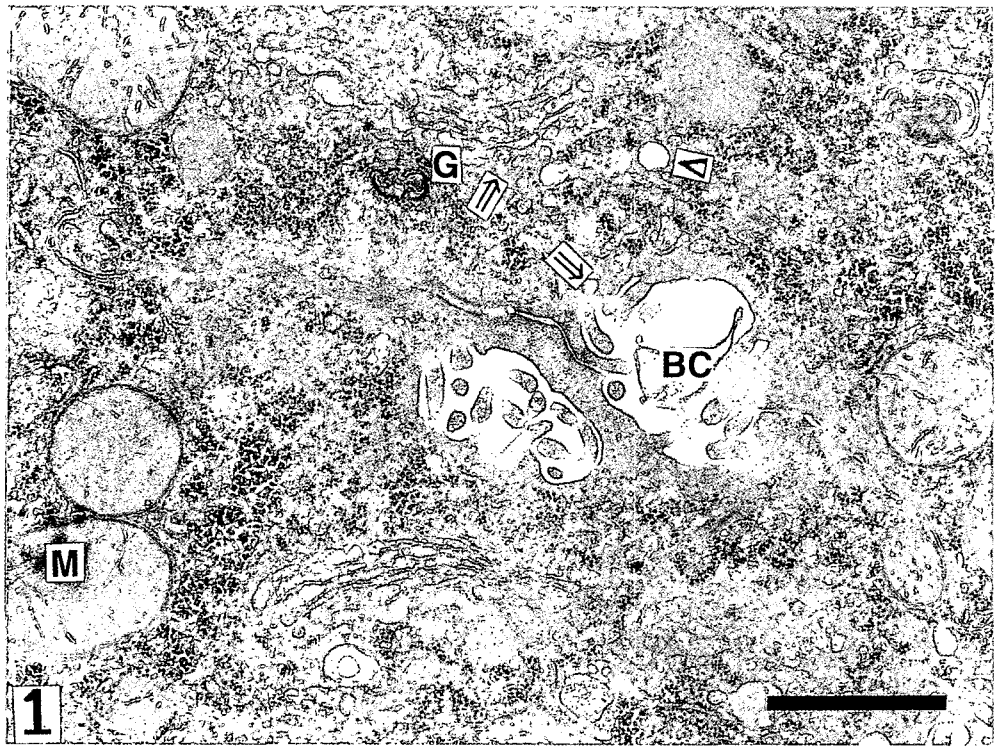
Fig. 2. Electron micrograph on the deep etching replica of hepatocytes from a rat after 20 min of taurocholic acid treatment. The cytosol is empty with vesicular remnants of organelles representing mostly mitochondria (M), vesicles (vacant arrowhead) and endoplasmic reticulum (ER). Meshwork of microfilaments are localized around the bile canaliculus (BC). Some of the microfilaments enter the microvilli to make the core filaments (arrowhead). Note that a close association is observed between microfilaments (arrow) and vesicles (vacant arrowhead). M: mitochondria. Bar=1 μm

Fig. 3. Electron micrograph on the deep etching replica of hepatocytes from a rat after 40 min of taurocholic acid treatment. Around the bile canaliculus (BC), a filamentous structure is observed, encasing it. Microfilaments (arrow) are directly attached to the cell membrane. Bar=1 μm

Fig. 4. Electron micrograph on the deep etching replica of hepatocytes from a rat after 20 min of taurocholic acid treatment. The mashwork of microfilaments are localized around the bile canaliculus (BC). Branching microfilaments (arrow) are attached to one another. They are also attached to the bile canalicular membranes and vesicles. A elongated vesicle (vacant arrow) is seen to be fused to canalicular membrane. Bar=0.5 μm

Fig. 5. Electron micrograph on the deep etching replica of hepatocytes from a rat after 20 min of taurocholic acid treatment. The microfilaments form a network around the bile canaliculus (BC). Vesicles appear to increase around the bile canaliculus Close association is observed between the microfilaments (arrow) and vesicles (vacant arrowhead). A vesicle (vacant arrow) is fused to the bile canaliculus. Bar=0.5 μm

Fig. 6. Electron micrograph on the deep etching replica of hepatocytes from a rat after 40 min of taurocholic acid treatment. The microfilaments run so as to encase the bile canaliculus (BC). Note that sporadic microfilaments (arrow) are found in association with vesicles on one side and with the canalicular membrane on the other side. Bar=0.5 μm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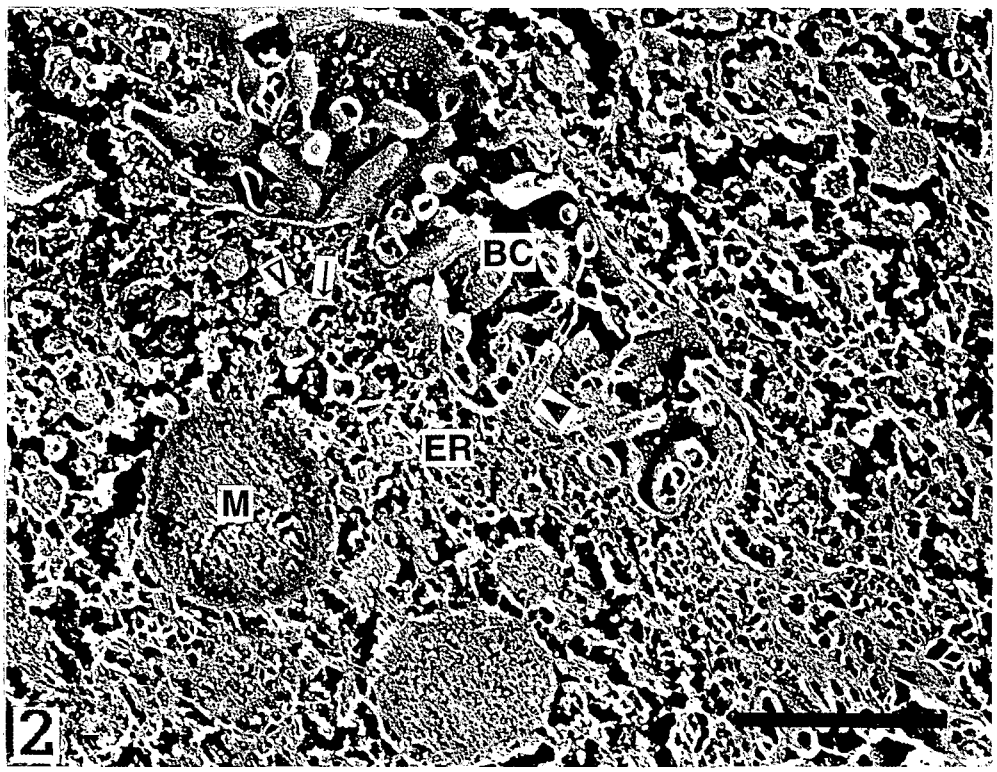
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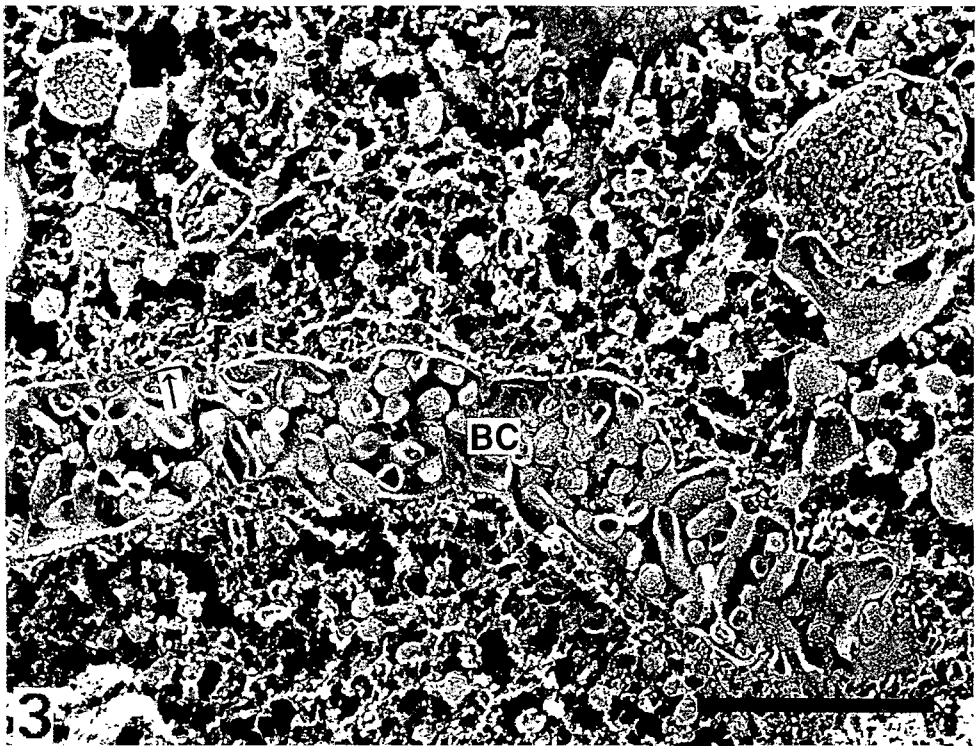
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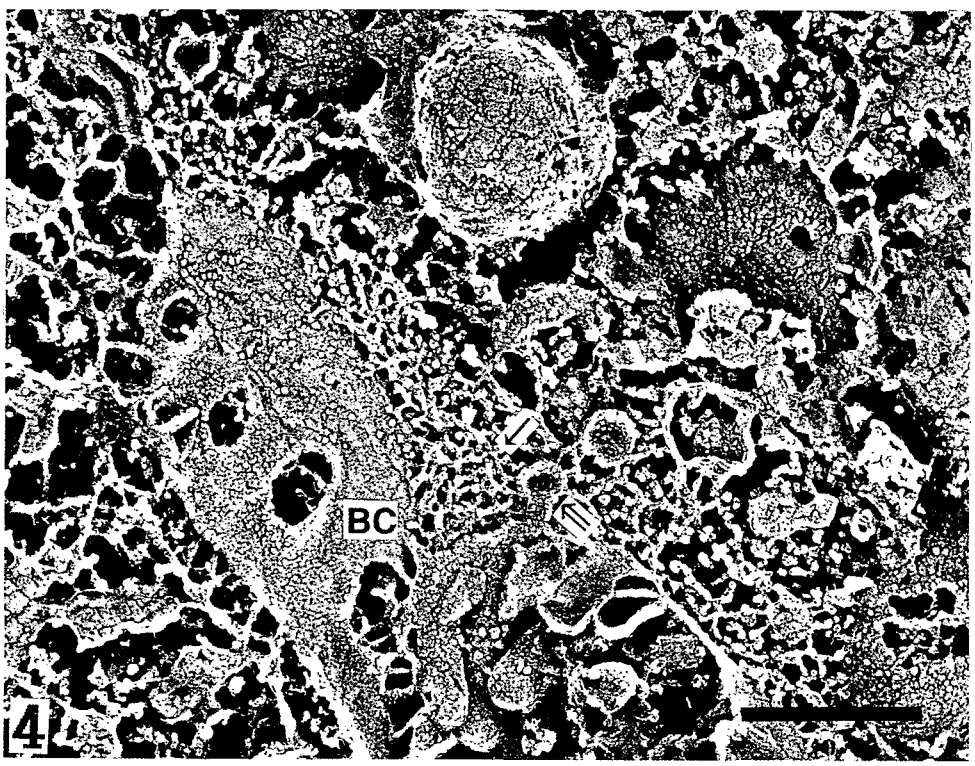
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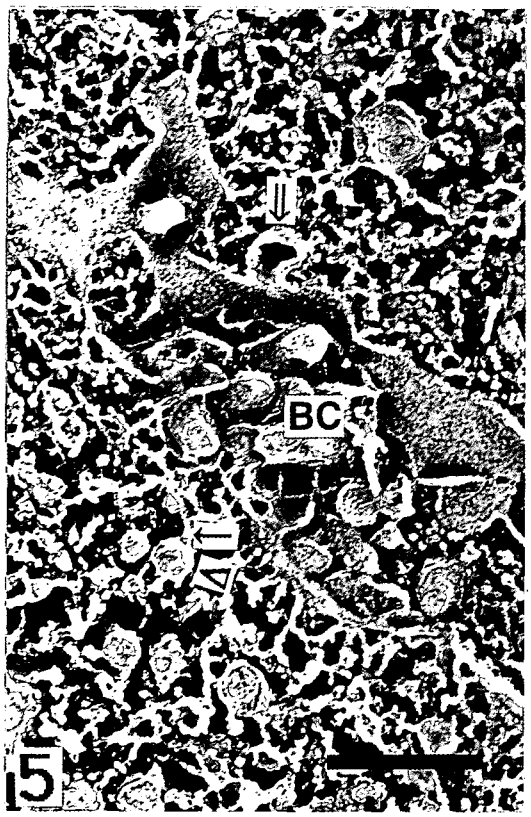
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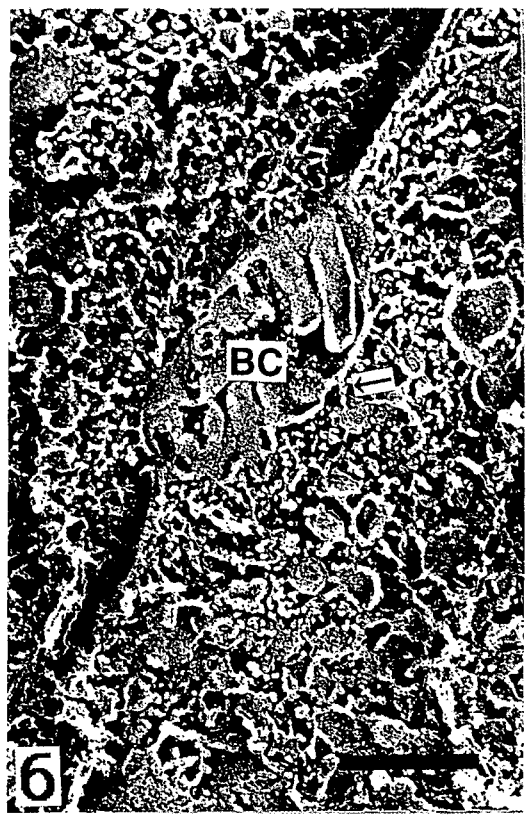
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