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MICROCORROSION CASTING IN NORMAL AND PATHOLOGICAL BILIARY TREE MORPHOLOGY

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Abstract

The organization of the intrahepatic biliary tree was studied in three dimensions by scanning electron microscopic (SEM) corrosion casts, in normal and cholestatic rat liver.

In the normal liver the observation revealed the features of the biliary passages from the bile canaliculi to the canaliculo-ductular junction, to the ductules and the bile ducts, confirming previous SEM observations.

In cholestatic liver, the modifications and the proliferation of bile ductules appear clearly.

Resin flow from canalicular to sinusoidal network was never observed.

The method was found to be very useful in the evaluation of the architecture of the intrahepatic biliary tree, under normal as well as under pathological conditions.

KEY WORDS: Corrosion casting; intrahepatic bile tree; liver; bile ducts; bile canaliculi; extrahepatic cholestasis.

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Introduction

During the last decade scanning electron microscopy (SEM) has substantially contributed towards defining the submicroscopic morphology and the tridimensional arrangement of the liver.

Many studies have dealt with the hepatocyte and the sinusoid structures (1,5,6,13,14,18).

Other studies were undertaken to observe the arrangement and the structure of the biliary tree (5,9,12,21).

These fundamental studies have contributed towards clarifying what is currently considered the anatomical subdivision of the intrahepatic biliary tree. A particular contribution has been made in opening the argument about intracellular diverticula of canaliculi (12) and in studying the extension of the contact cell surface bordering canaliculi (6).

Interesting images of the cell surfaces lining periportal ductules and ducts have been obtained (9).

Ductules were characterized by a narrow lumen (2 - 4 μm in width) lined by flat or cuboidal cells. Ductular cells show numerous microvilli, sparse cytoplasmic extensions, corresponding to "primary cilia", and rare cellular evaginations similar to blebs. Ducts are characterized by a larger lumen and denser cilia, 0.2 μm in thickness and 5 - 10 μm in length. A dense connective sheath surrounds the ducts.

These structures and their modifications have been thoroughly investigated in studies of experimental pathology.

Experimental extrahepatic cholestasis has proved to be a very useful experimental model for obtaining elementary liver damages. In fact, it led us to study hepatocyte alterations, canalicular dilatation, ductular proliferation, bile regurgitation, intrahepatic collagen fibrillogenesis and many other pathological features of the liver that are also present in other patho-

logical conditions.

In our previous studies (2,4), rat liver, after extrahepatic biliary obstruction, was observed by means of current SEM and transmission electron microscopic (TEM) techniques in correlation with basic histochemical and hemato-clinical methods.

We demonstrated proliferation of the intrahepatic biliary tree, an increased number of canaliculo - ductular junctions and an increase in the length of the bile canalicular network.

Furthermore, we found support for the theory of the transhepatocytic and ductular pathways of bile regurgitation (2) by retrograde infusion of ferritin into the biliary tree and by observing an intact cytoplasmic barrier separating the bile canaliculi from Disse's space. The apparently weak barrier between the sinusoids and the bile canaliculi lumina has attracted the interest of the researchers studying the onset of jaundice in extrahepatic cholestasis.

Nishi (19), in agreement with Metz et al. (10) and De Vos and Desmet (3), showed the SEM image of a rat bile canaliculus "widely" opening into the space of Disse 14 days after ligation of the common bile duct.

These observations have been supported by freeze etching studies of human patients with extrahepatic cholestasis (22,23).

The hypothesis of a direct communication between Disse's space and the canaliculi has been rejected in other studies that employed different techniques (2,8,25).

Studies employing TEM serial sections do not permit an easy overall view of a tridimensional structure . On the contrary, SEM allows tridimensional observation. But when parenchyme is under study, what is observed is a cross section of the organ obtained by cutting or breaking. This means that mechanical forces are applied to the tissue during specimen preparation, possibly causing artifacts or destruction of details in the area under study.

In the seventies the introduction of SEM microcorrosion cast technique provided the means for observing the tridimensional arrangement of hollow structures, either blood vessels (11) or ducts (16).

The SEM corrosion cast technique was first applied to the liver by Murakami et al. (15) to visualize the peribiliary portal system.

Afterward, using a similar procedure, Nopanitaya et al. (20) studied the terminal distribution of the hepatic artery and Kardon and Kessel (7) conducted a thorough study of the liver microcirculation.

In 1984 Murakami (17) tested a monomeric methacrylate injection medium to prepare microcorrosion casts of the biliary tree in the rat. Bile canaliculi, ductules and ducts were well reproduced. Canaliculi were consistently filled only in the periportal areas.

The present study was undertaken in order to demonstrate the usefulness of microcorrosion casting in the study of the morphology of the intrahepatic biliary tree, in normal and experimental conditions, and in order to contribute to the study of the possible direct connection between vascular and intrahepatic biliary trees.

Materials and methods

30 Wistar rats of both sexes and weighing 200-300 gr were subjected to a double ligation followed by interruption of the common bile duct.Another 10-sham-operated rats served as controls.

Surgical techniques.

The operation was performed with the aid of a Zeiss operating micro-scope at 10X.The rats were anesthetized with an i.p. injection of 2.5-5 ml. of 0.1%Farmotal (50 mg.Thiopental sodium and 30 mg. sodium carbonate) followed by an administration of an ether-oxygen mixture. The animals were then subjected to medial laparatomy during which the liver was displaced up and to the right, the common bile duct was exposed in an extrapancreatic position, a double ligation was performed with 6/0 silk wound thread and a cut made between the two sutures. With the abdominal layers closed, the rats were allowed to awake and were subsequently followed in their postoperative course. The rats were kept on a standard diet for 28 days. This lapse of time was judged necessary to produce a severe cholestasis with its full pathological manifestations.

At this point the casts of the biliary tree were obtained.

Cast preparation.

The rats were anesthetized and the common bile duct was exposed with the same technique as described above. In control animals a 0.8 mm. cannula was inserted into the common bile duct, after ligation of its distal part. In operated animals the cannula was inserted into the proximal stump of common bile duct. The bile was aspirated by means of a hand operated syringe. In order to somewhat open the dead-ended biliary tree, radial cuts about 0.5 cm in depth were performed every cm along the anterior margin of the liver. Then the biliary tree was gently washed for 30 sec with 1.5-2 ml of Ringer's solution.

Subsequently 1.5-2 ml of a 1% solution of glutaraldehyde in 0.1M cacodylate buffer at pH 7.3. was injected for 1 min. then, 1.5-2 ml of

Mercox Cl 2R resin, mixed with a standard amount of its catalyzer, were injected by a syringe under moderate finger pressure. Time was left for polymerization.

Then, the liver was cut into small fragments with a sharp razor blade.

The specimens were macerated in a 15% NaOH solution at room temperature for 24 h. The samples were rinsed in distilled water and then in 5% trichloro-acetic acid solution in order to free casts from tissue remnants.

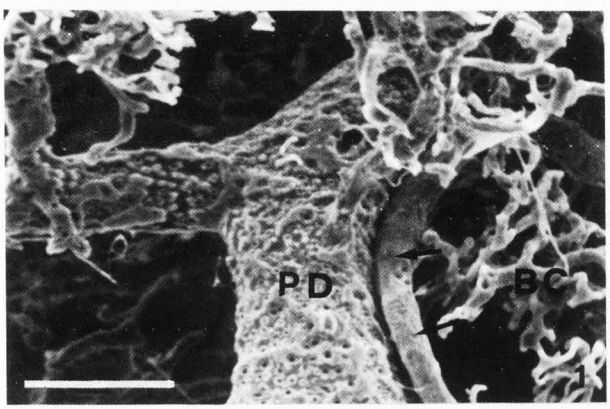
Samples were taken only from casts sufficiently injected. The best perfused areas were found mainly in the periportal regions: they measured 1 to 3 mm. in diameter.

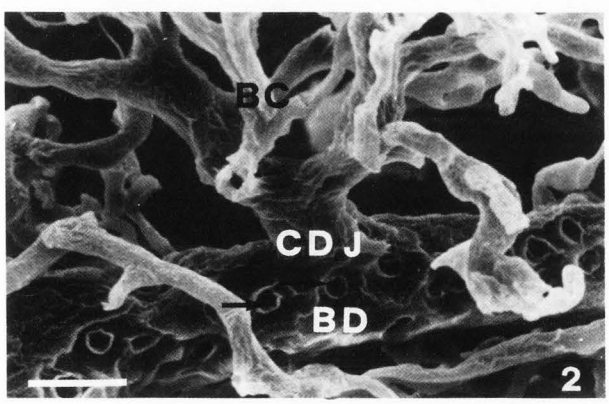
Finally, they were fixed to stubs and covered with gold in an Edwards Sputter and observed by a Cambridge 150 SEM, operated at 15-20 kV.

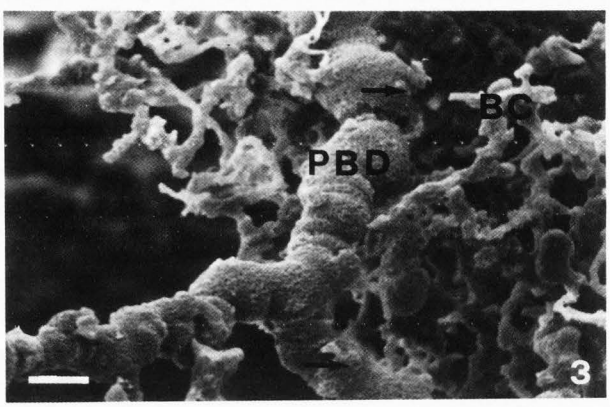
Results

Scanning electron microscopic observation of the cast of normal rat liver provided a three-dimensional detailed description of the biliary tree.

According to the classic description of biliary tree, the bile canaliculi formed a dense interconnecting capillary network, connected with larger tubules (bile ductules) located in perilobular areas (generally in portal tracts); finally, the bile ductules flowed into the portal ducts forming approximately a right angle, after







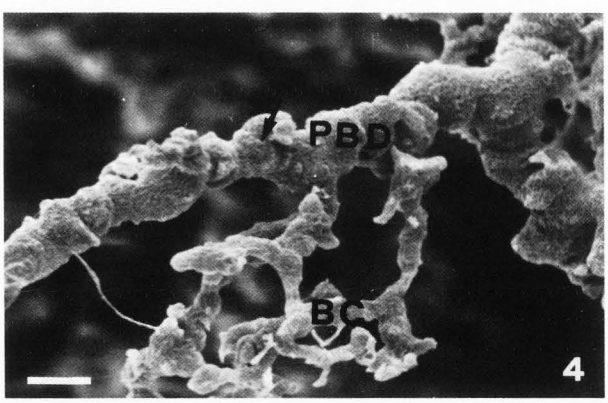


Fig. 1: Normal biliary tree.PD = Portal Bile Duct; BC = Bile Canaliculi; (arrows) bile Ductule; Bar = 100 μm

Fig. 2: Normal biliary tree. BC = Bile Canaliculi; CDJ = Canaliculo-ductural junction; (arrows) = Rounded impression probably due to the nuclei of epithelial cells. BD = Bile ductule; Bar = 10 μm

Fig. 3: Cholestatic biliary tree. PBD = Proliferating bile ductule; BC = Bile canaliculi; (arrow) = Lateral diverticuli and sacculations of proliferating bile ductules; Bar = 10 μm

Fig. 4: Cholestatic biliary tree. PBD = Proliferating bile ductuli; BC = Bile Canaliculi (arrow) = Sacculations and lateral diverticuli; Bar = 10 μm

a course in the perilobular space, running, sometimes, parallel to the bile ducts (Fig. 1).

The bile ducts and ductules exhibited a regular luminal diameter and their course was approximately straight.

At higher magnification, the cast surface of bile ducts and ductules showed the presence of rounded depressions which were absent on the cast surface of bile canaliculi. These rounded depressions, which appeared to be more numerous in the larger ducts, are probably due to the rounded nuclei of epithelial cells (2,4,9) crushed against the connective tissue around the structures.

These circular impressions appear broader than the diameter of cilia and cannot be attributed to membrane processes. We never observed these features in canalicular casts.

The canaliculo-ductular junctions can be identified by the funnel-shaped casts present near the bile canaliculi opening into bile ductules (Fig. 2).

The cast of biliary trees after cholestasis showed a homogeneous pattern of alterations. We observed a noticeable proliferation of bile ductules, which may also be frequently encountered in the centrolobular area (Fig. 3).

The network of bile canaliculi was more dense. At higher magnification bile ductules and bile canaliculi appeared widely dilated with tortuous course, numerous lateral sacculations and dead ends (Fig. 4), according to our previous TEM and SEM observations (2,4).

The cast of normal and pathological biliary trees did not exhibit evidence of the vascular one. In fact, we never observed resin regurgitation from the bile canaliculi into the sinusoids.

Discussion

In agreement with the indications given by Murakami (17) we found microcorrosion cast particularly useful to confirm and elucidate the tridimensional organization of normal biliary tree.

However, problems concerning the injection of a closed system must not be underestimated. Injection of any fluid in the dead-ended bile tree heightens its inner pressure. For this reason we tried to open the system by a series of cuts and we also lowered its pressure by suction of the bile and the perfused solutions. However, some artifacts are possible. For this reason we avoided studying the central part of the classic lobule where the pressure during injection was higher because of the presence of the dead ends of canaliculi. Nevertheless, both under normal and pathologic conditions, corrosion casts showed that the diameters of canaliculi, ductules and ducts were notably larger than in observations by SEM. This indicates a stretching of the bile tree lumen by the resin.

Our observations, corroborated by previous TEM and SEM studies (9, 12, 24), led us definitively to identify the architecture of bile canaliculi, canaliculo-ductular junctions, bile ductules and, in portal tract, bile ducts; moreover they enabled us to evaluate their tridimensional relationships.

On the other hand, the observation of cholestatic casts shows a disarrangement of the normal lobular structure,with an evident ductular proliferation.

The intralobular position of proliferating bile ductules confirms and clarifies previous observations (24).

The corrosion cast technique also provides further details on modifications of caliber, tortuous length, pocketing and side branching of proliferating bile ductules, corroborating our previous SEM and TEM observations (2,4). The method used obviously does not allow one to measure the actual diameters of ducts. We can, however compare different samples obtained from different animals when a standard procedure is followed.

In addition, the fact that both normal and cholestatic biliary tree casts never exhibit resin regurgitation into vascular sinusoidal bed, is in agreement with the hypothesis of the absence of direct communication between the bile canaliculi and the space of Disse, even in advanced cholestasis. This contention is supported by numerous authors (2, 8, 25) but is opposed by some SEM researchers (19). Furthermore, these findings confirm our SEM observations (2), demonstrating a hepatocytic cytoplasmatic strip (0.1 - 1 μm in thickness) always present between the biliary channel and the subendothelial space.

References

1. Brooks SEH, Haggis GH. (1973). Scanning electron microscopy of rat's liver. Application of freeze-fracture and freeze-drying techniques. Lab Invest, 29, 60-64.

2. Carpino F, Gaudio E, Marinozzi G, Melis M, Motta PM, (1981). A scanning and transmission electron microscopic study of experimental extrahepatic cholestasis in the rat. J Submicrosc Cytol 13 581-598.

3. De Vos R, Desmet VJ.(1978). Morphologic changes of the junctional complex of the hepatocytes in the rat liver after bile duct ligation. Br J Exp Pathol 59, 220-227.

4. Gaudio E, Carpino F, Marinozzi G, Melis M, Motta PM. (1981). Biochemical findings and ultrastructural correlations in experimental extrahepatic cholestasis.(Aspetti bioumorali e correlazioni ultrastrutturali nella colostasi extraepatica sperimentale). Epatologia, 27 (6), 221-233.

5. Grisham JW, Nopanitaya W, Compagno J (1976).Scanning electron microscopy of the liver: a review of Methods and Results. In: Progress in Liver Disease, vol. V, chapter 1, (Popper H, Shaffner F eds), Grune and Stretton, Inc, pages 3-27.

6. Grisham JW, Nopanitaya W, Compagno J,Nagel AEH (1975).Scanning electron microscopy of normal rat liver. The surface structure of its cells and tissue components. Am J Anat 144, 295-322.

7. Kardon RM, Kessel RG. (1980).Three Dimensional Organization of the Hepatic microcirculation in the rodent as observed by SEM of Corrosion cast. Gastroenterology 79, 72-81.

8. Koga A, Todo S. (1978).Morphological and functional changes in the junction of the bile canaliculi induced by bile duct ligation. Cell Tissue Res 195, 267-276.

9. Marinozzi G, Muto M, Correr S, Motta P. (1977).Scanning microscope observations of intrahepatic biliary tree. J Submicrosc Cytol 9, 127-143.

10. Metz J, Aoki A, Merlo M, Forssman WG. (1977).Morphological alterations and functional changes of intrahepatocellular junctions induced by bile duct ligation. Cell Tissue Res 182, 299-310.

11. Miodonsky A, Kus J, Tyrankiewicz R. (1980).-SEM study of tracheal vascularization in guinea pig. Arch Otolaryngol 106, 31-37.

12. Motta P, Fumagalli G. (1974). Scanning electron microscopy demonstration of cilia in intrahepatic bile ducts Z Anat Entwickl Gesch 145, 223-226.

13. Motta P, Porter KR. (1974). Structure of rat liver sinusoids and associated tissue spaces as revealed by scanning electron microscopy. Cell Tiss Res 148, 111-125.

14. Motta P, Fumagalli G. (1975).Structure of rat bile canaliculi as revealed by scanning electron microscopy. Anat Rec 182, 499-514.

15. Murakami T, Itoshima T, Shimada S. (1974).-Peribiliary portal system in the monkey liver as evidenced by the injection replica scanning electron microscope method. Arch Histol Japn 37, 245-260.

16. Murakami T. (1971).Application of the scanning electron microscope to the study of the fine distribution of the blood vessels. Arch histol Japon 32: 445-454.

17. Murakami T, Itoshima T, Kusuluma H, Ohtsuk A, Jones AL, (1984). Arch. histol. Japon 47, 223-237.

18. Muto M. (1975). A scanning electron microscopic study on endothelial cells and Kupffer cells in rat liver sinusoids. Arch Histol Japon 37, 286-369.

19. Nishi M, (1978). Scanning electron microscopic study on the mechanism of obstructive jaundice in rats. Arch Histol Japon 41, 411-426.

20. Nopanitaya W, Grisham JW, Aghajanian JG. (1978). Intrahepatic microcirculation: S.E.M. study of the terminal distribution of the hepatic artery. Scanning Electron Microsc 1978; II; 837-842.

21. Nopanitaya W and Grisham JW, (1975). Scanning electron microscopy of mouse intrahepatic structures. Exp Mol Pathol 23, 441-458.

22. Robenek H, Herwig J, Therann H. (1980). The morphologic characteristics of intercellular junctions between normal human liver cells and cells of patients with extrahepatic cholestasis . Am J Path 100, 93-133.

23. Robenek H, Therann H. (1981). A quantitative freeze fracture analysis of gap and tight junctions in the normal and cholestatic human liver 38, 39-56.

24. Schaffner F, Popper H. (1961). Electron microscopy study of normal and proliferated bile ductules. Am J Path 38, 393-410.

25. Tanikawa K. (1979). Ultrastructural aspects of the liver and its disorders. Igaku Shoin, Ltd, New York and Tokyo, 2nd ed, 176-186.