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A Scanning Electron Microscopic Study on the Sinusoidal Fenestrations in the Hepatic Lobule of Normal Rat

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간소엽내 동모양혈관내피창에 관한 주사전자현미경적 연구

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적 요

간소엽에 있는 동모양혈관내피창은 혈관과 간세포사이의 물질교환에 중요한 역할을 하는 부위로 알려져 있다. 그러나 이들 내피창에 관해서는 아직도 검토되어야 할 문제가 많이 남아 있다.

본 연구에서는 동모양혈관내피창 중 특히 큰창의 실재 여부와 창의 소엽내 분포상을 관찰하고 창의 크기와 분포상을 기초로 하여 내피창의 유형과 분포상을 재검토하고자 하였다.

실험동물은 식이조건을 일정하게 하기 위하여 최종 식이 투여 3시간 후에 관류고정하였으며 식에 따라 제작된 모든 조직표본은 주사전자현미경으로 관찰하였다.

그 결과 동모양혈관의 내면에서 여러가지 크기의 내피창이 관찰되었는데 이들은 작은창, 중간창 및 큰창의 3가지 유형으로 구분되었다.

작은 창은 낱개로 나타나거나 여러 개가 모여서 나타났으며 중간창은 낱개로 또는 작은창에 섞여서 나타났다. 큰창도 낱개로 나타나거나 작은창과 중간창에 둘러싸여 나타났다. 특히 중간창은 소엽의 중심대에 많이 분포하는 경향이 있어 새로운 유형으로 분류하였다. 큰창은 다른 유형의 창과 마찬가지로 그 윤곽이 평탄하였으며 세포질에 의해 분할된 상도 관찰되었으나 파열된 상은 찾아 볼 수 없었기 때문에 인공산물이 아닐 것으로 추정하였다.

소엽은 창의 분포에 따라 주변대, 중간대 및 중심대로 구분되었다. 주변대에서는 작은창만이 보이는 동모양혈관이 많이 관찰되었다. 중심대에서는 중간창과 작은창이 보이는 동모양혈관이 많이 관찰되었다. 중간대에서는 큰창과 중간창및 작은창이 다 같이 보이는 동모양혈관이 많이 관찰되었다.

이상의 소견으로 미루어 동모양혈관내피창은 소엽의 주변대에서 중간대로 가면서 점차적으로 커지다가 중간대에서 중심대로 가면서 작아지는 경향을 보이지만 중심대의 창은 주변대의 것에 비하면 큰 편이다.

Key words : Sinusoidal fenestration, Hepatic lobule, Rat

INTRODUCTION

Hepatic sinusoids have been studied by many investigators, because they are important sites where the materials are exchanged between the blood plasma and hepatocytes (Fawcett, 1955; Wassermann, 1958; Bennett, *et al.*, 1959; Wood,

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1963; Majno, 1965; Kuhn and Olivier, 1965; Burkel and Low, 1966; Papadimitriou and Walters, 1968; Schaffner and Popper, 1968; Ito and Shibasaki, 1968; Laschi and Casanova, 1968; Grubb and Jones, 1971; Wisse, 1972; Gemmel and Heath, 1972; Ito, 1973; Ogawa *et al.*, 1973).

From the scanning electron microscopic studies, the sinusoidal fenestration has been well demonstrated in the hepatic lobule of various animal species (Itoshima *et al.*, 1974; Muto, 1975; Nopanitaya and Grisham, 1975; Muto *et al.*, 1977). From these studies, the sinusoidal fenestration has been divided into two types; small and large, according to their sizes. However, Some investigators have denied the existence of the large fenestration (Wisse, 1970; Orci *et al.*, 1971; Brooks and Haggis, 1973; Eguchi *et al.*, 1976).

On the other hand, the data on the distribution of the fenestrations also differ from author to author (Ashworth and Sauders, 1960; Montesano and Nicolescu, 1978; Nopanitaya *et al.*, 1979; Vidal-Vanaclocha and Baebera-Guillem, 1985).

However, since the pattern of distribution of the sinusoidal fenestration is associated with its size, it is considered that the types and pattern of distribution of the fenestrations should be revaluated.

Thus this study was designed to examine the morphology and distribution of the sinusoidal fenestrations in the hepatic lobule of the normal rat.

MATERIALS AND METHODS

Normal albino rats (Wistar, male, 250~280 g) were used for this study. At 3 hr after the last feeding, the animals were anesthetized by intraperitoneal injection of sodium pentobarbital (Nembutal 0.015 g/g body weight) and perfused with Ringer solution through the heart under pressure of 120 cm H_2O for 2 hr followed by perfusion with 2% glutaraldehyde solution buffered with sodium cacodylate (pH 7.3) for 10 min. The inferior vena cava was widely opened at the beginning of the perfusion. After perfusion, the liver was removed and cut into small blocks. The tissue blocks were kept in the same fixative containing 2% tannic acid solution at 4 °C for 6 hr, and then post-fixed with 2% aquaous osmium tetroxide at 4 °C for 6 hr. They were rinsed in the distilled water for 12 hr, dehydrated in a series of graded ethanol, and substituted by isoamyl acetate. Then they were dried at the critical point in CO_2 and cracked. Finally the samples were sputtered with gold palladium in a vacuum evaporator (HUS-OGB) and observed by using the scanning electron microscope (S-800, Hitachi).

The liver lobule and sinusoids were photographed at low (×1500~2000) and high (×6000~8000) magnifications. The photographs were enlarged on printing papers at a final magnification. Low magnification electron micrographs were used for recording the location of sinusoids with respect to the zonation which define the hepatic lobule. The high magnification electron micrographs were used for the determination of diameter and number of each type of fenestrations in the given area of sinusoids. The sinusoids photographed has been clearly identified with respect to their locations in the area of lobule as compared to those in the low magnification pictures.

RESULTS

In the view of low power, the sinusoid appe-

ared as a space between the liver cell plates which were one cell in thickness, branched and anastomosed (Fig. 1) The inner surface of the sinusoid showed various size fenestrations. These fenestrations were usually round or oval in shapes. And they showed smooth edges. From the measurements, the sinusoidal fenestrations in the hepatic lobule of normal rats were divided into three types; small, medium-sized and large (Table 1). The small fenestrations appeared to be individually or gathered into clusters (Figs. 2 and 3).

The medium-sized fenestrations were either individually organized or in the cluster of small fenestrations (Figs. 4 and 5). The large fenestrations appeared to be either individually or accompanied by small or medium-sized fenestrations. They were also seen to be partitioned into two parts by slender cytoplasmic strands. However, neither interruption of cytoplasmic strands nor tearing of the fenestrations were encountered (Figs. 6 and 7). Through the large fenestrations, microvilli of the hepatocytes or collagen fibrils in the space of Disse could be observed.

From the distribution pattern of the sinusoidal fenestrations, the hepatic lobule could be divided into three zones; peripheral, intermediate and central (Tables 2 and 3). The sinusoid showing only small fenestrations (type I) were predominant (Figs. 2 and 3) in the peripheral zone, although the small fenestrations were distributed throughout the entire lobule. In the intermediate zone, the sinusoid providing with large fenestrations (type III) were abundant (Figs. 4 and 5). In the central zone, the sinusoids having the medium-sized fenestrations (type II) were frequently seen (Figs. 6 and 7). Based on the type of sinusoid, the zonation within the lobule was determined. The central and peripheral zones were 5~6 cells in thickness. Therefore the intermediate zone was large and the sinusoids observed in this zone were much more than other zones (Tables 2 and 3).

The fenestrations were not observed on the inner surface of the central vein and tributaries of the portal vein (Fig. 1).

Table 1. Diameter range of each type of fenestration observed in the hepatic lobule (μm).

Fenestrations / Diameter range	small	medium	large
	0.01~0.10	0.11~0.90	1.0~2.0

Table 2. Number of each type of sinusoid observed in each zone of the hepatic lobule.

Type of sinusoid / Zones	I	II	III	Total
Peripheral	107	34	17	158
Intermediate	24	35	112	171
Central	21	101	27	149
Total	152	170	156	478

Remarks:
I : type of sinusoid provided with only small fenestrations
II : type of sinusoid provided with small and medium-sized fenestrations
III : type of sinusoid provided with small, medium-sized and large fenestrations

Table 3. Proportion for each type of sinusoid in each zone of the hepatic lobule(%).

Sinusoids / Zones	I	II	III	Total
Peripheral	67.72	21.52	10.76	100
Intermediate	14.03	20.47	65.50	100
Central	14.10	67.78	18.12	100

DISCUSSION

Many investigators have divided the fenestrations into two types; small and large, according to their sizes (Muto *et al.*, 1977; Vonnahme and Müller, 1981). The morphology of sinusoidal fenestrations observed in this experiment was

similar to that described by previous investigators. However, the fenestrations could be divided into three types; small, medium-sized and large, based on the size and distribution pattern of the fenestrations.

The problem on the existence of large fenestration still remains to be settled.

Some investigators denied the existence of the large fenestrations (Wisse, 1970; Brooks and Haggies, 1973; Frenzel *et al.*, 1976) from the presence or ruptured cytoplasmic strands which subdivide the large fenestrations. The rupture of the cytoplasmic strands would be caused by hydrostatic pressure applied during perfusion fixation and the tearing of fenestrations would be resulted. However, many investigators have reported that the small and large fenestrations coexist (Itoshima *et al.*, 1974; Muto, 1975; Grisham *et al.*, 1975). Some others have suggested that the large fenestrations may be resulted from the physiological fusion of the small ones (Montesano and Nicolescu, 1978). Motta and Porter (1974), and Muto (1975) have reported that the large fenestrations measured from 1 to 2 μm in diameter. Another SEM study of perfusion fixed rat liver have shown that the large fenestrations ranging from 1 to 3 μm were present in the endothelial lining cells of the sinusoid. From the freeze fracture replica of perfusion fixed rat liver, Montesano and Nicolescu (1978) have described that the large fenestrations were more than 600 nm in diameters. The difference in the size of large fenestrations may be due to the physiological states of the liver, sampling techniques or any reasons that the large fenestration was excluded as an artifact.

From this study, cytoplasmic strands appeared to be attenuated. However, large fenestrations were less than 2.0 μm in diameter and neither interruption of cytoplasmic strands nor images showing the tearing of large fenestrations were observed. Therefore, the perfusion fixation performed from this study would be adequate to preserve the sinusoidal fenestrations and the size of the large fenestrations might be kept as real ones.

The distribution pattern of the large fenestrations differ from author to author (Itoshima *et al.*, 1974; Grisham *et al.*, 1976; Muto *et al.*, 1977; Oikawa, 1979; Vonnahme and Müller, 1987), although the small fenestrations are widely distributed throughout the entire lobule. Muto *et al.* (1977) and Vonnahme and Müller (1981) have reported that the large fenestrations occurred at random in the hepatic lobule of human and monkey respectively. Oikawa (1979) has reported that the large fenestrations are distributed equally throughout the lobule in the mouse.

Itoshima *et al.* (1974) has described that the large fenestrations are distributed in the central area of the lobule in the guinea pig. Grisham *et al.* (1975) has demonstrated that the large fenestrations are predominant in the peripheral area in the lobule of the rat. Muto (1970) have reported that the large fenestrations appeared to be numerous in the intermediate area and only the small fenestrations occupied the narrow area adjacent to the central vein or portal canals in the normal rat liver. Vidal-Vanaclocha and Barbera-Guillem (1985) have reported that the fenestrations were larger around the central vein than in the periportal area.

The data mentioned above may indicated that the distribution pattern of the fenestration differ according to the animal species, feeding or physiological states (Kuhn and Olivier, 1965; Wisse, 1970; Motter and Porter, 1974; Nopanitaya and Grisham, 1975). However, our data were partly similar to those observed by Muto (1970) and Vidal-Vanaclocha and Barbera-Guillem (1985)

who used same animal species.

From this evidence, it is assumed that the distribution pattern does not differ in the same animal species and same feeding states.

SUMMARY

Rat liver sinusoids were observed by scanning electron microscopy. The sinusoids were provided with fenestrations which were divided into three types; small, medium-sized and large. The small fenestrations were usually gathered into clusters. The medium-sized fenestrations were either individually organized or in the cluster of small fenestrations. The large fenestrations were usually accompanied by small or medium-sized fenestrations. The lobule was divided into three zones; peripheral, intermediate and central, according to the distribution pattern of the fenestrations. The sinusoid providing with small fenestrations (type I) were predominant in the peripheral zone. The sinusoid showing medium-sized fenestrations (type II) were frequently observed in the central zone. The sinusoid having large fenestrations (type III) were abundant in the intermediate zone.

This evidence indicate that the sinusoidal fenestrations become larger toward the intermediate zone from the peripheral zone of the lobule and progressively smaller toward the central zone. However, the fenestrations observed in the central zone seem to be larger than those seen in the peripheral zone of the lobule.

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FIGURE LEGENDS

Fig. 1. A portion of the hepatic lobule from normal rat. The hepatocytes (H) form one cell thick plates between which the sinusoids (double arrowheads) appear as branched and anastomosed grooves. C: central vein. P: branch of portal vein. Bar=4 μm

Fig. 2. A portion of the inner surface of sinusoid (type I) in the peripheral area of hepatic lobule (boxed area of A in Fig. 1). Small fenestrations (arrow) appear to be distributed, singly or gathered into clusters. Bar=1 μm

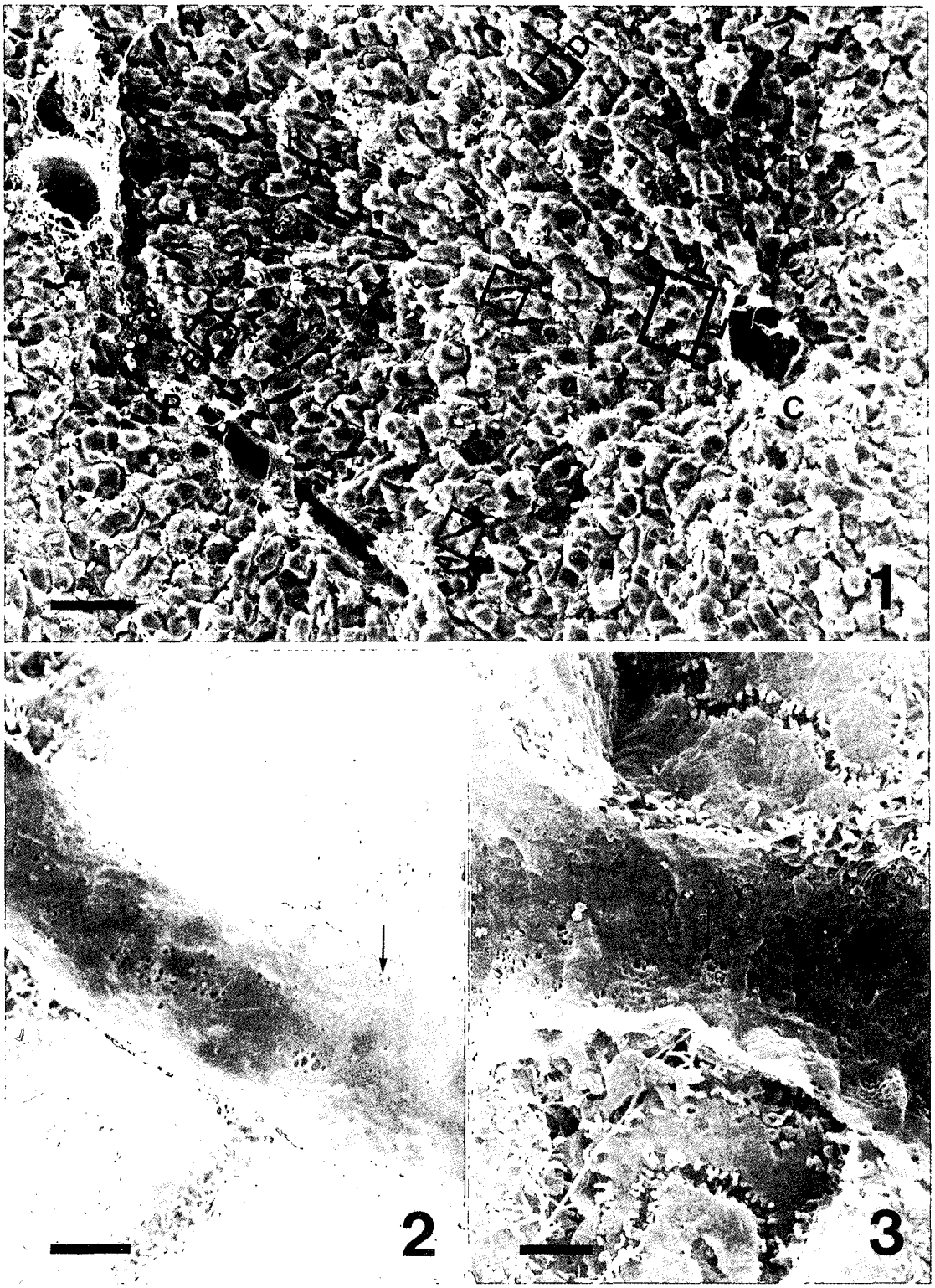
Fig. 3. A portion of the inner surface of sinusoid (type I) in the peripheral area of hepatic lobule (boxed area of B in Fig. 1). Small fenestrations (arrow) appear to be individually or gathered into clusters. Bar=1 μm

Fig, 4. A portion of the inner surface of sinusoid (type III) in the intermediate area of hepatic lobule (boxed area of C in Fig. 1). Large fenestrations (triple arrows) appear to be scattered. Throughout the large fenestrations, microvilli of the hepatocytes are seen. Small (arrow) and medium-sized (double arrows) fenestrations are also seen. Bar=1 μm

Fig. 5. A portion of the inner surface of sinusoid (type III) in the intermediate area of hepatic lobule (boxed area of D in Fig. 1). Small (arrow), medium-sized (double arrows) and large (triple arrows) fenestrations are seen. Bar=1 μm

Fig. 6. A portion of the inner surface of sinusoid (type II) in the central area of the lobule (boxed area E in Fig. 1). Medium-sized fenestrations (double arrows) are seen. Small fenestrations (arrow) appear to be individually or gathered into clusters. Bar=1 μm

Fig. 7. A portion of inner surface of sinusoid (type II) in the central area of the lobule (boxed area of F in Fig. 1). Medium-sized fenestrations (double arrows) are widely distributed. Small fenestrations (arrow) are gathered into clusters. Bar=1 μm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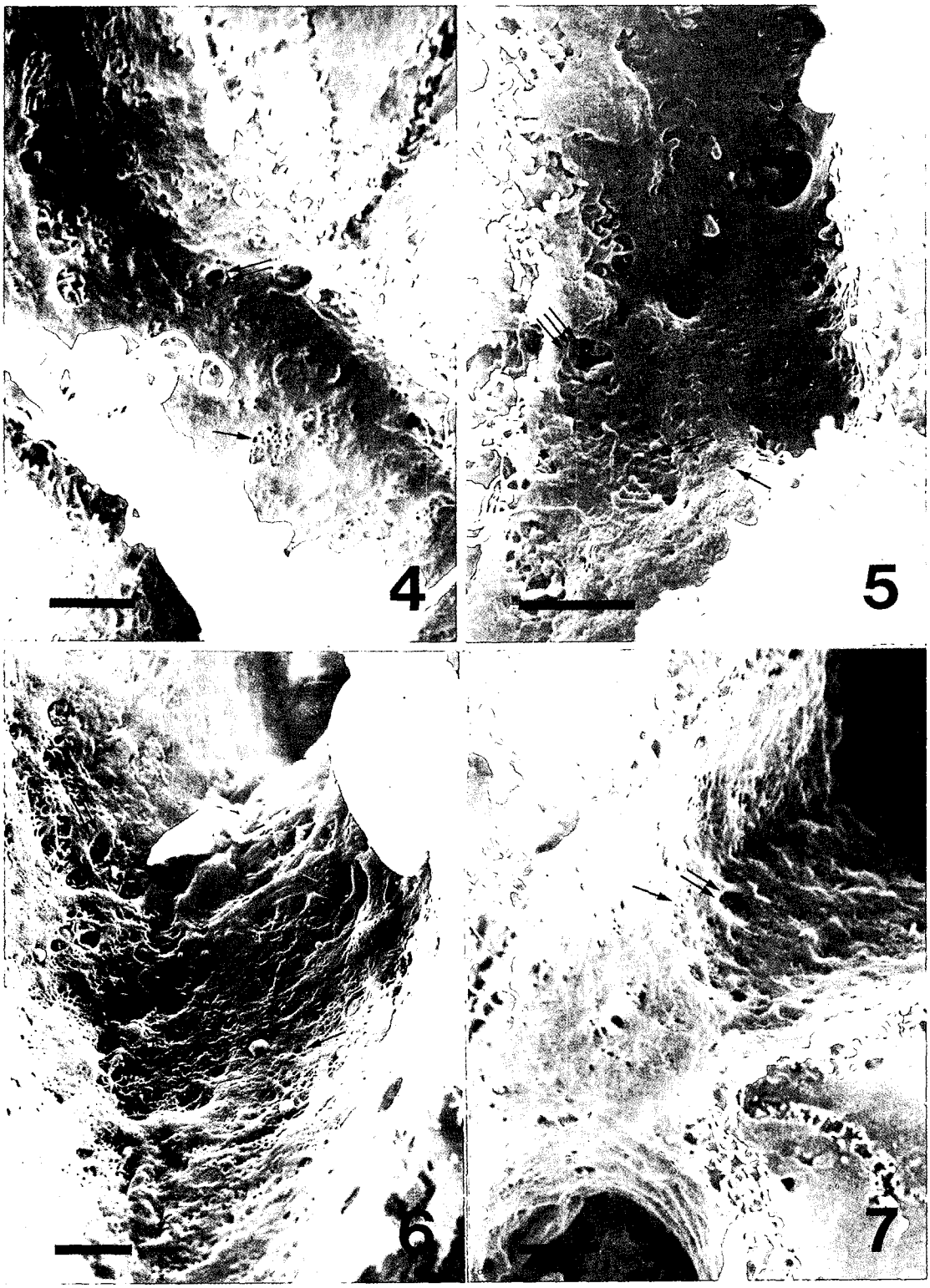
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