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Ultrastructural Changes in the Bile Canaliculi and the Lateral Surfaces of Rat Hepatocytes During Restorative Proliferation*

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Summary. Ultrastructural changes in the bile canaliculi and the lateral surfaces of rat hepatocytes during regeneration following a two-third partial hepatectomy were studied by transmission and scanning electron microscopy. A marked increase in microvilli, widening of the intercellular spaces, and invagination and indentation of cytoplasmic membranes were seen in the lateral surfaces of hepatocytes during the early period after the operation. The density of the microvilli on the lateral surfaces gradually decreased and intercellular spaces returned to normal within 24 h, whereas the bile canaliculi revealed dilatation and tortuosity with elongation of microvilli. Hepatocytes during mitosis became rounded and showed dispersed microvilli on the sinusoidal surface. The bile canaliculi of mitotic hepatocytes were continuous with those of adjacent hepatocytes. On the 2nd or 3rd day posthepatectomy, hepatic plates became more than one-cell thick and hepatocytes showed occasional acinar arrangements around the dilated bile canalicular lumina. These features gradually returned to normal by one week after the operation. This study revealed unique sequential changes in the bile canaliculi and the lateral surfaces of hepatocytes during regeneration after partial hepatectomy.

Key words: Hepatocytes – Bile canaliculi – Lateral surfaces – Scanning electronmicroscopy – Mitosis

Introduction

It is known that proliferation of hepatocytes is accompanied by a variety of modifications in cell membranes such as changes in the composition of lipids (Dyatlovitskaya et al. 1976; Bruscalupi et al. 1980) and glycopro-

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teins (Akasaki et al. 1975; Marcea et al. 1980), membrane fluidity (Bruscalupi et al. 1980), hormone receptors (Leoni et al. 1975; Leffert and Knoch 1980), ion permeability (Leffert et al. 1975), enzyme activities (Wright 1977) and the cellular junctions (Yee and Revel 1978). It has also been demonstrated that the bile canaliculi reveal significant morphological changes during regeneration after partial hepatectomy (Viragh and Bartok 1966; Gabbiani and Ryan 1974; Mori and Novikoff 1975; Pfeifer and Reus 1980), probably reflecting changes in bile-secreting activities (Leong et al. 1959) and/or reorganization of bile-secreting channels (Ogawa et al. 1979).

Although morphological changes in the hepatocyte membrane seem to provide insight into the regulation of cellular proliferation and functional changes in proliferating hepatocytes, they have been studied mainly by transmission electronmicroscopy (TEM) (Lane and Becker 1966; Stenger and Confer 1966; Viragh and Bartok 1966), and there have been few scanning electron microscopic studies (Grisham et al. 1976; Ogawa et al. 1979; Tomoyori et al. 1980). Therefore, this study was carried out to reveal the sequence of events occurring on the cellular surfaces of hepatocytes from the very early stage after a two-third partial hepatectomy to the time when the hepatic ultrastructure was restored to normal. Attention was focused particularly upon the morphology of the bile canaliculi and lateral surfaces of hepatocytes.

Material and Methods

Experiments were carried out using 100–150 g male Wistar rats given a chow diet (Oriental Yeast, Tokyo, Japan) and water ad libitum and maintained in a room kept on a 12 h light and 12 h dark cycle. The animals were two-third partially hepatectomized or sham-operated between 9 and 11 a.m. At various periods following the operation, beginning at 3 h and extending to, at the longest, 28 days, two or three rats were laparotomized under light ether anesthesia, after which the livers were perfused via the portal vein with physiologic saline and then with a fixative containing 2% glutaraldehyde and 4% formaldehyde in 0.1 M cacodylate buffer at the rate of 5 ml per min. The vena cava was cut simultaneously with the initiation of the perfusion.

For preparation of specimens for SEM, small pieces of the tissue dehydrated with ethanol, approximately 1 mm by 1 mm by 3 mm, were manually fractured, subjected to critical point drying with liquid CO_2, coated with gold-palladium, and viewed in an Hitachi S-430 scanning electron microscope. From each specimen, hepatocytes both in peripheral and central portions of the liver lobules were photographed.

To visualize the architectural pattern of the bile canaliculi under light microscopy, ATPase histochemistry was performed using the perfusion-fixed livers. Frozen sections, approximately 10 μm thick, were incubated in the medium of Wachstein and Meisel (1957) using adenosine triphosphate as the substrate. After 30 min incubation at 37 C, they were visualized in a diluted ammonium sulfate solution and mounted on a glass slide with glycerol.

Results

Manual fracture of the perfusion-fixed hepatic tissue enabled us to view the lateral surfaces of hepatocytes and the bile canaliculi, because most of the adjacent hepatocytes were fractured at the level of the intercellular space. The SEM features of the lateral surfaces of the hepatocytes in the sham-operated rats at any time after the operation (Fig. 1 A and B) were

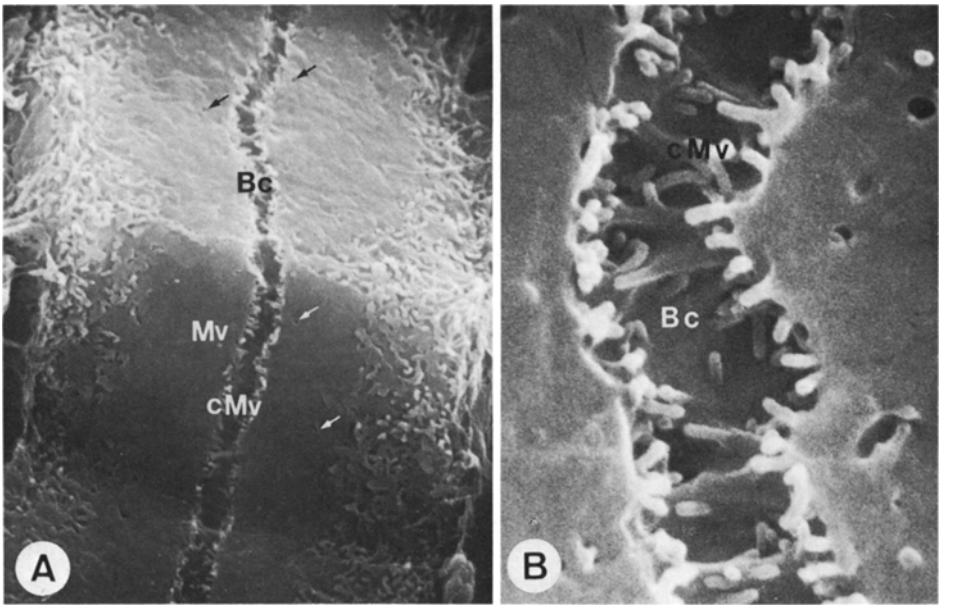
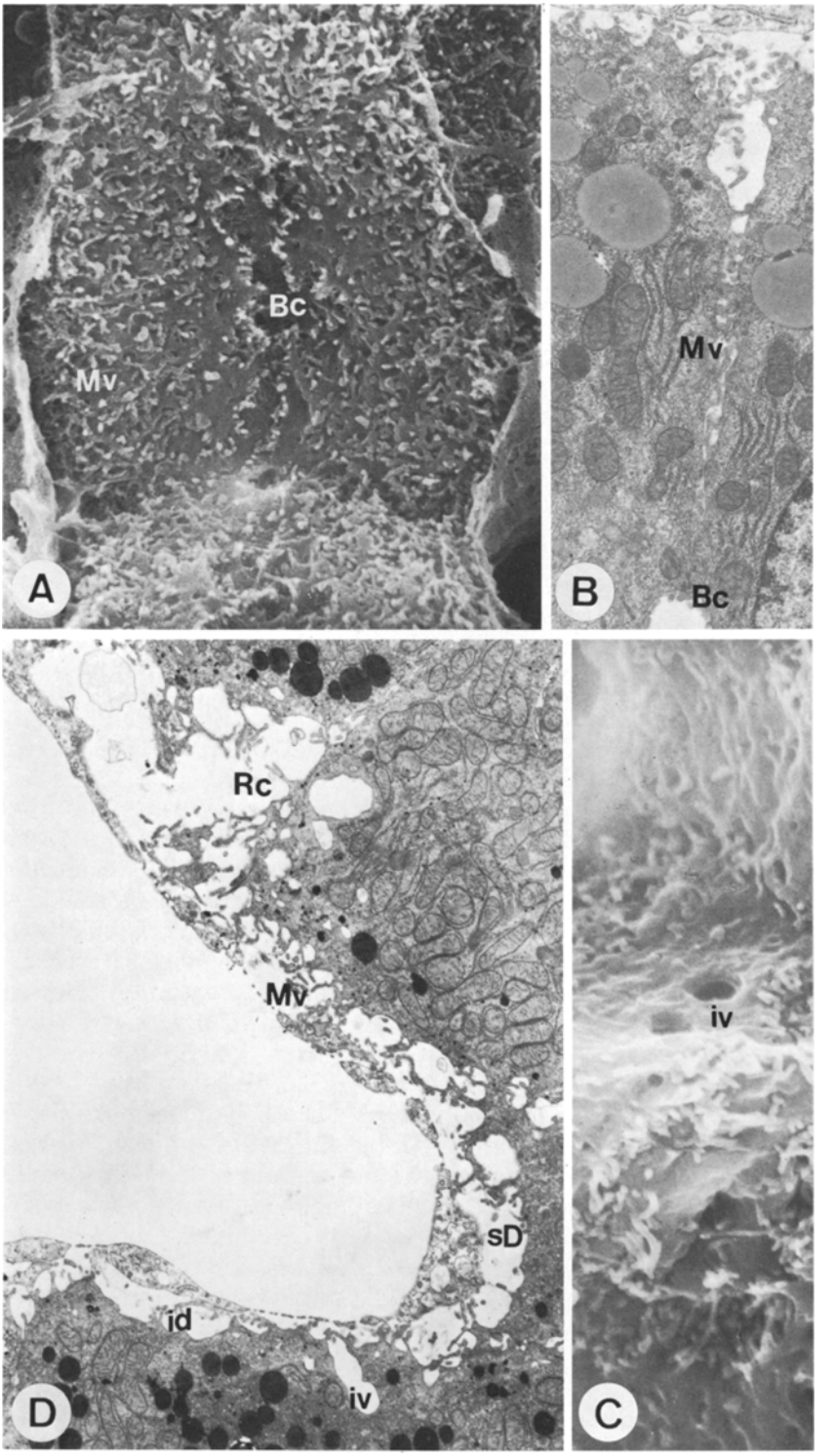


Fig. 1A, B. The liver of a sham-operated rat at 3 h after the operation. SEM of the fractured surface of the hepatic tissue. Canalicular microvilli (*cMv*). **A** Lateral surfaces around a hemi-bile canaliculus are smooth with ocassional pits (*arrows*) and short microvilli (*Mv*). **B** High magnification of a hemi bile canaliculus. Canalicular microvilli (*cMv*) localize predominantly at the edges of the lumen. **A** ×1,300; **B** ×4,500

essentially identical with those described previously (Compagno and Grisham 1971; Fujita et al. 1971; Brooks and Higgins 1973; Motta and Porter 1974; Grisham et al. 1975; Miyai et al. 1976; Jones and Schmucker 1977). However, a significant difference was noted between the morphology of hepatocytes in the central and peripheral parts of the liver lobule; that is, the peripheral hepatocytes revealed more microvilli in their lateral surfaces and wider bile canaliculi than the centrolobular hepatocytes. The organizational pattern of the bile canaliculi demonstrated by ATPase histochemistry was also similar to that of normal rat liver.

Early Changes (3–6 h after the Operation). The hepatocytes showed a marked increase in the density of microvilli in the lateral surfaces (Fig. 2A and B). Indentation and occasional deep invaginations of the lateral membrane, which probably correspond to the pinocytic vesicles induced by partial hepatectomy (Mori and Novikoff 1977), were also observed (Fig. 2C). The spaces of Disse and recesses among adjacent hepatocytes were markedly dilated (Fig. 2D). Indentation and invagination of the cytoplasmic membrane, and a decrease in the density of microvilli were seen in the sinusoidal surfaces of hepatocytes (Fig. 2D). Although bile canaliculi were slightly tortuous, the density of microvilli in the canalicular lumen did not change significantly (Fig. 2A). These changes were basically the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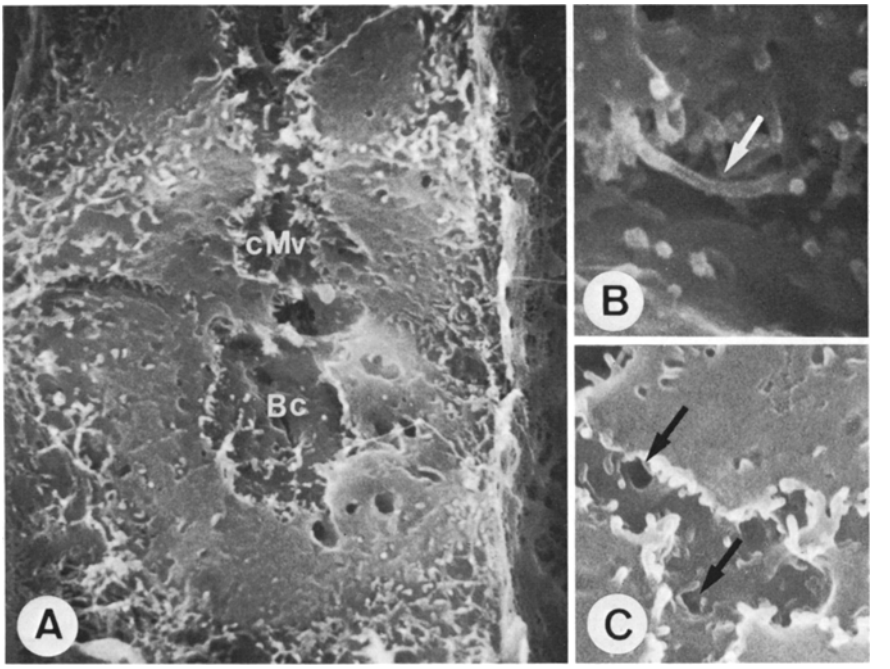
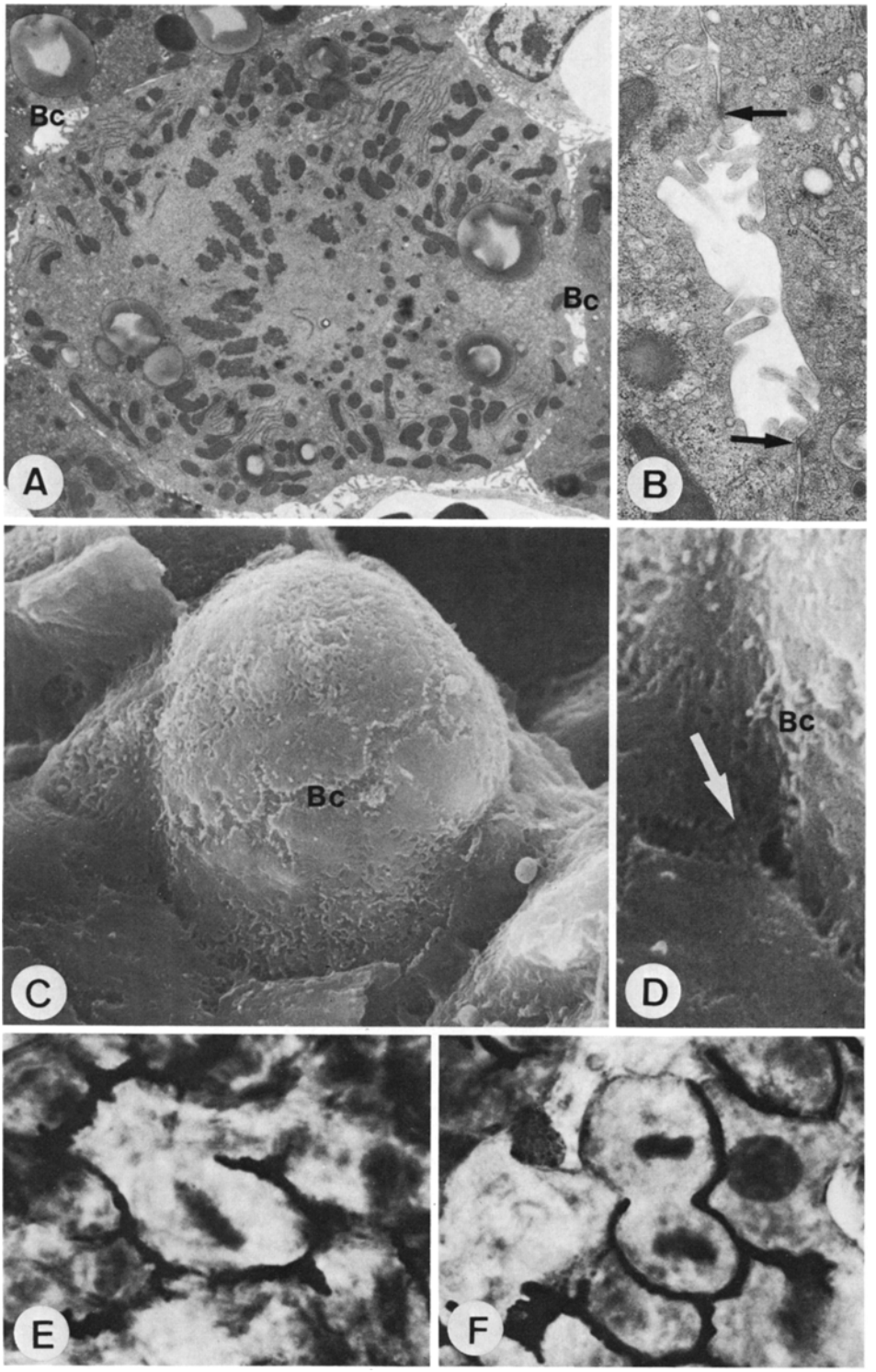


Fig. 3A–C. Twenty-four hours after the operation. **A** A bile canaliculus (*Bc*) shows marked dilatation. Canalicular microvilli (*cMv*) are localized mainly at the edge of the lumen and their density is not unlike that of the sham-operated rats. **B** An abnormally elongated microvillus (*arrow*) within a bile canaliculus. **C** Invagination of the canalicular membrane (*arrows*). **A** ×4,000; **B** ×17,000; **C** ×27,000

same when the livers were fixed at different perfusion rates (from 3 ml/min to 15 ml/min), suggesting that they were not artifacts due to over-perfusion. These changes were more prominent in the peripheral hepatocytes at 3 h after the operation, and were seen throughout the entire liver lobule at 6 h.

Changes during the Proliferating Phase (24–36 h after Operation). Dilatation of the intercellular spaces and an increase in the density of microvilli in the lateral surfaces were less prominent by 24 h after the partial hepatectomy (Fig. 3A). Indentation and invagination of the plasma membrane were also less frequent, whereas the bile canalicular lumen became markedly dilated (Fig. 3A). Although the density of canalicular microvilli was not altered, abnormally elongated microvilli were frequently found in the canalicular

Fig. 2A–D. Six hours after a two-third partial hepatectomy. **A** SEM of the rough lateral surfaces of hepatocytes showing a marked increase in microvilli (*Mv*). Bile canaliculus (*Bc*). **B** TEM of the dilated inter-cellular spaces and the rough lateral surfaces of adjacent hepatocytes. Microvilli (*Mv*). Bile canaliculus (*Bc*). **C** Invagination (*iv*) of the cytoplasmic membrane in the lateral surface of a hepatocyte. **D** TEM of the sinusoidal surface. The space of Disse (*sD*) and the recess (*Rc*) between two adjacent hepatocytes are markedly widened. Microvilli (*Mv*) are decreased in number, and indentation (*id*) and invagination (*iv*) of the cell membrane are seen. **A** ×4,700; **B** ×6,600; **C** ×17,000; **D** ×8,000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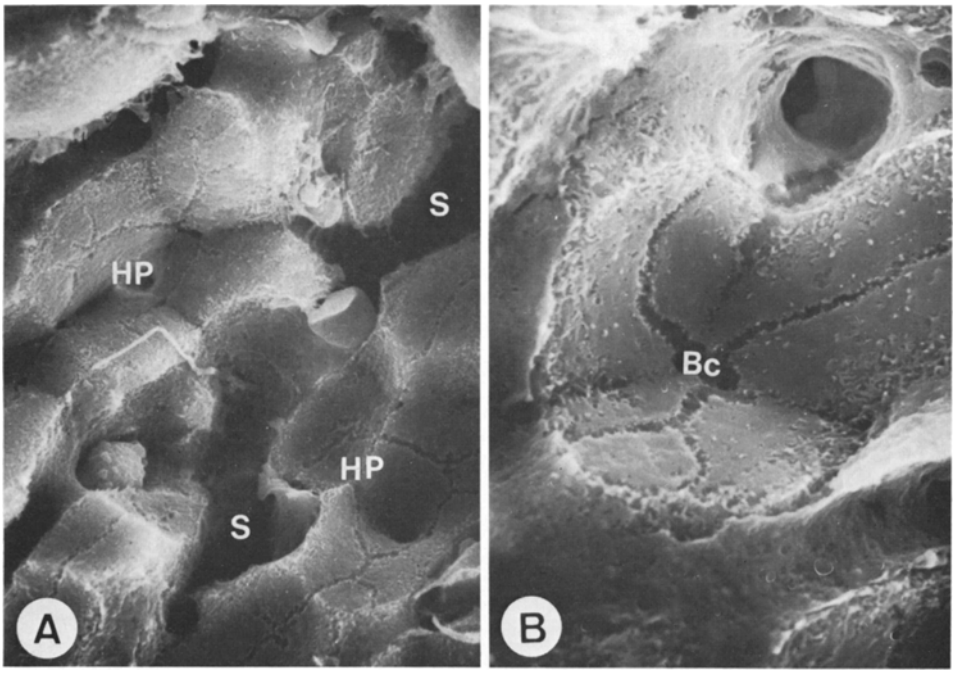

Fig. 5A, B. 72 h after the operation. **A** Hepatocytes are arranged two-cell-thick in hepatic plates (*HP*). Sinusoids (*S*). **B** Acinar arrangement of hepatocytes surrounding a dilated canalicular lumen (*Bc*). **A** ×1,700; **B** ×2,500

lumen (Fig. 3B). Occasional diverticulation of canalicular membranes was also encountered (Fig. 3C). These changes were more prominent in peripheral than in central hepatocytes.

The hepatocytes during mitosis, especially those in metaphase and anaphase, were shown without exception to be nearly round in shape by TEM (Fig. 4A). Therefore, the spherical hepatocytes seen by SEM were considered to be in mitosis (Fig. 4C). These hepatocytes showed dispersed microvilli in the sinusoidal surfaces and in the shallow and wide bile canaliculi (Fig. 4C). The canalicular lumina were obviously continuous with those of the adjacent hepatocytes (Fig. 4C and D). This continuity was also demonstrated by ATPase histochemistry (Fig. 4E and F). Tight junctions were

Fig. 4A–F. A mitotic hepatocyte at 31 h after the operation. **A** TEM of a mitotic hepatocyte during metaphase. The hepatocyte is nearly round in shape and shows two bile canaliculi (*Bc*) between the adjacent hepatocytes. **B** Higher magnification of A. Tight junctions (*arrows*) around a bile canaliculus. **C** SEM of a spherical hepatocyte, probably in mitosis, showing a shallow and dilated hemi-bile canaliculus (*Bc*). **D** Higher magnification of **C**. A bile canaliculus (*Bc*) on a mitotic hepatocyte is continuous with that of the adjacent hepatocyte (*arrows*). **E** A mitotic hepatocyte in metaphase. Bile canaliculi of a mitotic hepatocyte are continuous to those of the neighbouring hepatocytes. ATPase histochemistry. **F** A mitotic hepatocyte in telophase. Bile canaliculi also have continuity with those of adjacent hepatocytes. **A** ×2,200; **B** ×11,000; **C** ×3,300; **D** ×7,500; **E** ×900; **F** ×900

found around the bile canaliculi of mitotic hepatocytes (Fig. 4B) as in normal hepatocytes.

Changes during the Reorganizing Phase (48–72 h after Operation). During this period liver plates thickened, each plate being composed of more than one hepatocyte (Fig. 5A), and the sinusoids narrowed. These changes were more apparent in the peripheral portions of the liver lobules than in the central portions. The lateral surfaces of the hepatocytes contained few microvilli and were smooth (Fig. 5A and B). Indentation and invagination of the cell membrane were rarely seen on the lateral surfaces. The bile canaliculi still showed significant dilatation and tortuosity (Fig. 5B). There were occasional acinar arrangements of hepatocytes around the bile canaliculi (Fig. 5B). Most microvilli in the bile canaliculi were normal, with elongated ones being less prominent (Fig. 5B).

At one to four weeks after the operation, most hepatic plates had returned to one-cell thickness and the features of the hepatocyte surfaces had returned to normal except for occasional tortuosity of bile canaliculi.

Discussion

SEM observations of hepatocytes following a two-third partial hepatectomy demonstrated characteristic sequential changes in the cellular membranes. Early changes consisted of a marked increase in microvilli on the lateral surfaces, dilatation of the intercellular spaces, and invagination or indentation of the cell membrane. These changes may have been caused by various acute changes in the intrahepatic environment and metabolic changes in hepatocytes following the operation. One of the important factors probably related to these changes was an increase in the pressure of the portal vein after the operation (Benacerraf et al. 1957). As the pressure in the spaces of Disse was probably equivalent to that in the sinusoidal space due to the presence of numerous endothelial fenestrations (Wisse 1970, Ogawa et al. 1973), the increased portal pressure may have caused widening of the intercellular spaces between neighboring hepatocytes. The increase in microvilli on the lateral surfaces may thus have been a result of the widened intercellular spaces since it has been demonstrated that isolated parenchymal cells, including hepatocytes, in suspension have numerous microvilli on almost the entire cellular surface (Wanson et al. 1979).

However, it has been demonstrated that preneoplastic and neoplastic hepatocytes in man (Phillips et al. 1973) and rodent (Ogawa et al. 1979) show significant dilatation of the intercellular spaces and increased microvilli on the lateral surfaces. Studies have also shown that various biochemical changes (Akasaki et al. 1975; Leoni et al. 1975; Dyatlovitskaya et al. 1976; Wright 1977; Bruscalupi et al. 1980; Leffert and Knoch 1980) occur in association with restorative proliferation after partial resection of the liver. Therefore, early morphological changes may be a reflection of functional changes in the cellular membrane caused by various metabolic alterations.

The morphology of the bile canaliculi was significantly changed during regeneration, with marked dilatation, and tortuosity being observed and with some canaliculi revealing abnormally elongated microvilli. These findings were in agreement with previous TEM observations (Viragh and Bartok 1966; Gabbiani and Ryan 1974; Mori and Novikoff 1975; Pfeifer and Reus 1980) although its cause is not clear.

Recently, Pfeifer and Reus (1980) reported that there was no decrease in the number of canalicular microvilli per unit volume of hepatic parenchyma in association with the dilatation of canaliculi in regenerating liver, indicating that the dilatation may be due to production of new membrane proteins rather than to unfolding of the canalicular surface. They pointed out a major difference between the dilatation in regeneration and in early cholestasis, since in the latter condition is associated with a reduction or loss of the canalicular microvilli (Steiner and Carruthers 1961; Zaki 1966; Compagno and Grisham 1974; De Vos et al. 1975). Our observations made by SEM that microvilli at the edges of the dilated bile canaliculi were not significantly changed during regeneration are in agreement with the observations of Pfeifer and Reus (1980). Furthermore, the dilation was more pronounced in perilobular hepatocytes in regenerating liver whereas it was more conspicuous in centrilobular hepatocytes in early cholestasis (Wachstein 1963). These morphological changes in the canaliculi in regenerating liver indicates that the cause of the dilatation may not be related to cholestasis but rather to the functional load in bile secretion imposed on each hepatocyte during regeneration (Leong et al. 1959).

Our SEM study revealed the frequent appearance of rounded hepatocytes in the liver 30 h following the operation. These cells were considered to be in mitosis because of morphological similarity to hepatocytes observed during metaphase and anaphase by TEM. These hepatocytes revealed shallow and dilated bile canaliculi, but were obviously continuous with those of the adjoining hepatocytes. It has been shown that in the normal liver, bile secreting channels form a continuous anastomozing system which has no blind endings (Bhathal and Christie 1969; Elias and Sherric 1969). The observations made in the present study indicate that the continuity of the bile canaliculi is probably preserved even in dividing hepatocytes.

After the peak of mitosis, hepatic plates became more than one-cell thick and not infrequently dilated canalicular lumina surrounded by several hepatocytes were seen. It has been demonstrated that the division of hepatocytes occurs mainly around 30 h after partial hepatectomy, whereas that of sinusoidal-lining cells appears after the 3rd day (Fabrikant 1968). Thus, it seems likely that the relative increase in the ratio of hepatocytes to sinusoidal-lining cells causes thickening of the liver plates. Therefore, return to normality of the architectural pattern of hepatic plates in the late stage of regeneration may correspond with the restitution of the normal numerical ratio of parenchymal cells to sinusoidal-lining cells.

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