Three Dimensional Study of the Architecture of the Hepatocytes of The Normal Rat Liver by Scanning Electron Microscope.

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الخلاصة

تعتبر دراسة الكبد بالأبعاد ألثلاثيه من الأهداف ألمهمة في الماضي لحل الغموض الذي يحيط بوظائف الكبد ألمعقده . أجريت هذه ألدراسة بالمجهر ألألكتروني الماسح لخلايا الكبد وأظهرت بأن خلايا الكبد متعددة الوجوه تشكل صفائح من خليه واحده تتفرع و تلتقي وتحتوي كل خليه على ثلاثة وجيهات على الأقل . الأولى وجيهة ألقنيوات ألصفراويه و ألثانيه وجيهة الجيوب ألشعرية ألدموية وألثالثه وجيهة الخلايا ألكبديه ألملاصقه . عند كسر نموذج الكبد بالأصابع ظهرت خلايا الكبد واضحة بالأبعاد ألثلاثية فكان طول الخليه الكبديه 13 مايكرومتر تقريبا وعرضها 11 مايكرومتر تقريبا و هذه ألنتيجة مخالفه لما معروف في الكتب المنهجية للأنسجة و مطابقة للدراسات الحديثة لأنسجة الكبد الحية بواسطة المجهر ألألكتروني متحد ألبؤرة.

Abstract

The three dimensional study of the liver was an aim in the past to unraveling their complex functional mysteries . This scanning electron microscope study showed hepatocytes were polyhedral, multi faced, formed distinct plates of one cell thick. Three distinct facets of hepatocyte surfaces were clearly seen, canalicular surface, intercellular surface and sinusoidal surface. Livers fractured by fingers showed the three dimensions of hepatocytes clearly which were measured about 13µm and 11µm for length and width respectively. This result is on the contrary of the hepatocyte size that mentioned by the famous text books of histology and confirms the recent in vivo studies by the confocal electron microscope.

Introduction

The hepatocyte is probably the most versatile cell in the body. It is a cell with both endocrine and exocrine functions. It also synthesizes and accumulates certain substances, detoxifies others, and transport still other[1].

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It was appeared clear that three-dimensional study of hepatocytes was fundamental to unravel their complex functional mysteries.

Polyhedral hepatocytes form distinct plates (laminae) that are one cell thick in normal livers of adults of all species thus far studied by scanning electron microscope.[1,2,3,4,5,6,7,8]
In human plates are two cell thick until the age of five to six years, when the adult pattern of single cell plates is established.[9]
Plates appear to be continuous except where they are penetrated by sinusoids[6,7] .Plates are thus true muralia and not cords or bars. Hepatocytes are polyhedral, with six or more surfaces with approximately 20-30µm in diameter.[1,10] The surfaces are of three sorts; those exposed to perisinusoidal space; those exposed to the lumen of the bile canaliculus; and those in contact with adjacent liver cells[1]. The canalicular surface is formed by grooves in adjacent hepatocytes . Fractured surfaces disclose unroofed canaliculi (hemi canaliculi) [3,4.5.6.7]. Opened canaliculi measure from somewhat less than 0.5µm to a little more than 1.0µm in width.[6] Bile hemi canaliculi are bordered by short, thick microvilli . Canaliculi are typically located in the centers of hepatocellular plates,and many are straight. In other situations, canaliculi meander over the face of hepatocyte or branch
blindly on the surface of single cell. [6,7] Canalicular branches some times extend with 0.1µm of sinusoidal surfaces (space of Disse).[11]

As noted from reconstructions of serial thin sections studied by transmission electron microscope, direct connections between canaliculi and perisinusoidal spaces have also not been seen by scanning electron microscope.[12]

The so- called flat intercellular surface is not completely structureless, but contains a variety of pits and protrusions that vary some what in different species.[6,7,12]

Hepatocytes of all species contain a variable number of processes resembling microvilli but that are considerably thicker, together with a corresponding number of holes or pits of similar diameter.[3,4,6,7] They are relatively infrequent on rat hepatocytes , with only one or two on each intercellular cleft surface.[6]

The hepatocytic sinusoidal surface face sinusoids and form the hepatocellullar border of the space of Disse. Hepatocellular surfaces facing sinusoids are densely covered with microvilli.[3,4,5,6,7,8]

Materials and Methods

Ten 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 anaesthtic 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 edema 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.[13]

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. Five of the livers sliced into 1mm slices by avibratome, the other Five 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).

Results

Hepatocytes were polyhedral, multifaceted, formed distinct plates (laminae) which were one cell thick (fig1). The length and width of hepatocytes were approximately ranged from 13 to 11µm respectively (fig1,2). In favorably fractured spicements, plates were generally straight. Neighboring plates were separated by sinusoids (figs1,2).

Variations on this simple pattern were seen in (fig2) which shows, perforation in the muralium, occupied by short cross connections between adjacent sinusoids and the second variation was branching of muralium, to form two plates, each one cell thick.

Three distinct facets of hepatocyte surfaces were clearly visible with the scanning electron microscope (SEM):

1. Canalicular surface, opened, straight, central canaliculi were measured about 1.0µm in width. Microvilli were clearly concentrated at their lateral margins. Canaliculi were typically located in the centers of hepatocellular plates and in many areas they were straight and unbranched(fig1,2). In other situations, canaliculi meandered over the face of hepatocytes (fig3,4). Blindly ending canalicular branches some times extended close to sinusoidal surfaces (fig4), but direct connections between canaliculi and perisinusoidal spaces were not seen. Canaliculi were appeared along the hepatic plates, both in transversal and in perpendicular directions forming a meshwork (fig5).
2. Intercellular surface , cell surfaces laterally bordering canaliculi were the smoothest areas of hepatocyte membrane. Although these areas contained attachment complexes, the latter were not visible at the resolution attainable with SEM. Numerous holes and small protrusions were present on the flat surfaces (figs3,4). Protrusions were often located close to holes, protrusion was frequently present on the lip of a hole (fig4).
3. Sinusoidal surface. Hepatocellular surfaces facing sinusoids were densely covered by microvilli which largely filled the perisinusoidal space of Disse. At the sharply angled corners of hepatocytes, perisinusoidal space were contiguous with canaliculi (fig2,4).

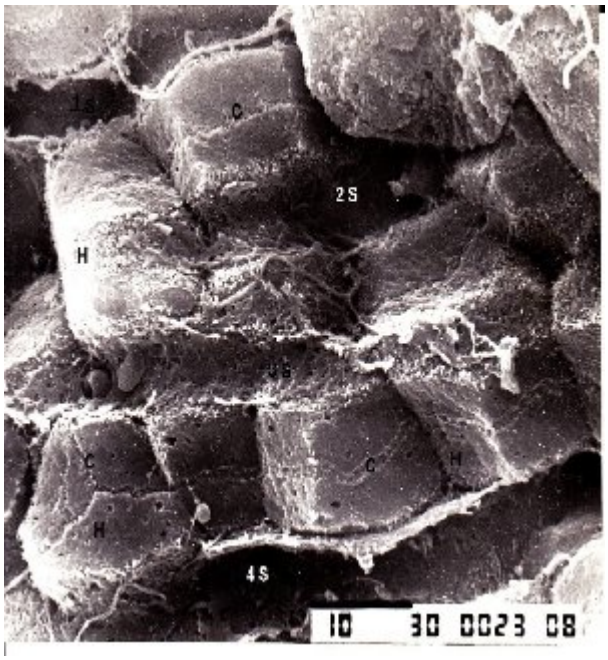

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Fig1: SEM of fractured liver spicement, showing the polyhedral, multifaceted hepatocytes which form one cell thick plates (muralia) separated by sinusoids, bile canaliculi run in the middle of hepatocytes. H:hepatocyte S:sinusoid C:bile canaliculus

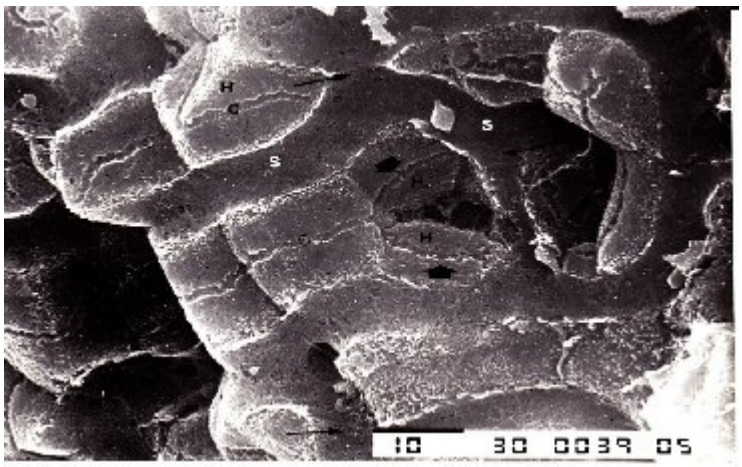

Fig2: SEM of fractured liver spicement shows
i. branching of muralium to form two plates, each one cell thick (arrow heads).
ii. cross connections between sinusoids , perforating the muralium (arrows). H:hepatocyte S:sinusoid C:bile canaliculus

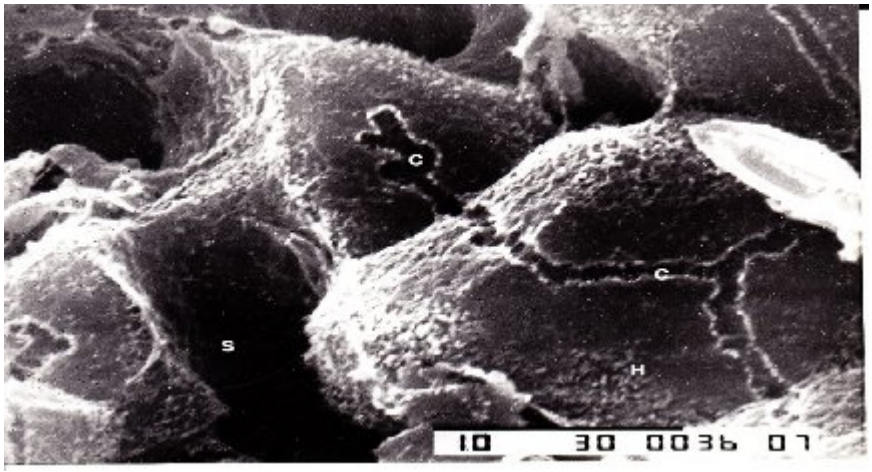

Fig3: SEM of fractured liver spicement shows, bile canliculi meander over the face of hepatocyte. H:hepatocyte S:sinusoid C:bile canaliculus

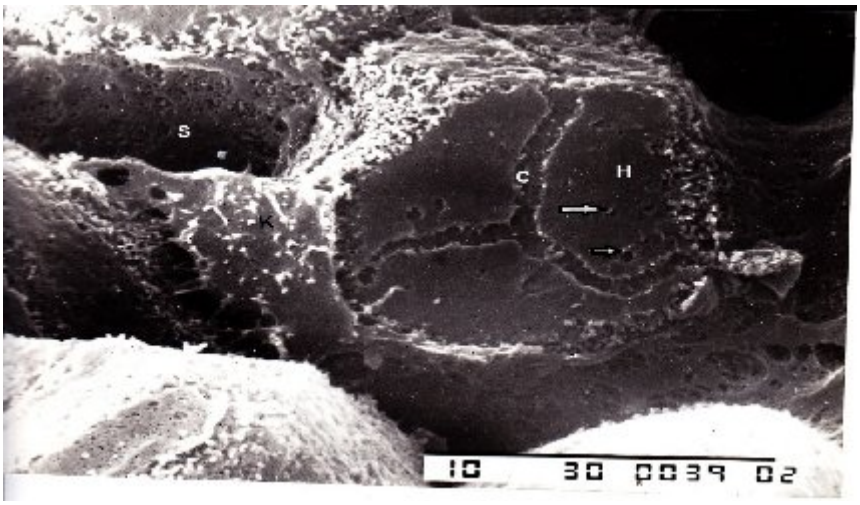

Fig4: SEM of sliced liver shows, branching bile canaliculi which end very close of perisinusoidal space of Disse. Note, protrusions and holes (arrows) H:hepatocyte S:sinusoid C:bile canaliculus K:Kupffer cell

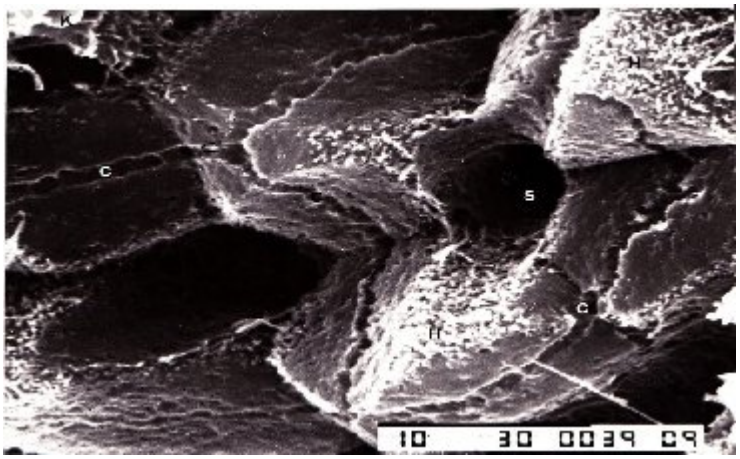

Fig5: SEM of fractured liver spicement shows, canaliculi in both longitudinal and transverse directions forming a meshwork.
H:hepatocyte S:sinusoid C:bile canaliculus.

Discussion

The assessment of tissue architecture is essential part of the understanding of physiology and disease of human organs.[14]

The three dimensional study of the liver was an aim in the past, so that the tissue of the liver have been extensively studied. As shown by introduction, there is general agreement that hepatocytes form laminae that are one cell thick in normal livers of adult of all species thus far studied by scanning electron microscope. This study confirms plates are true muralia of one cell thickness and not cords or bars in rats. The livers fractured by fingers were showed clearly and by three dimensional view the individual hepatocytes (fig1), which appeared polyhedral, having three sorts of surfaces, canlicular surface, the smooth intercellular surface which contains numerous small protrusions and holes of the same size which may increase the coherence of hepatocytes. The third surface is the sinusoidal surface. These results confirms established knowledge as shown in introduction. [1,2,3,4,5,6,7] This study was showed hepaocytes, measured about 13µm for length and 10µm for width. This result contradicts the established knoledges that mentioned in famous text books of histology, Leslie P.etal (2001) [10] and Anthony I. etal (2010). [1] At the same time in agreement with Vanessa Campo-Ruiz etal (2005), who described the size of hepatocyte

ranges from 12.8 to 13.1µm by the first in vivo study of the liver by confocal electron microscope.

Plates appeared to be continuous except where they were penetrated by sinusoids, this result in agreement with the study of Motta P, etal (1975).

Canaliculi typically occupy the centers of the intercellular face of hepatocyte, and usually are as straight as the plates of which they are apart. However, when the hepatocytes become sharply angulated, canaliculi meander over the face of individual hepatocytes and they some times have branches that end blindly near the sinusoidal surfaces with out apparently directly connecting with space of Disse of the sinusoid. This finding confirms the result of (Compagno and Grisham (1974) ; Motta and Fumagalli (1975). And also confirms study of Matter etal (1969) from utilizing reconstructions of transmission electron microscope photographs, obtained from serial thin section which shows tips of canaliculi branches come close to the space of Disse, with out apparently directly connecting with space of Disse. In these areas bile regurgitation may occur under experimental or pathological conditions[15]. Canaliculi were running along the hepatic plates, both in transverse and longitudinal directions. This finding confirms the result of Vanessa Campo-Ruiz etal (2005).

Lastly, it is believed- with due modesty-, the illustrations produced are in some cases of better quality than published illustrations.

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