

Scanning Electron Microscopy of the Canals of Hering of the Normal Rat Liver and by Corrosion Casts

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الخلاصة:

اظهر المجهر الالكتروني الماسح نوعان من الاتصال بين القنوات الصفراوية والانابيب الصفراوية في الفار.
١- عدة قنوات صفراوية تتجه لتكوين مايعرف بالامبورة .بعض الامبورات تحاط بخلايا كبدية والبعض الآخر يحاط بصورة رئيسه بالخلايا الكبدية وقسما منها يحاط بالقليل من الخلايا القنويه الصفراء وتكون الخلايا الكبدية والخلايا القنويه الصفراء متلاصقة
٢- تتصل القنوات الصفراوية بالقناة الصفراوية على هيئة تفرعات جانبية. في هذه الحالة، فان جزء من قناة هيرينك يتالف من خلايا كبدية في منطقه الاتصال.
وعند استخدام المجهر الالكتروني الماسح باستعمال الماده المتصلبه (ميروكس) المحقونه بالقنوات الصفراوية، اظهر قناة هيرينك على انها قناة عمياء (مغلقة) ناشئة اصلا من الفصيص كتجمع للقنوات الصفراوية لتكوين الامبورة النازلة إلى القناة البابية والتي تتزايد في الحجم وهي تستلم μ القنوات الصفراوية كتفرعات جانبية.

Abstract :

Scanning electron microscopy (SEM) revealed two types of bile canalicular-ductular junctions in rats.

1- Several bile canaliculi converged to form an ampulla .The ampulla was about 3x6 micro meter. Some ampulla walled mainly by hepatocytes and partly by a few ductular cells.

2- Bile canaliculi connected directly to bile ductule as aside branches. In this case, a part of the wall of the canal of Hering was formed by the hebatocytes at the junction of the bile canaliculus with the canal of Hering.

Scanning electron microscopy (SEM) of corrosion cast revealed canal of Hering as a blind canal originated from the hepatic lobule as a collection of bile canliculi forming ampulla which descended toward the portal tract increasing in size and receiving bile canaliculi as a side branches.

Introduction:

Bile canaliculi are 1-2 μ m in diameter tissue spaces between hepatocytes. Bile canaliculi become wider near the portal zone, where they may be seen emptying into the intralobular canals of Hering.^(1,2)

Since the first description of Hering , the transition part of the bile canaliculus to the bile ductule has been extensively studied by light microscopy^(3,4,5,6) , transmission electron microscopy^(7,8,9,10,11) and scanning electron microscopy^(12,13,14,15).

This part has been described by many synonyms, ampulla, passage de Hering , duct of Hering, intermediate pieces, canalicular-ductular junction, bile pre-ductule or canaliculo-ductular junction¹⁶. Bile canaliculi usually empty into bile ductules , forming an ampulla just before the junction .Sometimes bile canaliculi may also be connected directly with the bile ductules without any sacculation or dilatation .^(16,17)

The canal of Hering are lined partly by cholangiocytes(bile duct cells) and partly by hepatocytes.^(17,18).The junction between the bile canaliculi and the bile ductule is very interesting for liver pathology. The junction was designated as the "Achilles heel" of the biliary system. Obstructive jaundice was explained by bile leak at this junction⁽¹⁶⁾. The canal of Hering have been speculated to harbor intraorgan stem cells of the liver,

perhaps forming the hepatic stem cells "niche" and have been demonstrated to proliferate in disease state^(18,19,20)

Materials and Methods:

Six livers of adult rats, of an inbred albino-swiss strain weighing 200-240 gms, were used in this study. The animals were killed by an overdose of anaesthetic ether. Mammalian Ringers solution containing 0.4% xylocaine as vasodilator, was infused through a 19G needle in the left ventricle to wash out blood from the vascular system. A small opening was made in the right atrium to allow out flow of the blood and the perfusate. The perfusion was carried out through the systemic circulation because there is less chance of over loading the hepatic circulation and causing oedema than with perfusion via the portal vein.

When vascular wash-out was complete, the fixative of A 3% glutaraldehyde solution in millonig's phosphate buffer, PH 7.2-7.4, osmolality 550 mos/I. was used. About 800 ml of the fixative was perfused over a period of thirty to forty- five minutes, under a gravitational pressure of 130 Cm of solution.

The liver was then removed and immersed intact in fresh fixative for a further 72 hours , after which it was rinsed in buffer several times. Three of the livers sliced into 1mm slices by avibratome, the other three livers fractured by the fingers. Then osmicated for one hour by 1% osmium tetroxide in phosphate buffer, followed by rising in several changes of buffer solution. They were then dehydrated through 100% acetone for one hour and three changes of 100% acetone each for one hour. Specimens were mount on stubs using double-side tape, critical point dried and coated with gold, then screened in the-scanning electron microscope(JEOL JSM T300).

Another Six livers of adult rats, of an inbred albino-swiss strain weighing 200-240 gms, were used for corrosion cast in this study. After ligation of the cystic duct, a diluted mixture of commercially available methacrylate injection medium (mercox dainippon –ink co., ltd) was injected into the common bile duct. Injection pressure was kept under 10 Mm Hg .Mercox resin diluted with methyacrylate monomer (3:1).^(21,22) because mercox has high viscosity and is not always suited for full injection into liver. The injected liver was then removed, placed for 30 minutes in warm water bath (60-70 C) After polymerization of resin , the tissue was macerated in 20% NaOH for 24 hours, washed for 12 hours in water, and dried in air. Small pieces of casts were mounted on metal stubs using double-side tape, critical point dried and coated with gold, then screened in the-scanning electron microscope (JEOL JSM T300).

Results:

This study had two aspects .The first one was 3 dimensional study of canalicular-ductular junction (canal of Hering) and the second was corrosion cast study of the canalicular- ductular junction.

Scanning electrone microscope (SEM) makes very clear that the bile canaliculus is formed by a groove passing between adjacent hepatocyt. The bile canalicular-ductular junction has been rarely observed under SEM.

This study showed that bile canaliculi either gathered to form an ampullary dilation at the-periphery of lobule (Fig1). The ampulla walled by hepatocytes only because hepatocytes were devoid of cilia and the bile canaliculi were running between them or bile canaliculus entering canal of Hering as side branches poured into the canal of Hering without sudden changes of the luminal size and without ampullary dilation .In

this case, at the junction of the bile canaliculus with the canal of Hering, a part of the wall of canal of Hering was formed by the hepatocytes(Fig2).

Cross section(C.S.) of canal of Hering showed clearly the canal was walled by hepatocytes and bile ductular cells which abutted on each other directly. The hepatocytes were covered by microvilli and devoid of cilia, in contrast to ductular cells which had one long cilium for each(Fig3). Longitudinal section(L.S.) showed canal of Hering forming trough like structure. The inner side walled by hepatocytes while the outer side walled by bile ductular cells which always adjacent to the connective tissue of the portal tract.

The second aspect was SEM study of corrosion cast of the canaliculer-ductuer junction the commercially available methacrylate (mercox), whose viscosity was lowered by the supplement with methacrylate monomer, was readily injected through the common bile duct into entire biliary tract. The casts were appropriately brittle readily micro dissected with sharpened needle forceps.

Although slight extravasations Happened, but the architecture of the liver lobules were clearly seen as well as bile ducts of the portal tracts of the liver lobule(Fig4). Canal of Hering appeared to be a blind canal originated from the hepatic lobule as a collection of bile canaliculi (ampulla)which descended toward the portal tract increasing in size and receiving bile canaliculi as side branches.(Figs5,6)

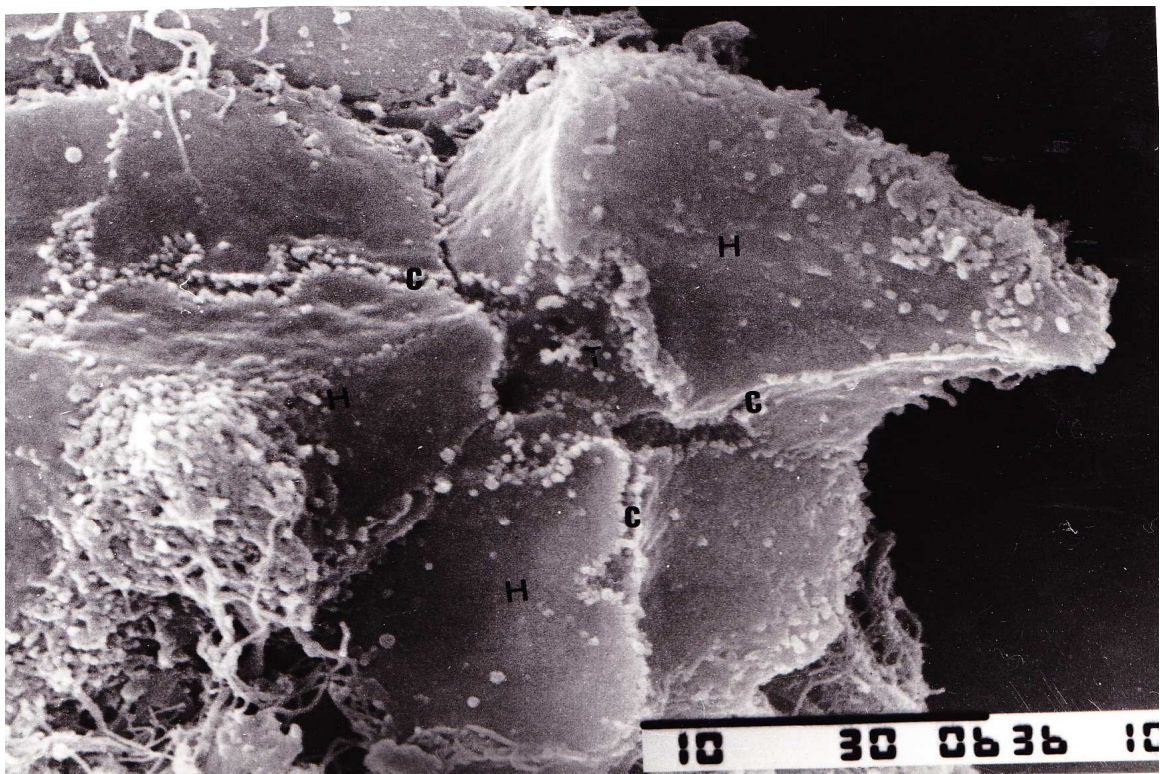


Fig.1: SEM of liver lobule, showing bile canaliculi (C) converging toward an ampulla in the center. Hepatocytes(H) surround the ampulla.

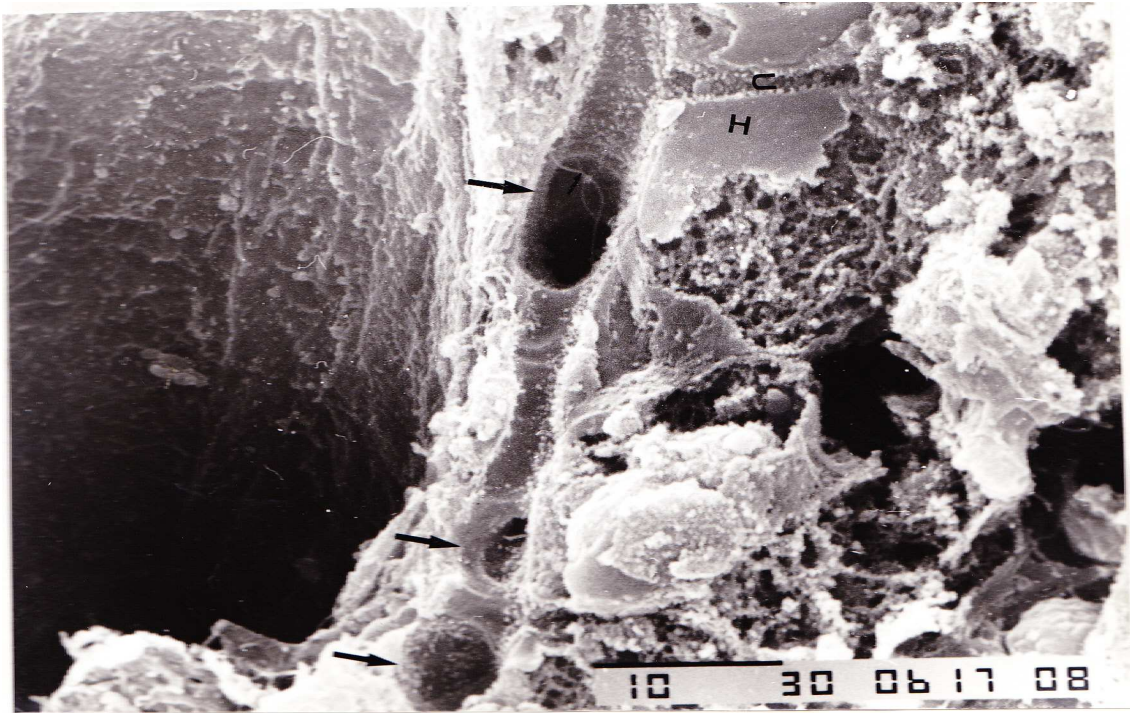


Fig.2: SEM of the limiting plate, showing canaliculus opens directly and separately through hepatocyte (H) into canal of Hering. The wall beside the portal vein consists of ductular cells(arrows) which contain cilia(small arrow).

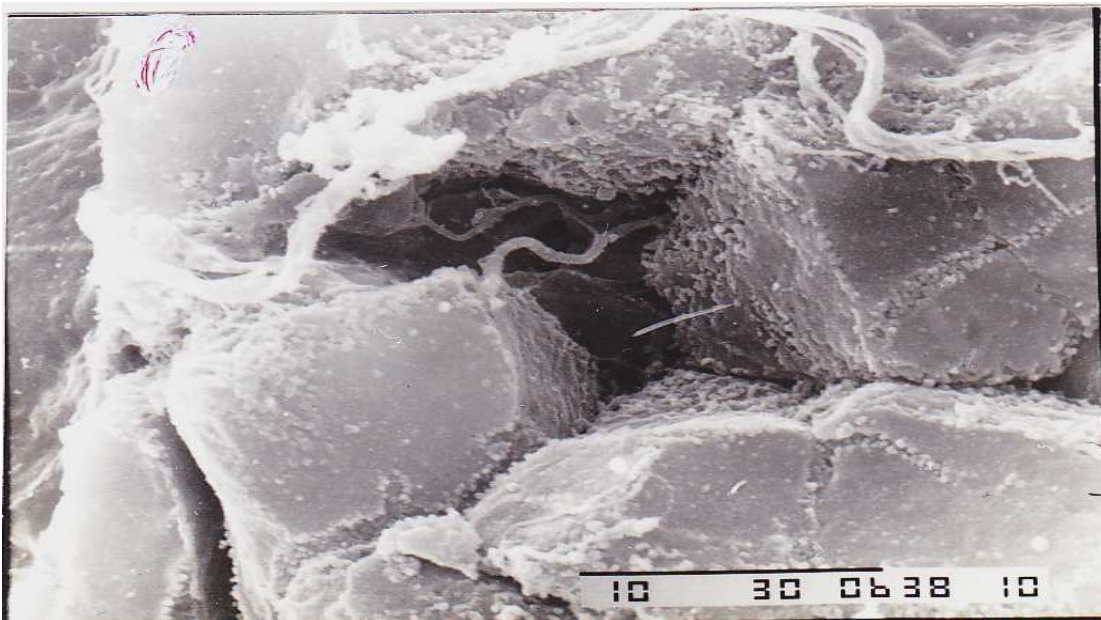


Fig.3: SEM , showing canal of Hering which surrounds by hepatocytes, contain canaliculi and ductular cells, contain cilia. The ductular cells are adjacent to portal vein.



Fig.4: SEM, showing general architecture of hepatic lobules by corrosion cast of biliary tract. L: hepatic lobule



Fig.5: SEM of corrosion cast, showing some bile canaliculi of the hepatic lobule open into a larger ductule which interpreted as canal of Hering , leaving the lobule to portal tract. C:canaliculi, A:ampulla, H:canal of Hering , D:bile duct.



Fig.6: SEM of corrosion cast, showing bile canaliculi open separately and directly into canal of Hering. C:canaliculi, H:canal of Hering , D:bile duct.

Discussion:

Recent studies showed that canal of Hering not only a passage conduct bile from biliary canaliculi in the lobule to the portal tract. Theise etal 1999, demonstrated that the canals of Hering contain hepatic progenitor cells with the capacity to regenerate hepatocytes and bile duct cells in livers with massive hepatic necrosis. ^(18,23)

As mentioned by introduction, the transition part of the bile canaliculus to the bile ductule has been extensively studied by light microscopy, transmission electron microscopy and scanning electron microscopy. The bile canalicular-ductular junction has been rarely observed under scanning electron microscopy. seven junction were observed on over 50 blocks. ⁽¹⁶⁾

This SEM study demonstrated two kinds of bile canalicular-ductular junction .

- 1- Several bile canaliculi converged to a central area of 3-6 micro meter in diameter, forming an ampulla which walled by hepatocytes. some hepatocytes that located beside the connective tissue of portal tracts were small cuboidal or pyramidal. Other ampulla walled by hepatocytes and bile ductular cells. The hepatocytes abutted on the bile ductular cells directly, in agreement with all transmission electron microscopy investigators ^(6,9,10,11) where junction lumen was surrounded by both cell types.
- 2- A bile canaliculus connected directly to the bile ductule as aside branch without forming the- ampulla dilation. The bile ductulal cell has a single cilium as in man. ^(13,14)

Although there were slight extravasations of corrosion cast, the architecture of the liver lobules were clearly seen due to filling of bile canaliculi and portal tract bile ducts by corrosion casts.

The formation of canal of Hering appeared to begin in the hepatic lobule due to convergence of several bile canaliculi to form an ampulla which appeared as the beginning of the canal of Hering which then descend toward the portal tract receiving bile canaliculi separately as aside branches without dilation .

Although canals of Hering were extensively studied, the description of canal of Hering explicitly or implicitly suggest that they, extend at least focally, beyond the limiting plate into the lobule was rare. Most description are vague on the matter, albeit inaccurately, characterized the-canal of Hering as invariably stopping at the limiting plate.⁽²³⁾

This study is a consistent with Theise etal study 1999, confirming the notion of inter lobular extension of the canal of Hering, correcting a common misconception.

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