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Abstract

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KEYWORDS: ito cell, fat-storing cell, lithocholic acid, liver cell necrosis, scanning electron microscopy

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SCANNING ELECTRON MICROSCOPY OF ITO'S FAT-STORING CELLS IN THE RAT LIVER

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Abstract. The whole body including extended processes of Ito's fat-storing cells was observed by scanning electron microscopy in rat liver injured with lithocholic acid (LCA). Necrotic foci developed in the midlobular zone 48 h after LCA administration. Demonstration of Ito cell bodies around the foci was probably facilitated by easy detachment of hepatocytes from Ito cells. The body and the processes were located mainly between the sinusoidal endothelium and hepatocytes; sometimes they were between hepatocytes. Ito cells often were proximate to collagen fiber bundles and sometimes were attached to them. The cell body was flatly round or elliptic, 7 to 12 μm in diameter. Its surface was finely undulated with microvillous projections about 0.1 μm in length. Branching patterns of the processes resembled a fern-leaf mantling the sinusoidal endothelium. The trunks of the processes were about 2 μm in diameter and 20-30 μm in length. These processes tapered, branching into thinner processes, with the most peripheral being 0.1 μm in diameter. Ito cells and their branching processes likely strengthen sinusoidal walls and control blood flow in the sinusoids.

Key words : Ito cell, fat-storing cell, lithocholic acid, liver cell necrosis, scanning electron microscopy.

Ito cells located in the space of Disse, store fatty droplets and vitamin A. These cells are thought to be related to liver fibrosis (1-5). Ito cells are attenuated and have numerous thin processes which extend into the Space of Disse, which renders thin-section observation of the complete cell difficult (6-10). Ito cell processes protruding through the sinusoidal fenestrae have been observed by scanning electron microscopy (SEM) (11-13). SEM has revealed cell bodies with attenuated processes in human liver biopsies (14, 15). However, animal cells have not been observed in detail (16, 17). In this paper, SEM observation of rat Ito cells are described.

MATERIALS AND METHODS

Six Sprague-Dawley male rats weighing 200 g each were used. Lithocholic acid (LCA) (250 mg/100 g body weight) dissolved in 2 ml of liquid paraffin was administered through a stomach tube after 24 h of fasting. The liver was studied 1, 3, 6, 12, 24, and 48 h after the administration. Under ether anesthesia, heparin (500 units) was injected into the heart to prevent blood coagulation. A polyethylene tube (1.6 mm in diameter) was inserted into the thoracic aorta. The liver was perfused with 500 ml of Ringer solution, and perfusion-fixed with 100 ml of 1 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at a pressure of 150 mmHg. The liver was removed and cut into blocks ($1 \times 2 \times 3$ mm), which were fixed overnight in the glutaraldehyde solution. The blocks were stained by the revised tannin-osmium method (18), dehydrated in a graded series of ethanol, and dried in an HCP-1 critical point dryer (Hitachi, Japan) using liquid carbon dioxide. The dried specimens were fractured with a scalpel under a stereomicroscope (19), thinly sputter-coated with platinum-palladium in an ion coater (Eiko IB-3, Japan), and observed under SEM (JSM-U3, Japan) (accelerating voltage 15 kV). Some perfusion-fixed blocks were transferred into formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for light microscopic evaluation.

RESULTS

Light microscopy revealed round or elliptic necrotic foci (100 to 200 μ m in diameter) in the midlobular zone of the liver 48 h after LCA administration (20).

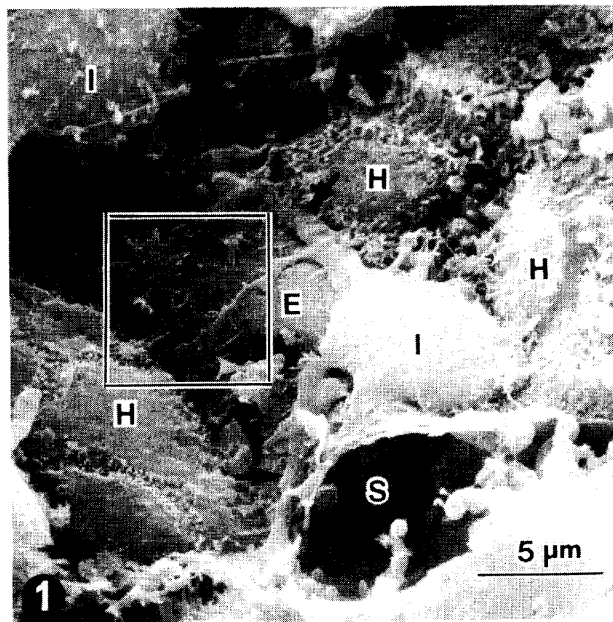


Fig. 1. The Ito cell in the center is attached to the perisinusoidal surface of the sinusoidal endothelium and has processes (arrows). The body of the Ito cell has a smooth surface with fine undulations. Another Ito cell is observed in the upper left corner. Abbreviations: I Ito cell; E endothelial cell; H hepatocyte; S sinusoidal lumen; B bile canaliculus. 48 h after LCA administration.

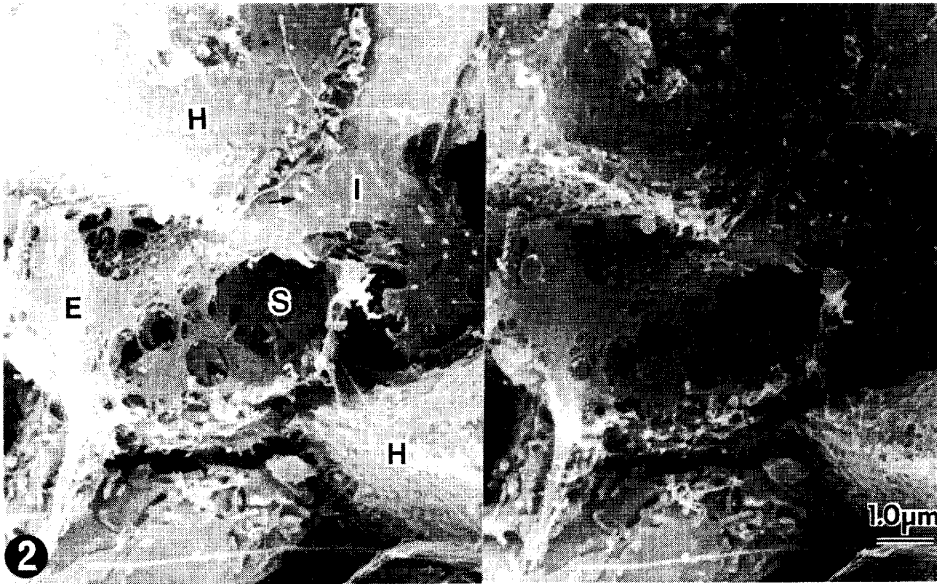


Fig. 2. A stereoscopic pair showing an Ito cell body and its processes mantling the sinusoidal endothelium. Thin microvillous projections (arrows) are scattered over the Ito cell. Abbreviations as in Fig. 1. 48 h after LCA administration.

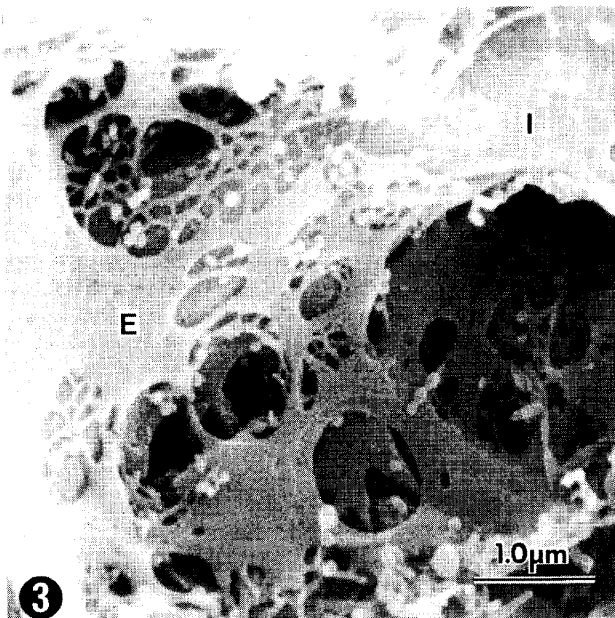


Fig. 3. Closer view of the central part of Fig. 2. An Ito cell's processes are observed through the fenestrae of the sinusoidal endothelium. Abbreviations as in Fig. 1. 48 h after LCA administration.

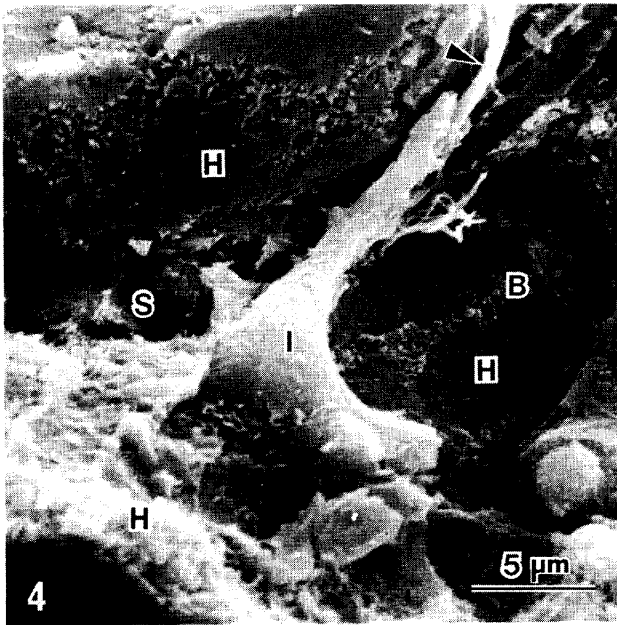


Fig. 4. Ito cell body is located between hepatocytes. A collagen fiber bundle (arrowhead) is held by a process of the Ito cell. Abbreviations as in Fig. 1. 48 h after LCA administration.

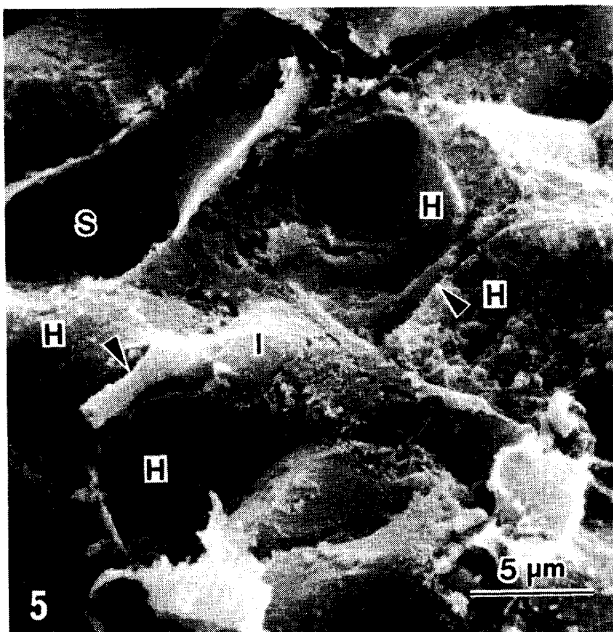


Fig. 5. Ito cell body and processes (arrowheads) are located between hepatocytes. Abbreviations as in Fig. 1. 48 h after LCA administration.

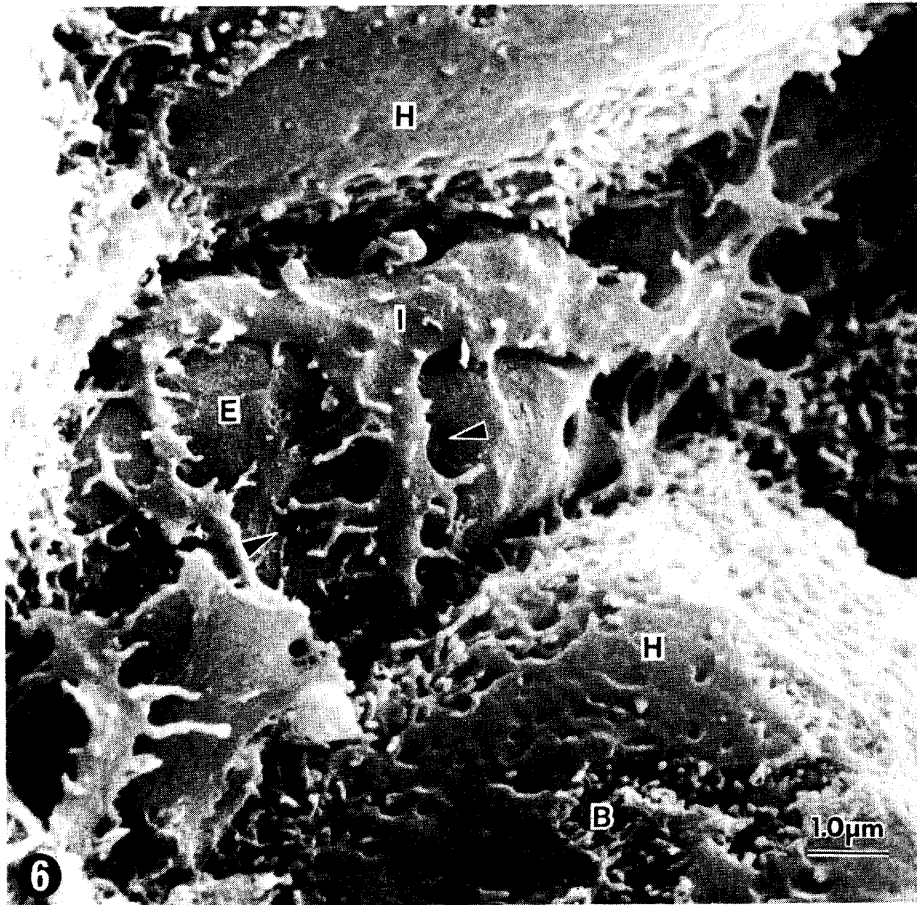


Fig. 6. Ito cell processes form a fern-leaf mantle on the sinusoidal endothelium. Thinner processes branch from thicker ones. Arrowheads indicate the fenestrae of the sinusoidal endothelium. Abbreviations as in Fig. 1. 48 h after LCA administration.

Liver architecture and hepatocyte appearance were normal outside of the foci. Since liver necrosis was not observed in rats studied within 48 h after LCA administration, all figures of the liver were taken 48 h after LCA administration.

By SEM, Ito cell bodies were often observed around the foci (Fig. 1), but few were seen in other zones. Ito cell processes were easily detected protruding through the sinusoidal fenestrae throughout the liver tissue (Figs. 2, 3). The cells within the foci were destroyed, and identification of Ito cells was difficult.

The Ito cell bodies were flat and round, or elliptic, and 7 to 12 μm in diameter. The appearance of the cells was relatively uniform. The cell body was usually located between sinusoidal endothelium and hepatocytes attached to the

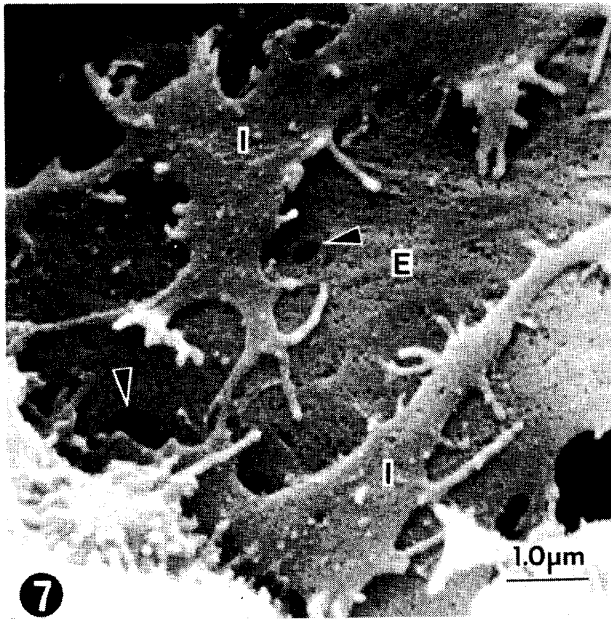


Fig. 7. Closer view of the square in Fig. 1. Processes like a fern-leaf are attached to the endothelial wall. The most peripheral processes are $0.1 \mu\text{m}$ in diameter. Arrowheads indicate fenestrae of the sinusoidal endothelium. Abbreviations as in Fig. 1. 48 h after LCA administration.

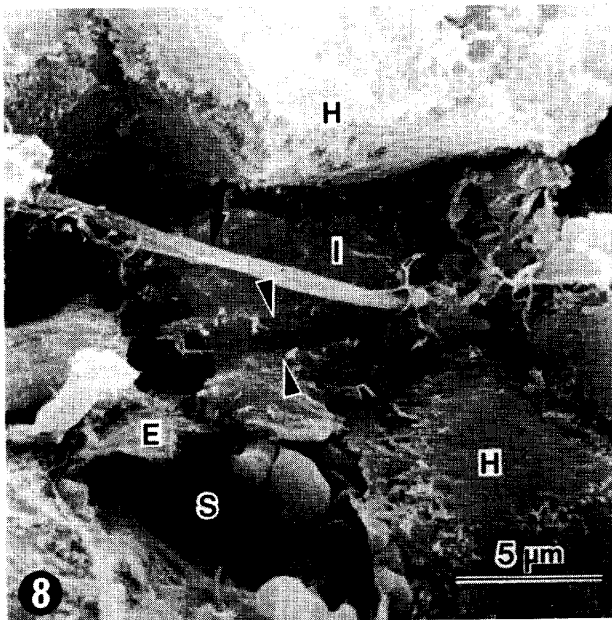


Fig. 8. Ito cell body in the space of Disse is attached to a bundle of collagen fibers (arrow). Note a slender groove (arrowheads) for holding the fiber. Abbreviations as in Fig. 1. 48 h after LCA administration.

endothelium (Fig. 1) ; occasionally they were located between hepatocytes (Figs. 4, 5). The surface was smooth except for fine undulations (Fig. 1). Thin microvillous projections approximately $0.1\ \mu\text{m}$ in length sparsely populated the body and main processes (Figs. 2, 6). Spherical bulgings, which have been noted after vitamin A administration (4), were not seen. The trunks of main processes were approximately $2\ \mu\text{m}$ in diameter and $20\text{--}30\ \mu\text{m}$ in length. The processes tapered, giving off smaller processes in a fern-leaf pattern ; these branches mantled the sinusoidal endothelium (Figs. 1, 6, 7). The most peripheral processes were $0.1\ \mu\text{m}$ in diameter. Processes also extended between hepatocytes (Fig. 5). Ito cells often were noted close to collagen fiber bundles. Some cells were attached to, but did not envelope, the bundle (Figs. 4, 8).

DISCUSSION

The whole body, including extended processes, of Ito cells was observed by SEM. Such observation had proven difficult in the past (11-13), as the space of Disse is hard to expose. Three dimensional reconstructions from transmission electron microscopic photography have not yielded satisfactory models (8-10).

Ito cells of rabbits, rats, hamsters, and mice have been reported to possess similar morphology and cytoplasmic content (16). Human Ito cell bodies, however, have extended processes, like cilia, and present a greater variety of shapes (14, 15). It is not clear whether this morphology is a species difference or whether the human cells were pathologically altered.

The hepatotoxic effects of LCA have been described (21-23), but Ito cell involvement in the injury has not been investigated. In the present SEM specimens, the space of Disse revealed Ito cell bodies around the midlobular zonal necrotic foci (20). Ito cell bodies were rarely seen in other zones. Liver cell degeneration induced by LCA injury probably facilitated detachment of hepatocytes from Ito cells and endothelium during fracturing for tissue preparation. The Ito cells were considered to be intact, because the necrotic foci induced by LCA were clearly demarcated. The present Ito cells may be available as a control for subsequent studies.

The bodies and processes of rat Ito cells were rather uniform in shape. The processes branched regularly to form a fern-leaf pattern, which mantled the hepatocytic surface of the sinusoidal endothelium. They resembled the pericyte described by Zimmermann (24). Ito cells likely strengthen sinusoidal walls and control blood flow in the sinusoids (25, 26).

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