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Surface Features of Cirrhotic Liver*

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Summary. The surface features of single cells and of multicellular tissue units in cirrhotic rat livers have been studied by scanning electron microscopy (SEM). Cirrhosis of the liver was produced in rats by simultaneously treating them with carbon tetrachloride and sodium phenobarbital. Connective tissue septa consisted of a loose mesh-work of fibers in which fibroblasts were embedded. The arrangement and surface features of hepatocytes in cirrhotic nodules differed from those found in parenchyma of normal livers. Hepatocytes in cirrhotic nodules universally formed plates two cells thick. The portion of the hepatocyte surface covered by microvilli was greatly increased in cells from cirrhotic livers, and this was reflected in a corresponding reduction in the area occupied by the smooth-surfaced narrow intercellular space. Canaliculi between hepatocytes in cirrhotic livers were reduplicated and frequently branched. Hepatocyte surfaces covered by microplicae and flattened microvilli, typical of connective tissue-facing surfaces in normal livers, were greatly increased in cirrhotic livers corresponding to the increase in connective tissue. Where hepatocytes directly contacted fibroblasts (and not fibers), their surfaces were entirely smooth. Sinusoidal endothelial cells in cirrhotic livers contained only isolated, relatively sparse pores, and they lacked both sieve plates (pore complexes) and large fenestrations.

Key words: Scanning electron microscopy – Cirrhosis – Liver – Rat.

Introduction

Surface features of cells in normal liver and the three-dimensional organization of multicellular tissue units have been elucidated by studies employing scanning electron microscopy (SEM). These studies have provided new and expanded insights into the structure of this organ (Brooks and Haggis, 1973; Motta

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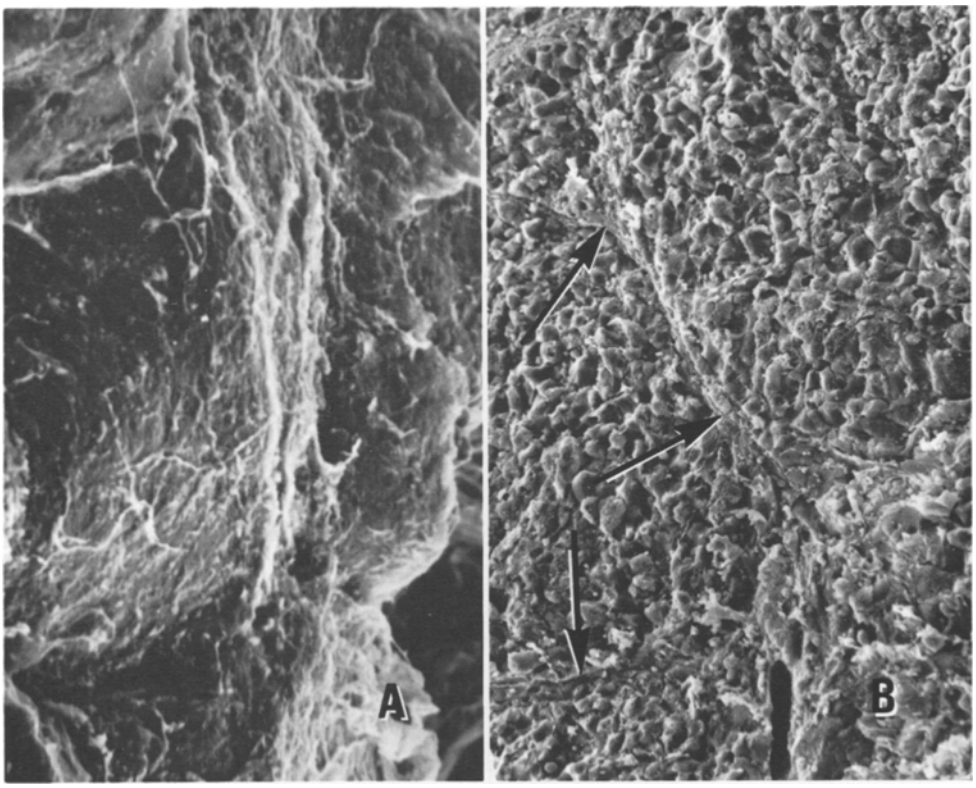

Fig. 1. A Cirrhotic liver fractured between parenchymal nodules. Nodules are covered with connective tissue septae, seen here en face. Scanning electron micrograph. ×165. **B** Intranodular fracture plane of a cirrhotic liver illustrating thin bands of connective tissue forming septae (arrows) between parenchymal nodules. Scanning electron micrograph. ×275

and Porter, 1974; Grisham et al., 1975, 1976; Motta and Fumagalli, 1974, 1975; Nopanitaya and Grisham, 1975). In the pathological realm, canalicular surfaces of hepatocytes have been examined following experimental occlusion of the common bile duct (Compagno and Grisham, 1974; Layden et al., 1975; Vial et al., 1976) and following administration of cholestatic bile acids (Layden et al., 1975). Additionally, the luminal surfaces of intrahepatic bile ducts have been viewed after extrahepatic biliary obstruction (Brooks et al., 1975). Many other pathologic lesions of liver also are associated with alteration of cellular surfaces and changed relationships of cells in multicellular aggregates. In this study, we have utilized SEM to examine cellular surface ultrastructure and supracellular organization of rat liver made cirrhotic by concurrent treatment with phenobarbital and carbon tetrachloride.

Materials and Methods

Cirrhosis was produced in male rats of Wistar strain, weighing 100–125 g, by a modification of the method of McLean et al. (1969). Throughout the experimental period, animals were fed a

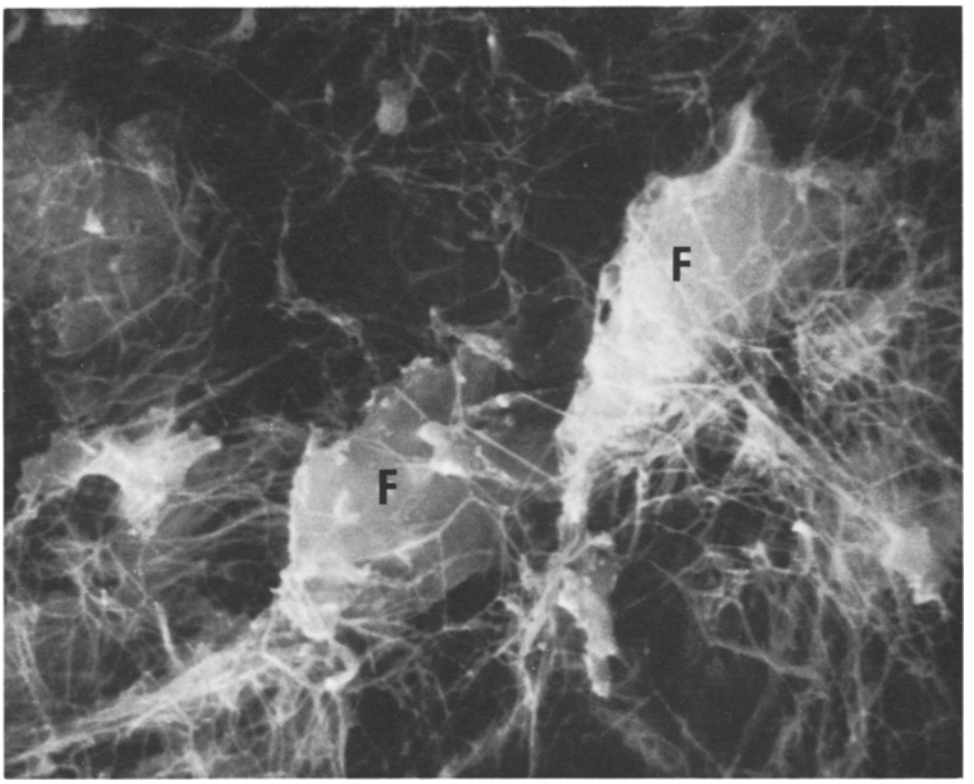

Fig. 2. A closer view of a septum showing cells (fibroblasts) (*F*) enmeshed in collagen fibers. Scanning electron micrograph. ×7000

diet of Purina laboratory chow ad libitum and were offered water that contained 0.5 g sodium phenobarbital/L. After receiving this regimen for one week, groups of rats were exposed to air saturated with carbon tetrachloride (CCl_4) in a chamber (71 × 45 × 45 cm; 144 L capacity) similar to that used by McLean et al. (1969). Compressed air (8 l/min) was passed into the chamber through a train of two 250 ml bottles, each containing 30 ml CCl_4. The chamber was equilibrated with air saturated with CCl_4 for one minute. Gas-flow was then turned off, rats were placed in the chamber, and CCl_4-saturated air was administered for 1.5 min. Animals were left in the chamber for another 1.5 min prior to being returned to their cages. The gassing procedure was repeated twice a week (Monday and Thursday) for 5 weeks (10 doses). Animals were killed one day after the tenth gas exposure. Rats given only sodium phenobarbital in drinking water for 6 weeks were used as controls.

All animals were killed by decapitation and their thoracic and abdominal walls were opened. Livers were fixed by perfusing them via the left ventricle with a 4% solution of paraformaldehyde in phosphate buffer. Fixed liver samples were prepared for SEM by the techniques described by Grisham et al. (1976). Tissues were fixed and processed by conventional methods for transmission electron and light microscopy.

Results

All animals in the experimental group (phenobarbital in drinking water plus CCL_4) were irritable and had unthrifty fur. Two out of 40 rats died after the

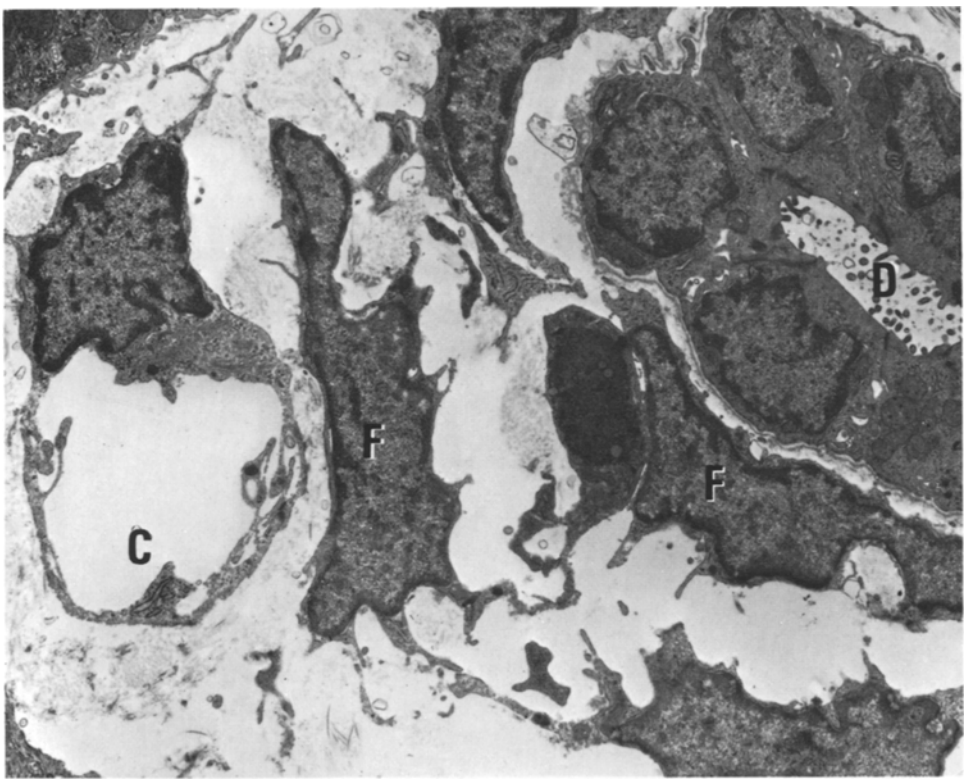

Fig. 3. Ultrastructure of a sectioned internodular septum from a cirrhotic liver conforms that it contains fibroblasts (*F*), and collagen, as well as capillaries (*C*), and a small bile duct (*D*). Transmission electron micrograph. ×7300

eighth gassing. Animals given phenobarbital were normal in appearance and none of them died. The average body weight of the experimental rats (237.5 ± 28.6 g) was less than that of control rats treated only with phenobarbital (277.0 ± 22.5 g); this difference was statistically significant ($p = < 0.005$).

The gross structure of the livers and spleens of animals in experimental and control groups differed strikingly. Rats fed phenobarbital and treated with CCl_4 had yellow-brown, nodular livers; livers of control rats treated only with phenobarbital were dark reddish-brown and their surfaces were smooth. Weights of livers in control and treated animals did not differ significantly, but the spleens of experimental animals (1.6 ± 0.1 g) were almost three times as large as those of control groups (0.6 ± 0.1 g) ($p < 0.0001$). One to four ml of yellow ascitic fluid was commonly found in the peritoneal cavity of each experimental rat, but none was noted in any of the control rats.

Light microscopic findings in the livers of all rats fed phenobarbital and exposed to CCl_4 were consisted with those described by McLean et al. (1969). Cirrhosis with bile duct proliferation and prominent nodular parenchymal regeneration characterized the livers of all experimental rats. Livers of the group of animals treated with phenobarbital alone were only slightly congested.

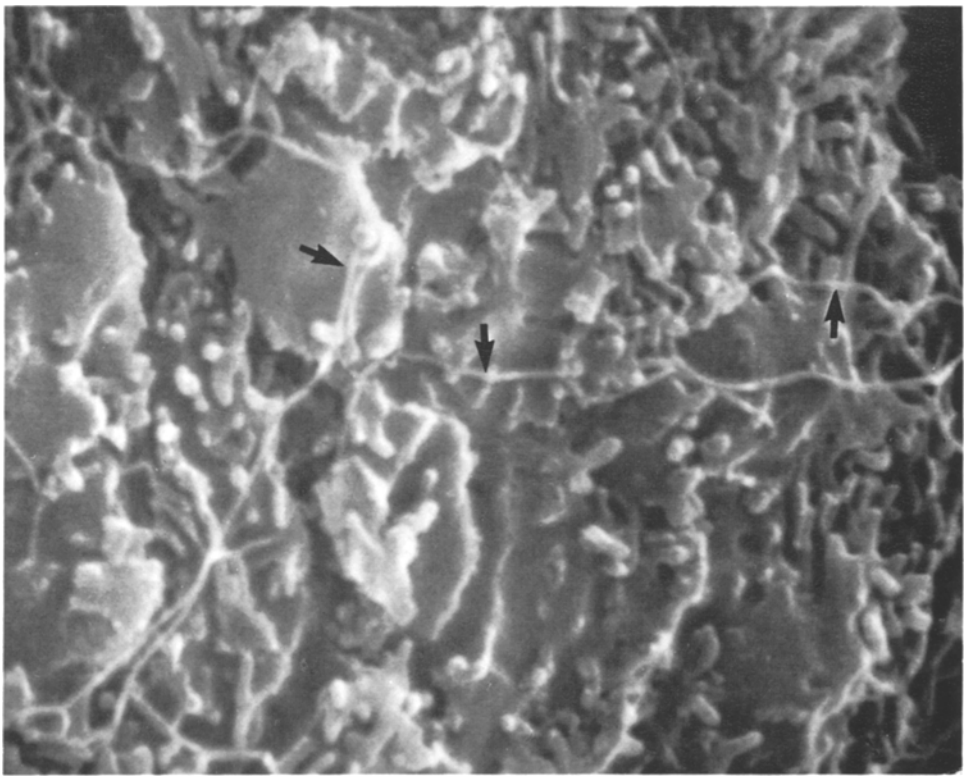

Fig. 4. The surface of a hepatocyte abutting connective tissue fibers contains flattened microvilli and microplicae, interspersed with linear, smooth-surfaced indentations. A few collagen fibers are visible (arrows). Scanning electron micrograph. ×12,000

By SEM the livers of control animals were normal in every respect and demonstrated the features previously described by us and other investigators (Brooks and Haggis, 1973; Motta and Porter, 1974; Grisham et al., 1975, 1976; Motta and Fumagalli, 1974, 1975). TEM of thin sections showed that the smooth endoplasmic reticulum was greatly increased in amount, compatible with the long exposure to phenobarbital.

SEM clearly delineated the nodular aggregates of hepatocytes separated by thin septa of connective tissue (Figs. 1A and 1B). Closer views of connective tissue septae demonstrated cells embedded in a meshwork of fibers (Fig. 2); TEM showed that these cells were fibroblasts (Fig. 3). Connective tissue fibers were small (about 10 to 30 nm) and highly branched (Fig. 2). Hepatocytic surfaces facing connective tissue contained irregularly shaped microvilli, varying from finger-like to leaf-shaped (microplicae); smooth-surfaced, linear indentations were also presents on these surfaces (Fig. 4). Surfaces of hepatocytes directly contacting fibroblasts were smooth and free of microvilli (Fig. 5A); this observation was corroborated by TEM (Fig. 5B).

Hepatocytes in parenchymal nodules typically were aggregated into plates two cells or more thick (Figs. 6A and 6B), unlike the arrangement in normal

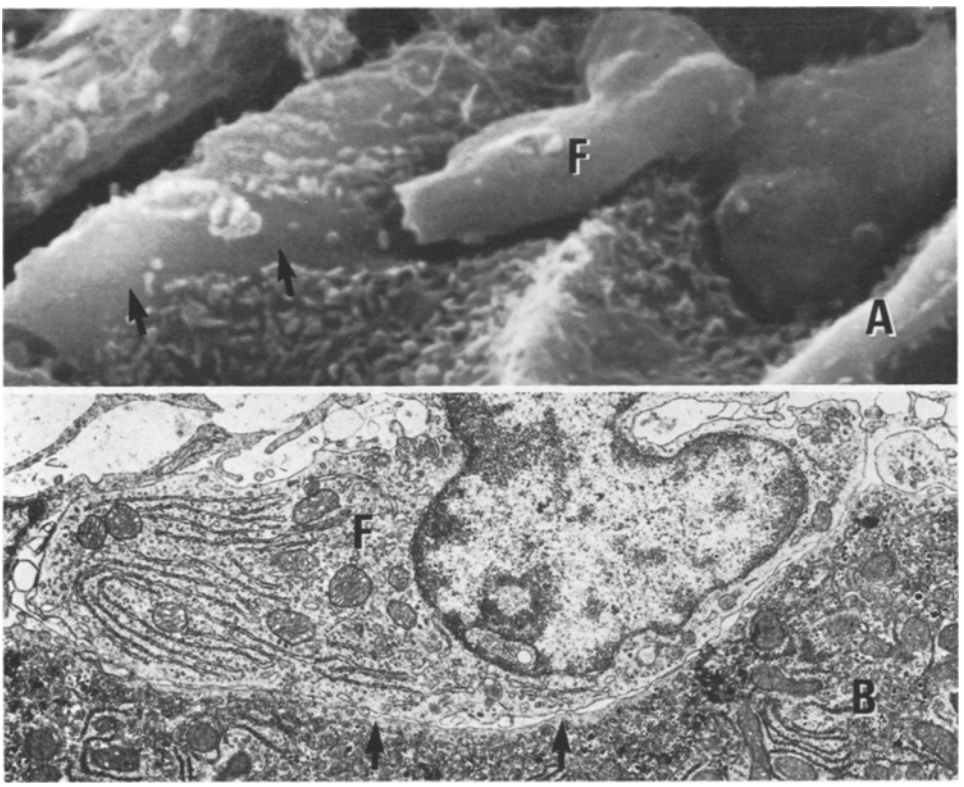

Fig. 5A and B. The hepatocyte surface directly touching fibroblasts (*F*) is smooth and devoid of microvilli (arrows) as indicated by both scanning and transmission electron microscopic study. **A** Scanning electron micrograph. ×7000. **B** Transmission electron micrograph. ×8300

livers where they occupy plates one cell thick. Canaliculi occupied a central position in bicellular plates, with branches that penetrated between individual cells towards sinusoids (Fig. 6B). On the surfaces of individual hepatocytes, canaliculi were highly branched and ramified (Fig. 7). TEM of thin sections corroborated the branching of canaliculi, demonstrating numerous canalicular cross-sections between adjacent hepatocytes (Fig. 8). Noncanalicular surfaces of hepatocytes were almost completely occupied by microvilli, and the flat intercellular space which normally borders was greatly reduced in width (Figs. 9A and 9B).

In many areas sinusoidal endothelium contained only isolated single pores (Fig. 10A); affected endothelial cells lacked both sieve plates and large fenestrations. TEM of thin sections corroborated this observation, showing sinusoids to be lined with nearly continuous endothelium (Fig. 10B), but a subendothelial basement membrane was not found. Capillaries in connective tissue septa were composed of a continuous endothelial tube, subtended by a complete basement membrane.

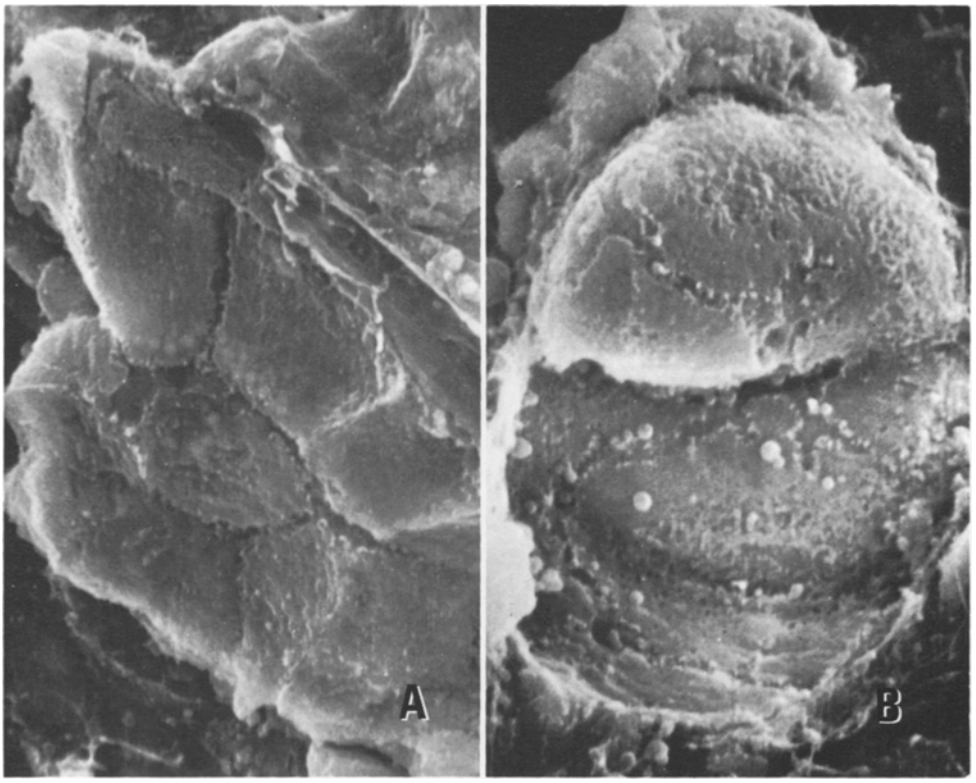

Fig. 6A and B. Two-cell thick hepatic plates in cirrhotic livers are seen here in longitudinal (**A**) and transverse (**B**) fracture planes. Note the wide, branched bile canaliculi between hepatocytes. Scanning electron micrographs. **A** ×3500, **B** ×4000

Discussion

This study of the surface morphology of the cirrhotic liver by SEM extends and clarifies knowledge of the structural derangements that characterize this disorder. Nodules of hepatocytes were segregated by connective tissue septa into units lacking identifiable afferent and efferent vessels and bile ducts. Within the nodulars units of parenchyma hepatocytes always formed plates more than one cell thick. In cross section such multicellular aggregates resembled tubules. Surfaces of individual hepatocytes were also greatly altered from that which typifies normal liver. Bile canaliculi were highly branched and ramified over the surfaces of single cells. In normal livers branched bile canaliculi are not prominent, occurring mostly in the vicinity of portal tracts (Grisham et al., 1975, 1976; Nopanitaya and Grisham, 1975). Unpublished observations from our laboratory concerning the surface structure of regenerating and neoplastic hepatocytes indicate that branching and reduplication of bile canaliculi, as noted here in hepatocytes of cirrhotic liver, is associated with hepatocellular proliferation.

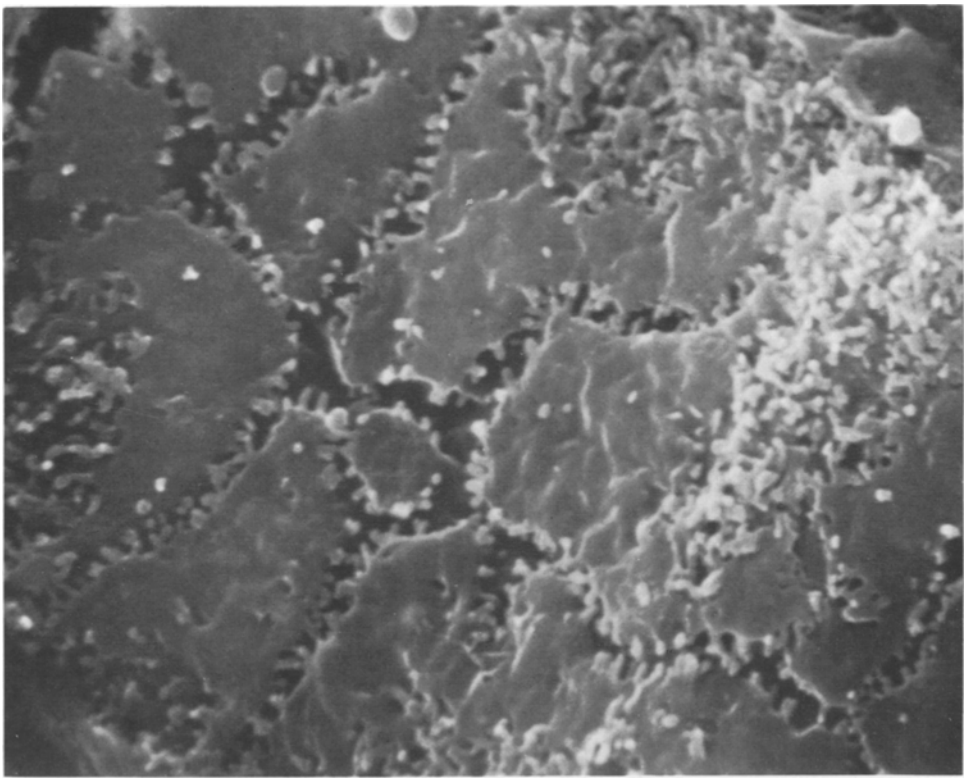

Fig. 7. High magnification view of a bile canaliculus which is highly branched, forming the outline of a candelabrum. Microvilli of this bile canaliculus are marginated. Scanning electron micrograph. $\times 10{,}000$

Areas of the hepatocytic surface densely covered with microvilli, characteristic in normal liver of surfaces facing sinusoids (Grisham et al., 1975, 1976; Motta and Porter, 1974), were greatly increased in cirrhotic livers. Even surfaces that abutted other hepatocytes contained microvilli. As a result of this change in the cellular surface membrane, the extent of the flat intercellular surfaces lateral to bile canaliculi, which are separated from adjacent cells by a space of about 10–15 nm and which contain attachment complexes, was greatly reduced. This alteration has previously been noted in hepatocytes of cirrhotic livers by TEM (Phillips and Steiner, 1965). It has been speculated that this extension of the space of Disse between adjacent hepatocytes in plates two cells thick increases the absorptive surface of this cell and compensates for its being located at a greater than normal distance from a sinusoid (Phillips and Steiner, 1965).

In normal livers the surfaces of hepatocytes in limiting plates, which face connective tissue of portal tracts and large hepatic veins, are structurally distinct, containing leaf-shaped microvilli and microplicae (Grisham et al., 1976; Nopanitaya and Grisham, 1975). In cirrhotic livers this type of hepatocellular surface

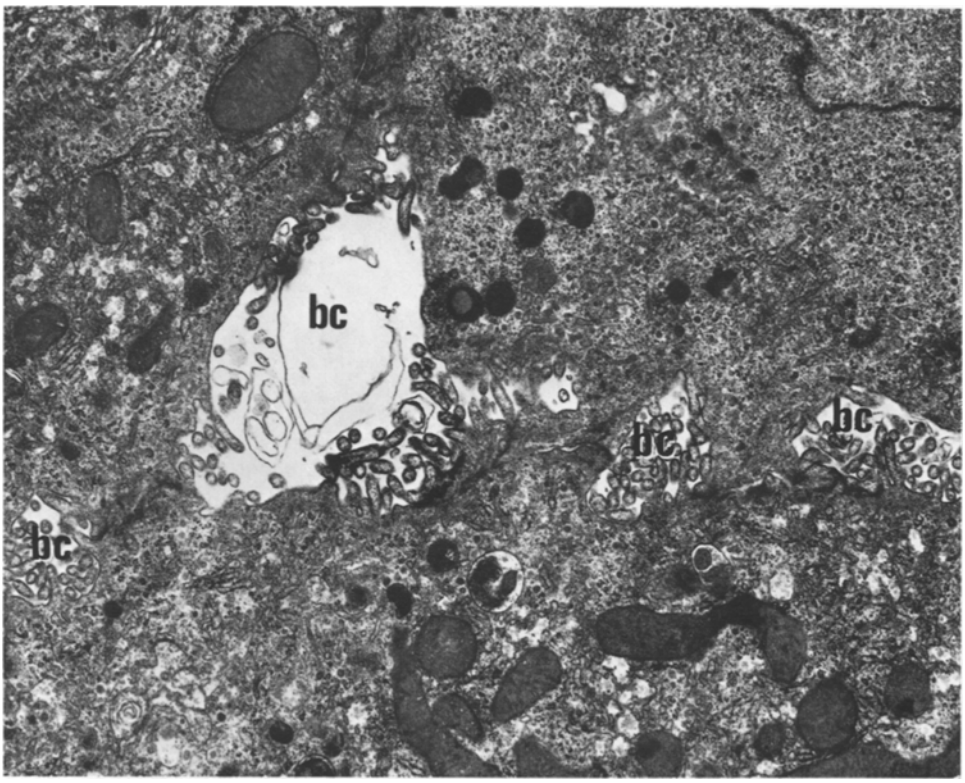

Fig. 8. Multiple cross-sections of bile canaliculi (*bc*) in thinly sectioned tissue corroborate canalicular branching. Transmission electron micrograph. × 12,000

is expanded, occupying all hepatocytes facing connective tissue septa. This observation, coupled with the findings in normal livers, suggests that this surface feature is probably induced by adjacent connective tissue. It seems unlikely that it reflects an absolute difference between hepatocytes that occupy limiting plates and those that do not.

Connective tissue septa are composed of a meshwork of fibers that encase fibroblasts, bile ducts and capillaries. Fibers, closely apposed to the surface of fibroblasts, are more delicate than are those found in portal tracts and along sinusoids of normal livers, measuring about 10–30 nm, as compared to the normal 50 to 100 nm (Stenger, 1965). Variation in fiber diameter may be related to the "maturity" of collagen (Stenger, 1965).

Sinusoidal endothelium was greatly altered in cirrhotic livers. Both sieve plates composed of groups of pores and large fenestrations were lacking. Recent studies in this laboratory indicate that the relative number and distribution of pores, pore complexes and fenestrations are dynamically related to conditions of sinusoidal blood flow and composition (Nopanitaya et al., 1976a and b). The changes described here are compatible with the so-called capillarization

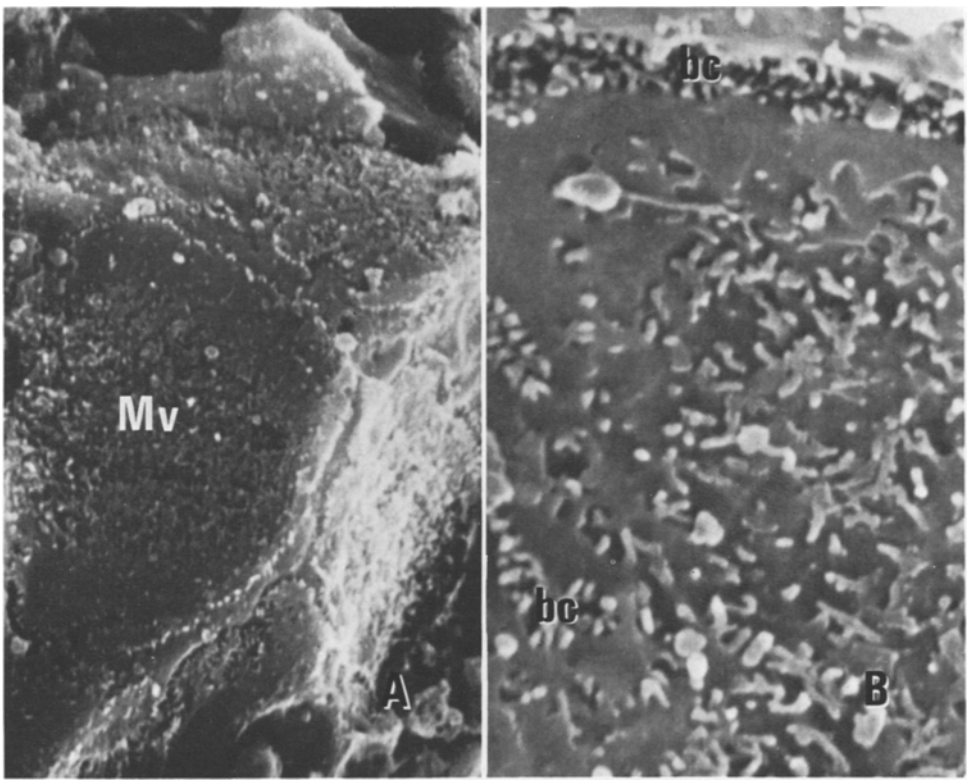

Fig. 9. A The intercellular surface of a hepatocyte from a parenchymal nodule is covered with microvilli (*Mv*). Scanning electron micrograph. ×3500. **B** A high magnification view of the surface of an intranodular hepatocyte illustrating that the flat membrane bordering bile canaliculi (*bc*) is greatly narrowed. Most of the surface of this hepatocyte is covered with microvilli. Scanning electron micrograph. ×11,200

described by TEM (Schaffner and Popper, 1963; Stenger, 1966), although sinusoids in the cirrhotic livers of this study lacked the basement membrane previously described in cirrhotic livers. Small vessels in connective tissue septa appeared identical to usual capillaries (Bennett et al., 1959).

This study demonstrates that surfaces of hepatocytes and sinusoidal endothelial cells in cirrhotic livers differ considerably from those of normal liver; it also reiterates that the manner in which hepatocytes are aggregated into plates and tissue units is markedly abnormal in cirrhosis. Most of the surface alterations probably reflect changes in cellular microenvironments or represent adaptive changes to altered physiologic conditions, such as blood and bile flow. Further studies will be required to clarify specifically the relationships between these morphological changes and the altered functional capacity of the cirrhotic liver.

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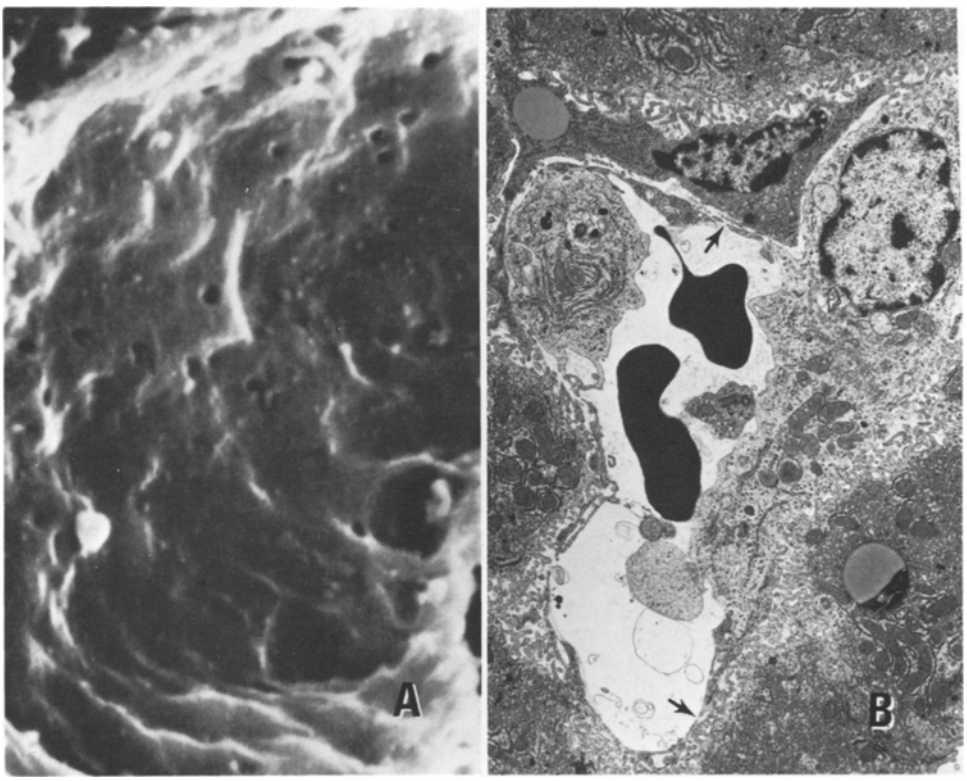

Fig. 10. A An endothelial cell from a sinusoid in a parenchymal nodule contains isolated pores; pore complexes (sieve plates) are not seen. Scanning electron micrograph. ×13,000. **B** A thin section of a similar sinusoid confirms that only a few pores (arrows) are present in the endothelial cells. Transmission electron micrograph. ×4500

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