www.jpnim.com Open Access eISSN: 2281-0692
Journal of Pediatric and Neonatal Individualized Medicine 2014;3(2):e030206
doi: 10.7363/030206
Published online: 2014 Jun 14

Mini Atlas

Scanning electron microscopy in liver biopsy interpretation in children: a mini atlas

Cristina Mocci[1], Terenzio Congiu[2], Daniela Fanni[1], Clara Gerosa[1], Mina Komuta[3], Peter Van Eyken[4], Gavino Faa[1], Alessandro Riva[4]

[1]Department of Surgical Sciences, Division of Pathology, University of Cagliari, Italy
[2]Department of Morphology, University of Varese, Italy
[3]Department of Pathology, University of Leuven, Belgium
[4]Department of Pathology, Ziekenhius Oost Limburg (ZOL), Genk, Belgium

Abstract

We have extensively studied the ultrastructural picture of the liver by scanning electron microscopy (SEM) but these studies were not used, up to now, in clinical practice because they were considered to be mainly a means of research in the 3D structure of liver specimens. Our new technique allows us to introduce ourselves to the 3D structure of intracellular organelles, making it possible to study them in normal and pathologic conditions. We used a very small part of the liver biopsies from 5 children aged 3 to 8 years old, who underwent a liver biopsy for diagnostic purposes. The specimens were fixed and processed according to our modification of the OsO_4 maceration method of Tanaka and Mitsushima. Liver biopsies fixed for 20' in a mixture of glutaraldehyde and paraformaldehyde, postfixed in 1% OsO_4 for 2 h, cut with a tissue sectioner and then macerated in 0.1% OsO_4 for 60 h at room temperature. Specimens were dehydrated in graded acetone, critical point dried and coated with gold palladium. To selectively remove cell components, some specimens were subjected to ultrasound treatment (25 Hz for 1') prior to dehydration. To demonstrate the hepatic stroma, some aldehyde-fixed specimens were submitted to maceration with NaOH 1N according to Ohtani method. With this method, all cells were removed, allowing the visualization of collagen fibers. Observation was carried out by an Hitachi S4000 Field Emission SEM (Hitachi High-Technologies Co., Tokyo, Japan) operated at 20 kV. We are showing the results of our new technique applied to the liver tissue. These data open, in our opinion, a new field in the research of nuclear pathology, with possible intriguing data on pathological nuclear pore changes in the setting of different liver diseases.

Keywords

SEM, osmic maceration, liver biopsy, mitochondria, bile canaliculi, nucleus.

Corresponding author

Cristina Mocci, Department of Surgical Sciences, Division of Pathology, University of Cagliari, Italy; email: cristina_mocci@libero.it.

How to cite

Mocci C, Congiu T, Fanni D, Gerosa C, Komuta M, Van Eyken P, Faa G, Riva A. Scanning electron microscopy in liver biopsy interpretation in children: a mini atlas. J Pediatr Neonat Individual Med. 2014;3(2):e030206. doi: 10.7363/030206.

Introduction

The ultrastructural picture of liver by scanning electron microscopy (SEM) has been extensively described in different diseases in several studies [1-3]. The observation of the specimens started with a low power view, 3X, showing the overall structure of the biopsy, with its margins, it continued with a 3,000X magnification view that showed the biliary canaliculi from the inside and from the outside, and went on to a 60,000X view to show us the mitochondria in their different shapes and environment. Also the nuclear membrane and its pores were seen. The introduction by Tanaka and Mitsushima [4] of the OsO_4 maceration method changed the approach to the study of hepatic diseases by SEM, allowing the visualization of cellular organelles. The use of OsO_4 maceration method was restricted at the very beginning mainly to the study of tissues from experimental animals due to technical difficulties in processing the specimens. The modification introduced by Riva et al. allowed a faster and a simpler tissue processing that could be applied easily to human biopsies [5]. The aim of this study was to analyze, by SEM, the cytoplasm of liver cells in children, applying the osmium maceration method (developed in our laboratory) [5], which allows the tridimensional study of intracellular organelles in human biopsies.

Materials and methods

Five patients affected by liver disorders underwent liver biopsy from the right lobe. Liver specimens, from the liver biopsies, were fixed and processed according to our modification of the OsO_4 maceration method of Tanaka and Mitsushima [4]. In brief, liver specimens were fixed for 20' in a mixture of glutaraldehyde and paraformaldehyde, were postfixed in 1% OsO_4 for 2 h, cut with a tissue sectioner and then macerated in 0.1% OsO_4 for 60 h at room temperature. Specimens were dehydrated in graded acetone, critical point dried and coated with gold palladium. To selectively remove cell components, some specimens were subjected to ultrasound treatment (25 Hz for 1') prior to dehydration. To demonstrate the hepatic stroma, some aldehyde-fixed specimens were submitted to maceration with NaOH 1N according to Ohtani et al. [8]. With this method, all cells were removed, allowing the visualization of collagen fibers. Observation was carried out by an Hitachi S4000 Field Emission SEM (Hitachi High-Technologies Co., Tokyo, Japan) operated at 20 kV.

Results

At very low power, SEM allows a panoramic view of the biopsy sample (**Fig. 1**). At this enlargement, it is possible to evaluate the margins of the needle biopsy core. Moreover, the finding of portal tracts and terminal veins, separated by the hepatocytes in the absence of bridging septa, allows the observation of an important element which is the preserved liver architecture. At higher power, portal tracts appear as distinct entities characterized by a collagen background homing portal veins and hepatic artery branches (**Fig. 2**). At this enlargement, bile ducts are not clearly evidenced. In contrast, terminal veins (also called central veins) appear as one single structure with a large lumen, characterized by a fenestrated endothelium allowing the confluence of periterminal sinusoids. The analysis of the interior of the vein evidences the prominent nuclei of the endothelial cells covering the vein lumen. Bile ducts appear as tubular structures irregular in shape, differing from the other portal structures for the presence of an epithelial lining (**Fig. 3**). Ductal epithelial cells are characterized by an oval nucleus and a scant cytoplasm. Bile ducts are embedded in a collagen milieu. Kupffer cells appear as irregularly-shaped cells, characterized by a folded cell membrane and by the presence of multiple arms extending from the cell body towards the sinusoidal borders (**Fig. 4A**). The interior of Kupffer cells, after osmium maceration, is characterized by an high number of cytoplasmic vesicles representing lysosomes and fagolysosomes, all typical of macrophages (**Fig. 4B**). By SEM after maceration, following removal of the cytosol, it is possible to analyze the cytoplasmic architecture, including the cytoplasmic organelles, such as mitochondria, and endoplasmic reticulum (**Fig. 5**). At higher power, mitochondria appear

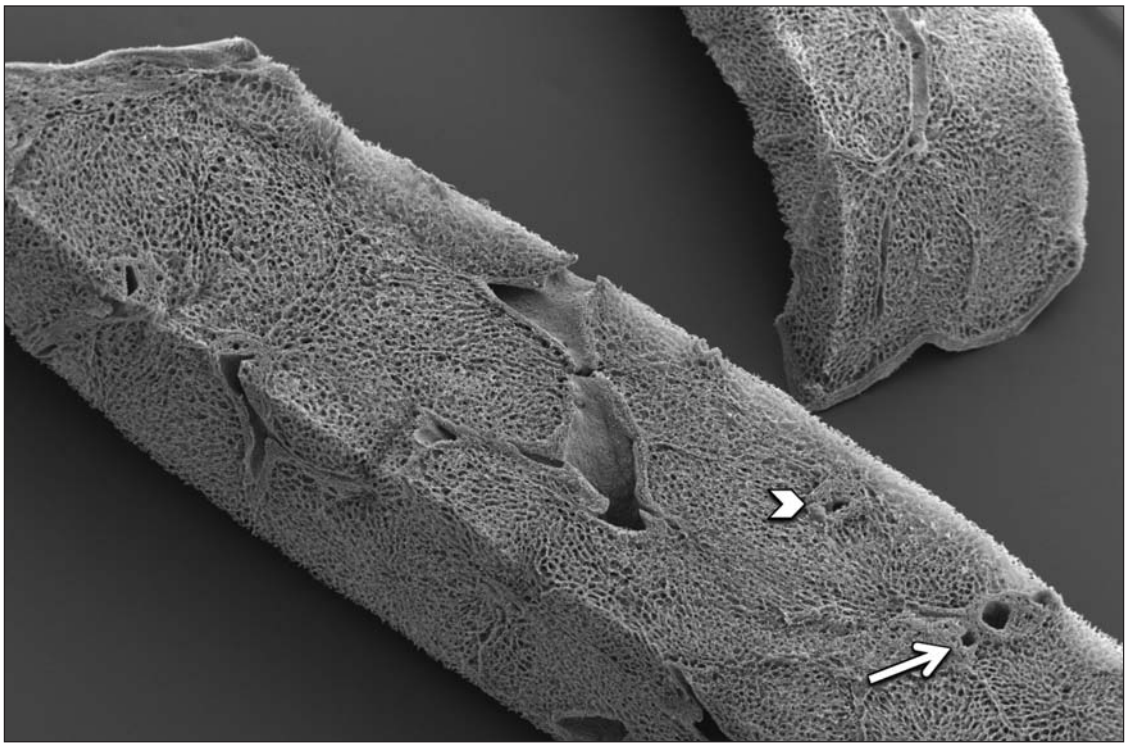

Figure 1. Low power (31X) view of a liver biopsy SEM with osmic maceration with portal tracts (arrow) and terminal veins (arrowhead), separated by the hepatocytes in the absence of bridging septa.

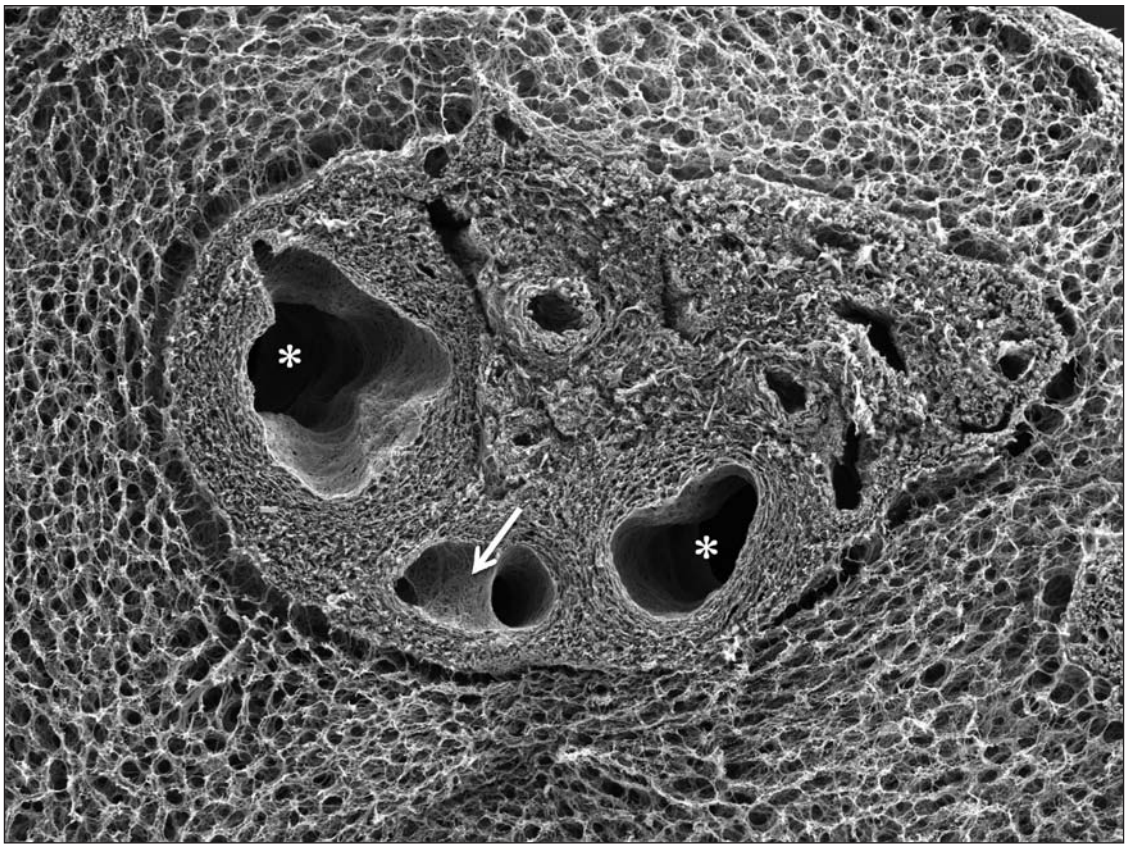

Figure 2. At higher power, portal tracts appear as distinct entities characterized by a collagen background homing portal veins (asterisks) and hepatic artery branches (arrow).

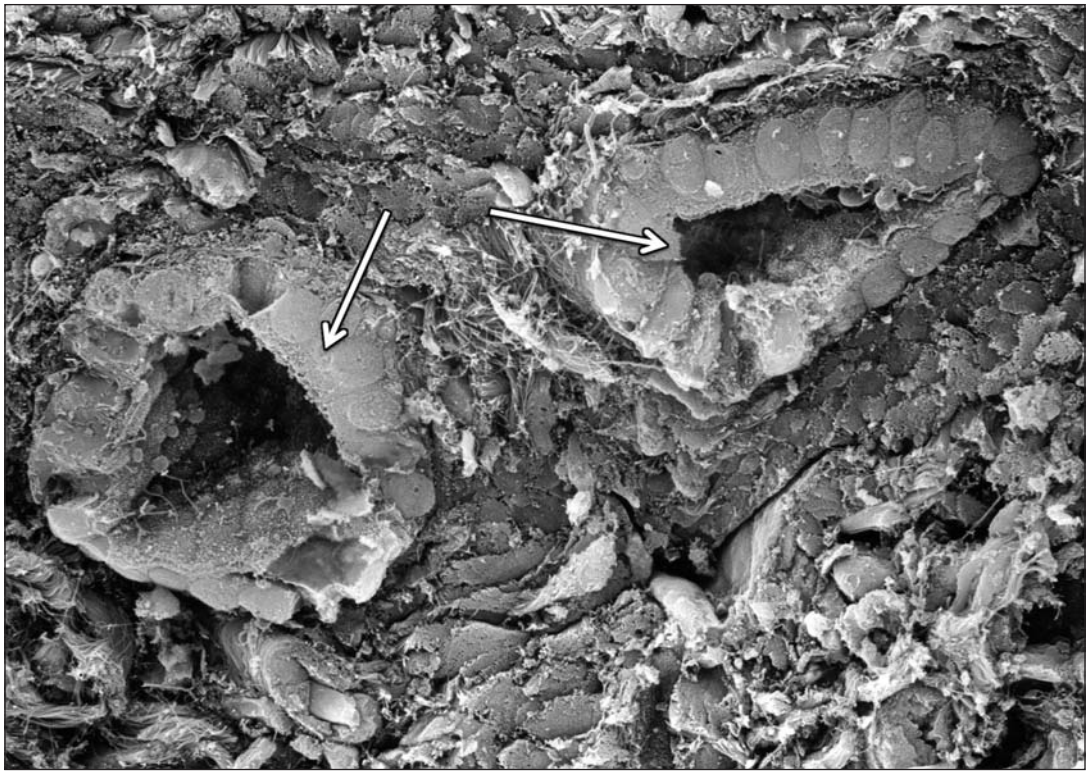

Figure 3. Two biliary ducts at a portal space with a clear epithelial lining (arrows) (600X).

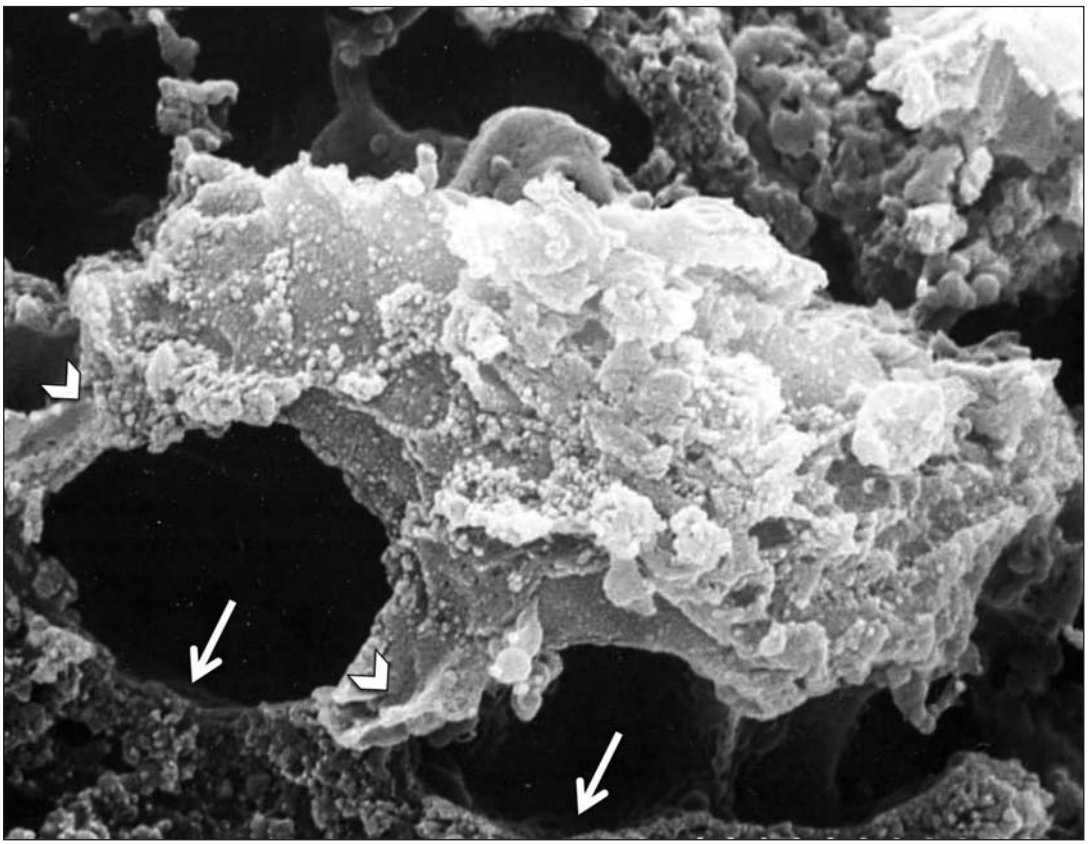

Figure 4A. Kupffer cells appear as irregularly-shaped cells, characterized by a folded cell membrane and by the presence of multiple arms (arrowheads) extending from the cell body towards the sinusoidal borders (arrows).

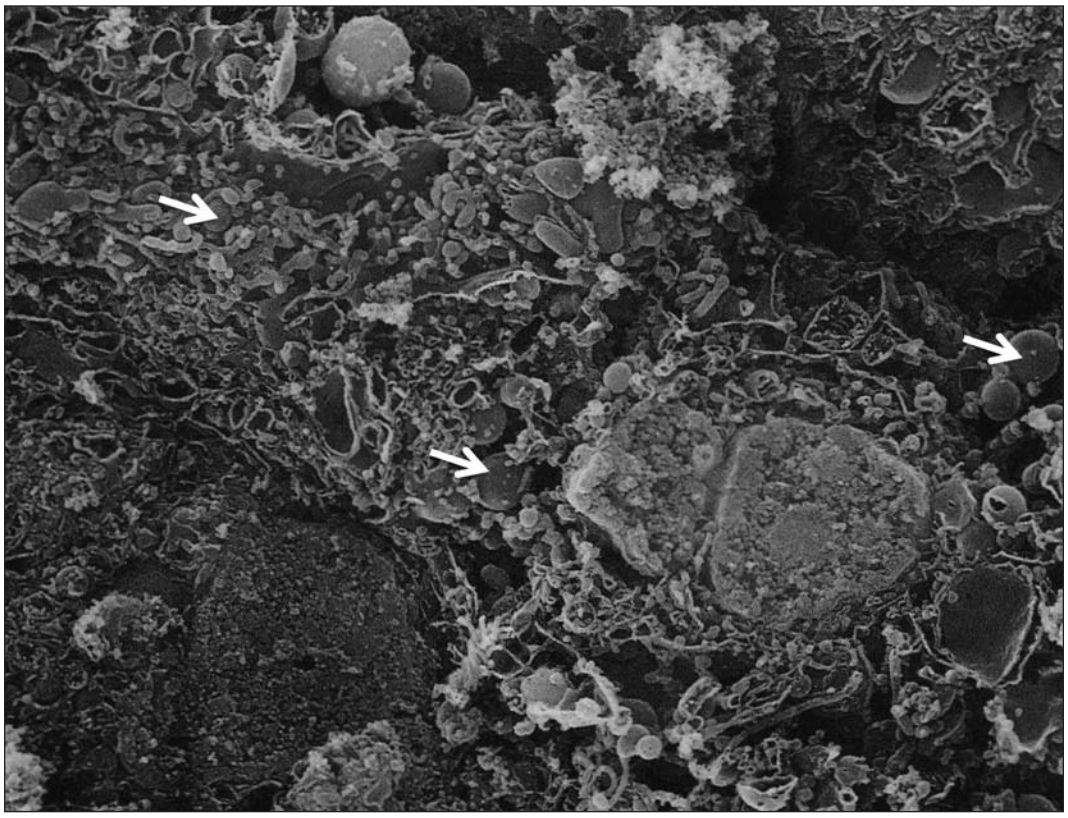

Figure 4B. The interior of Kupffer cells, after osmium maceration, is characterized by a high number of cytoplasmic vesicles (arrows) representing lysosomes and fagolysosomes, all typical of macrophages (6,000X).

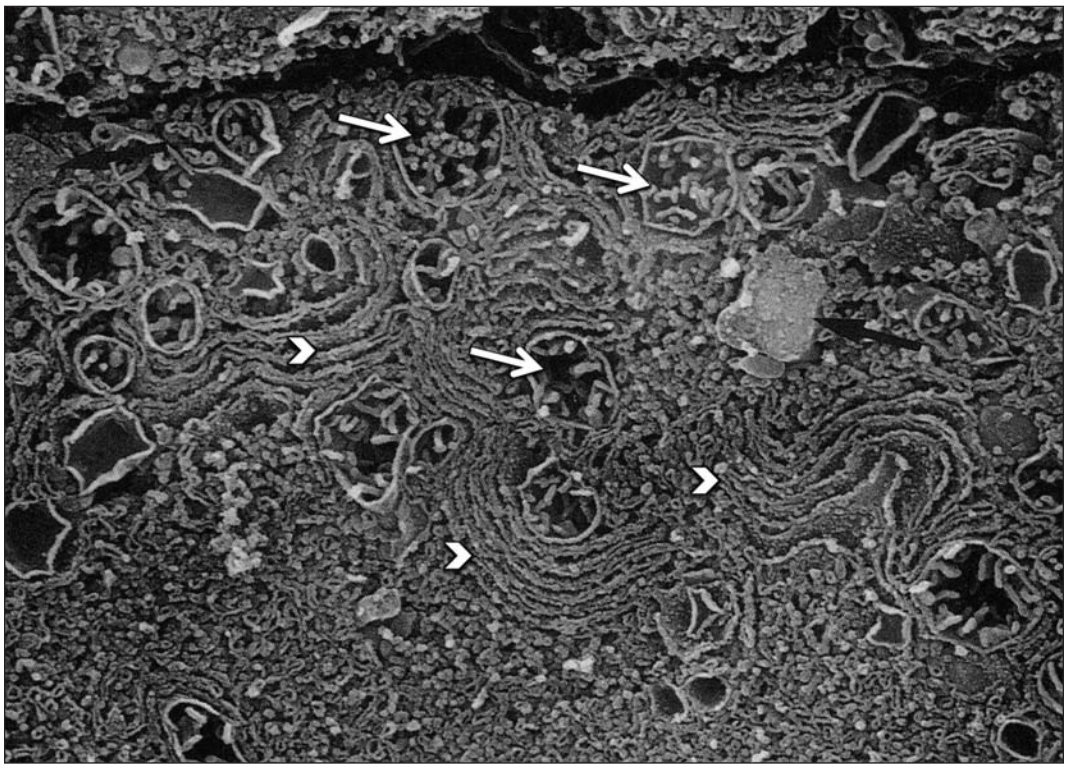

Figure 5. Following removal of the cytosol, it is possible to analyze the cytoplasmic architecture, including the cytoplasmic organelles, such as mitochondria (white arrows), and endoplasmic reticulum (arrowheads) (8,000X).

as tubular structures branching into the cytoplasm and intermingled between the endoplasmic reticulum (**Fig. 6A**). At this enlargement, thanks to the maceration method, it is possible to have a 3D image of mitochondria that are suggestive for one tubular glomerular-type structure. At higher power (**Fig. 6B**), our method allows a fine study of the interior of the mitochondria, allowing a better evaluation of cristae volume and architecture. Golgi apparatus appears as a sponge-like body, surrounded by multiple vesicles putatively containing export proteins, some of which may be observed when emerging from the trans-Golgi network (**Fig. 7**). The osmium maceration method allows the 3D study of the sinusoidal lumen and, in particular, of the sinusoidal wall (**Fig. 8**). The cell membrane of sinusoidal cells is characterized by the presence of fenestrae. By our method, it is possible to analyze the diameter of sinusoidal fenestrae and their shape. When large fenestrae are present in the field, the Disse space structures may be analyzed. Biliary ducts may be studied in deep with the maceration method. At low power (**Fig. 9A**), our technique allows the study of the extension of biliary canaliculi from one hepatocyte to the next, clearly evidencing the continuity of the biliary structures extending along the liver trabecules. At higher power (**Fig. 9B**), after detaching two hepatocytes by our technique, the external side of the hepatocyte cell membrane is characterized by the emergence of multiple extensions, probably representing the adhesion structures between one hepatocyte and the next. Detachment of the neighboring hepatocyte allows the 3D study of the interior of the biliary canaliculus: at this power, it is possible to analyze the number and the distribution of biliary villi, other than the extension of the canaliculus towards the different zone of the hepatocytic membrane (**Fig. 9B**). At high power, our method allows the ultrastructural study of biliary cell villi (**Fig. 9C**). The granular structure observed at the bottom of the figure putatively represents the components of the tight junctions of bile canaliculi. After removing the entire cytoplasm of hepatocytes, by our method it is possible to analyze the hepatocyte cell membrane from its interior side, following the extension of bile canaliculi (**Fig. 10A**). At higher power (**Fig. 10B**), bile canaliculi seen from the interior of hepatocytes appear as sponge-like structures, each hole corresponding to the origin of a single villus. Multiple small vesicles are seen in strict contact with the canaliculus, possibly representing the marker of the transport of substances from the hepatocytic cytoplasm into the biliary lumen. The study of the hepatocytes' nuclei is also highlighted by our method. The exterior side of the nuclear membrane (**Fig. 11A**) shows marked irregularities and infoldings of the nuclear membrane, that is characterized by the presence of multiple holes representing the nuclear pores. When nuclei are cut and chromatin removed, it is possible to observe the interior side of the nuclear envelope (**Fig. 11B**). Nuclear pores appear to be unevenly distributed throughout the nuclear membrane, also showing marked differences regarding their diameter. At high power, the complex structure of nuclear pores is better revealed (**Fig. 11C**): each pore is surrounded by numerous globular subunits, different in shape and dimensions, introducing a previously unrecognized variability in the structure of nuclear pores.

Discussion

The preliminary data here reported on the usefulness of SEM applying the Riva's maceration method in the analysis of liver biopsy show that this new technique might represent a useful tool in liver biopsy interpretation. The most relevant advantages of this technique, as compared to traditional transmission and scanning methods, are here highlighted.

This method, paralleling the classical scanning methods, allows the study of liver specimens at very low powers, overlapping the scanning study with optical data, as clearly shown in **Fig. 1**. The study of the borders of the liver biopsy core may have some relevance in the interpretation of small liver biopsies, particularly in pediatric patients, due to the low size of the needle utilized. In fact, a sharp border, similar to that shown in **Fig. 1**, is suggestive for a preserved architecture, whereas an irregular border is suggestive for a disrupted architecture, due to the presence of bridging septa. This evaluation is not allowed by transmission electron microscopy (TEM). Our maceration method allows the 3D study of the interior of the cytoplasm of the hepatocytes. In our opinion, on the basis of these preliminary data this datum represents the major advantage of our technique. By scanning the cytoplasm, after removing the cytosol, we were able to obtain a new vision of the cytoplasmic organelles, as well as of the nuclei. Mitochondria, appearing at TEM as multiple roundish bodies, revealed by this method a tubular branching structure, each body possibly representing the section of a mitochondrial tip

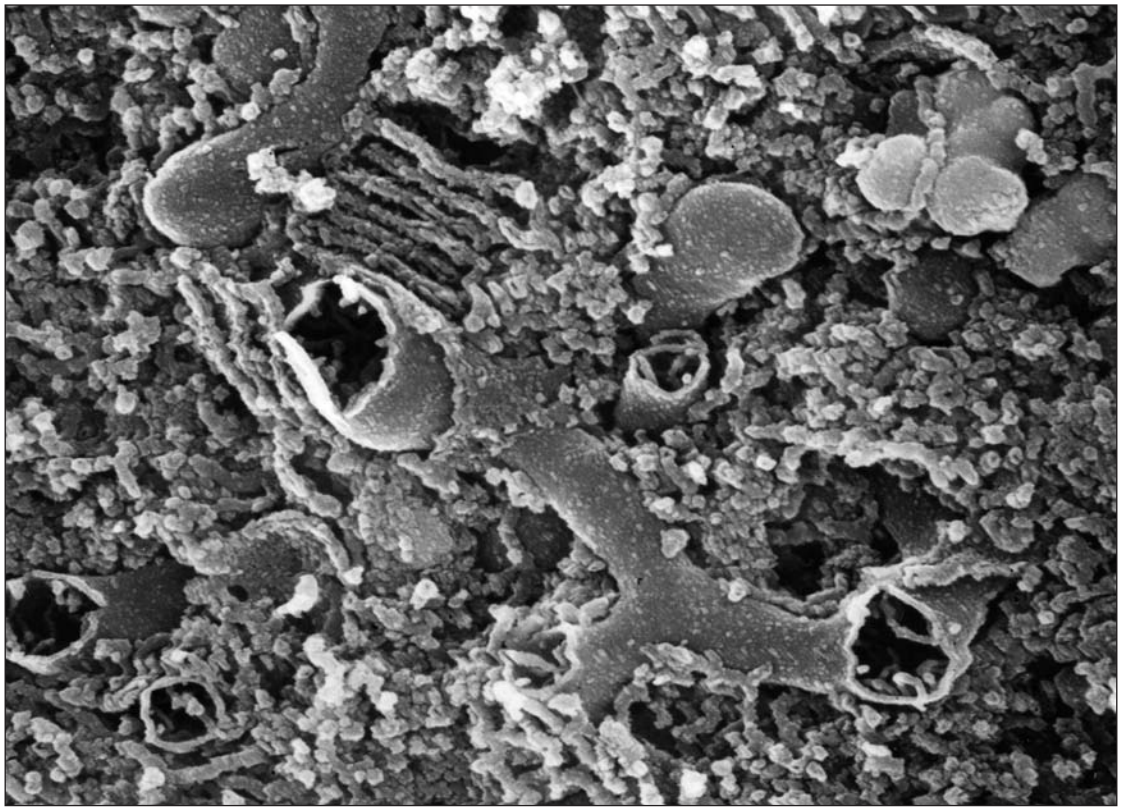

Figure 6A. At higher power (10,000X), mitochondria appear as tubular structures branching into the cytoplasm and intermingled between the endoplasmic reticulum.

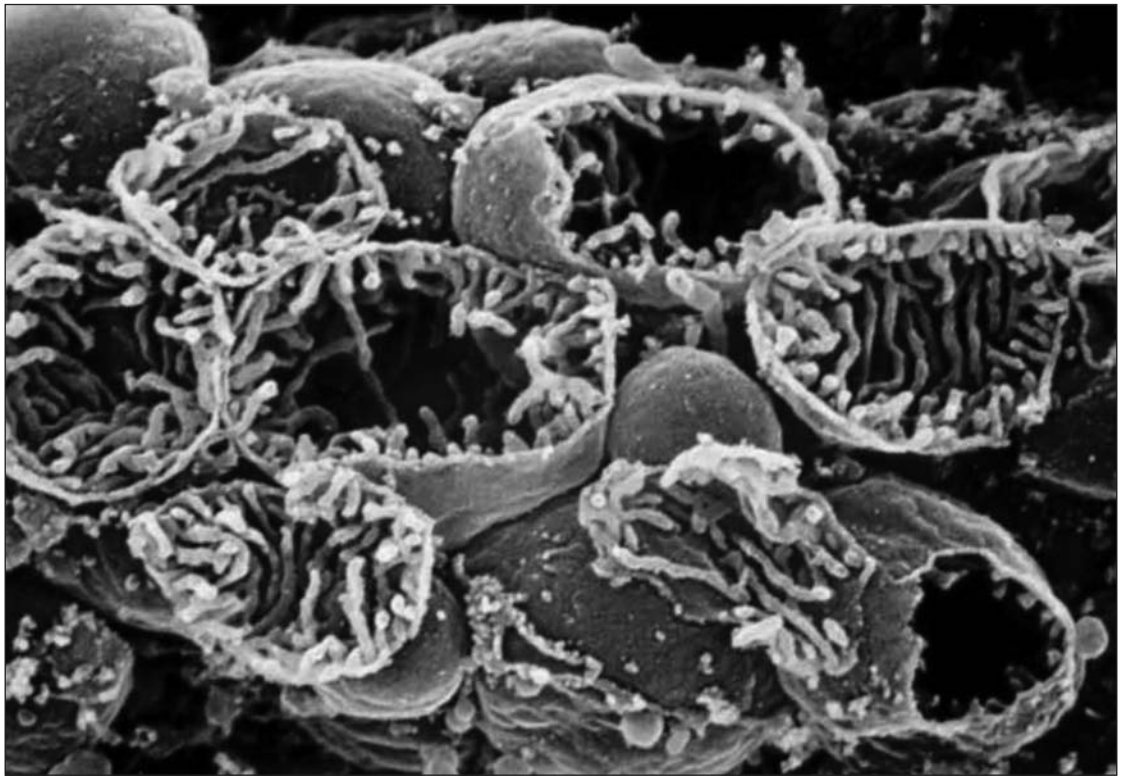

Figure 6B. At higher power (13,000X), interior of the mitochondria is presented, allowing a better evaluation of cristae volume and architecture.

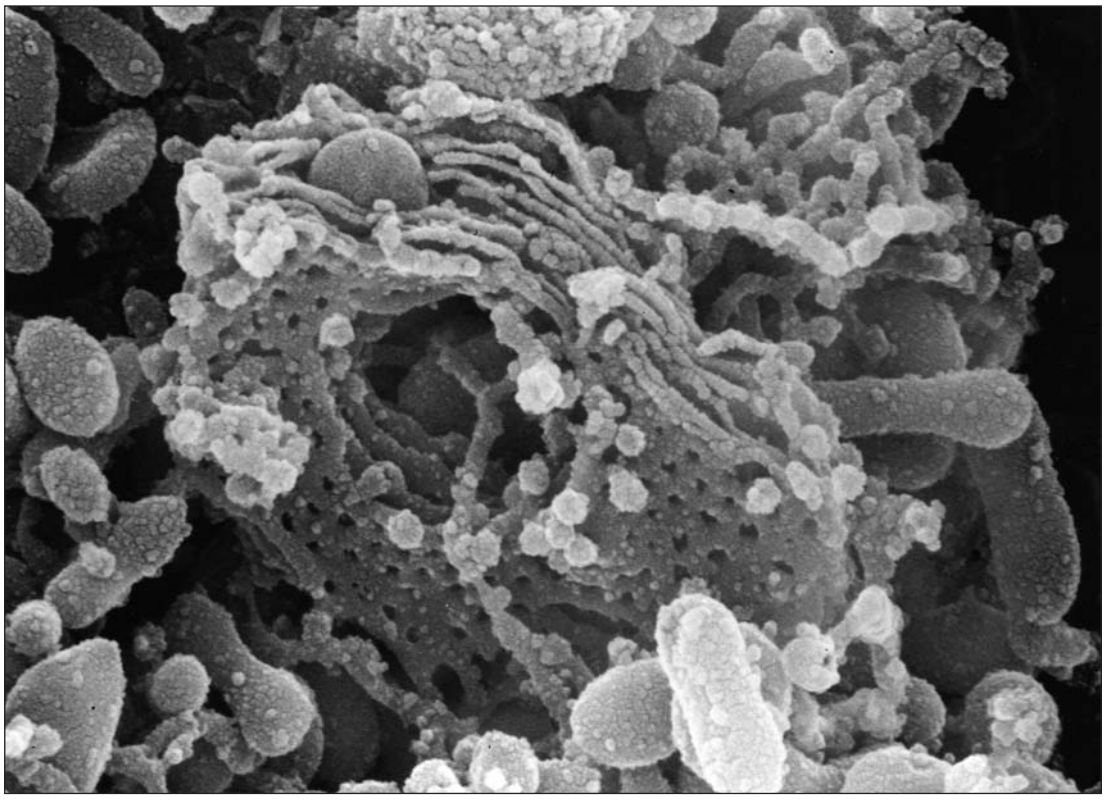

Figure 7. Golgi apparatus appears as a sponge-like body, surrounded by multiple vesicles putatively containing export proteins, some of which may be observed when emerging from the trans-Golgi network (15,000X).

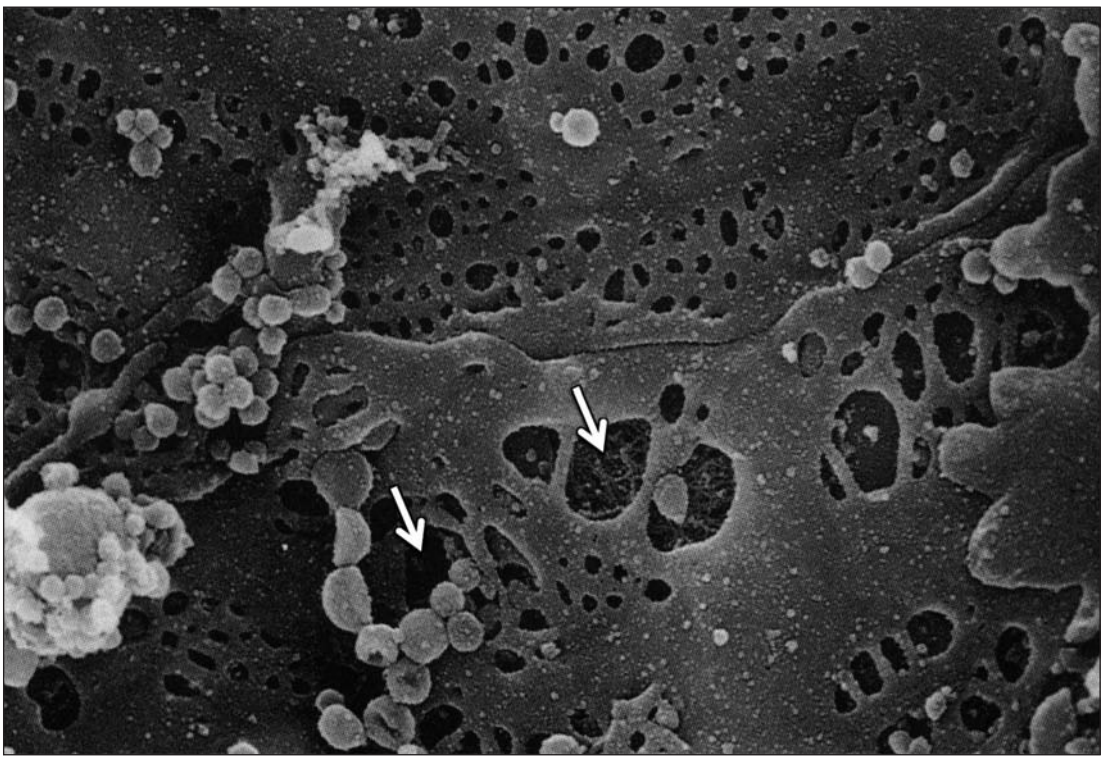

Figure 8. The osmium maceration method allows the 3D study of the sinusoidal lumen and, in particular, of the sinusoidal wall, characterized by the presence of fenestrae. When large fenestrae are present in the field, the Disse space structures may be analyzed (arrows) (11,000X).

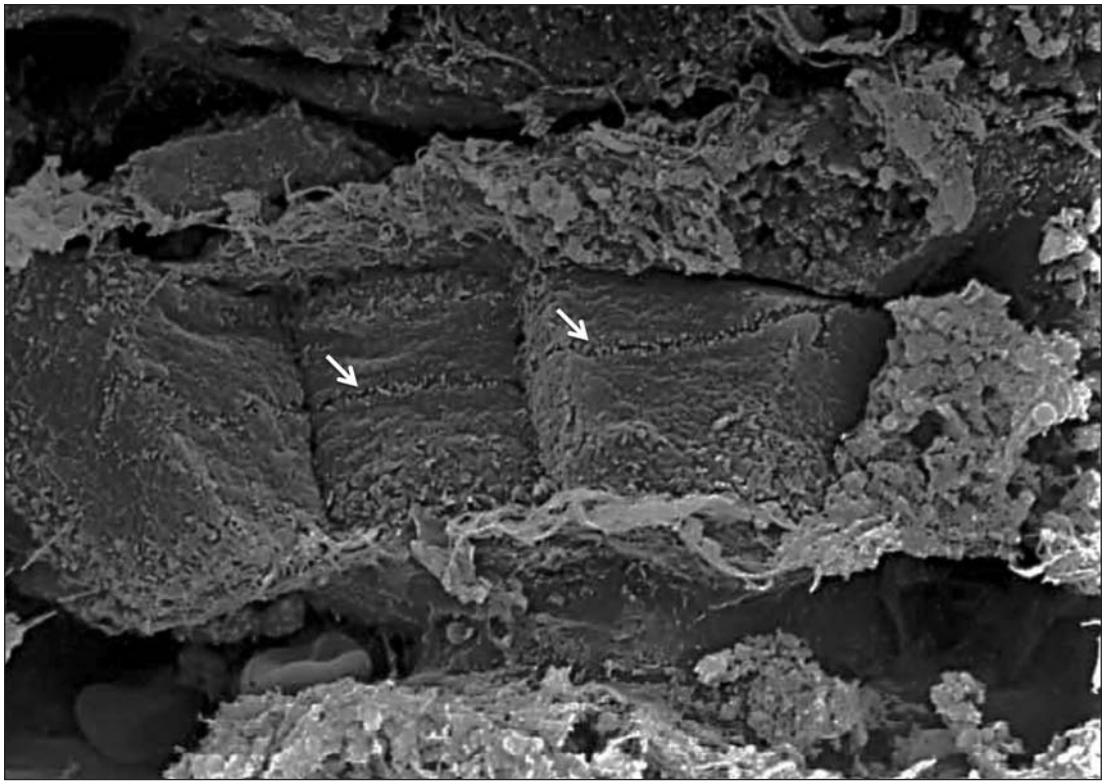

Figure 9A. This technique allows the study of the extension of biliary canaliculi from one hepatocyte to the next (arrows), clearly evidencing the continuity of the biliary structures extending along the liver trabecules.

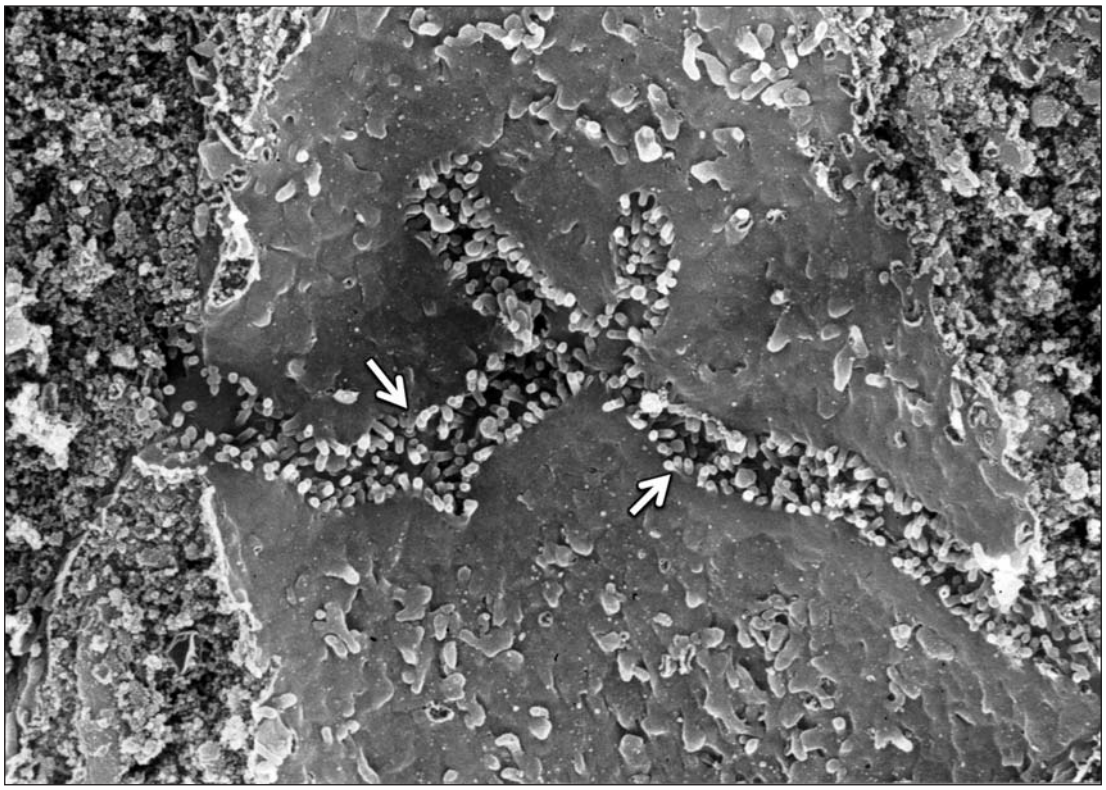

Figure 9B. Detachment of the neighboring hepatocyte allows the 3D study of the interior of the biliary canaliculus: at this power, it is possible to analyze the number and the distribution of biliary villi (4,000X).

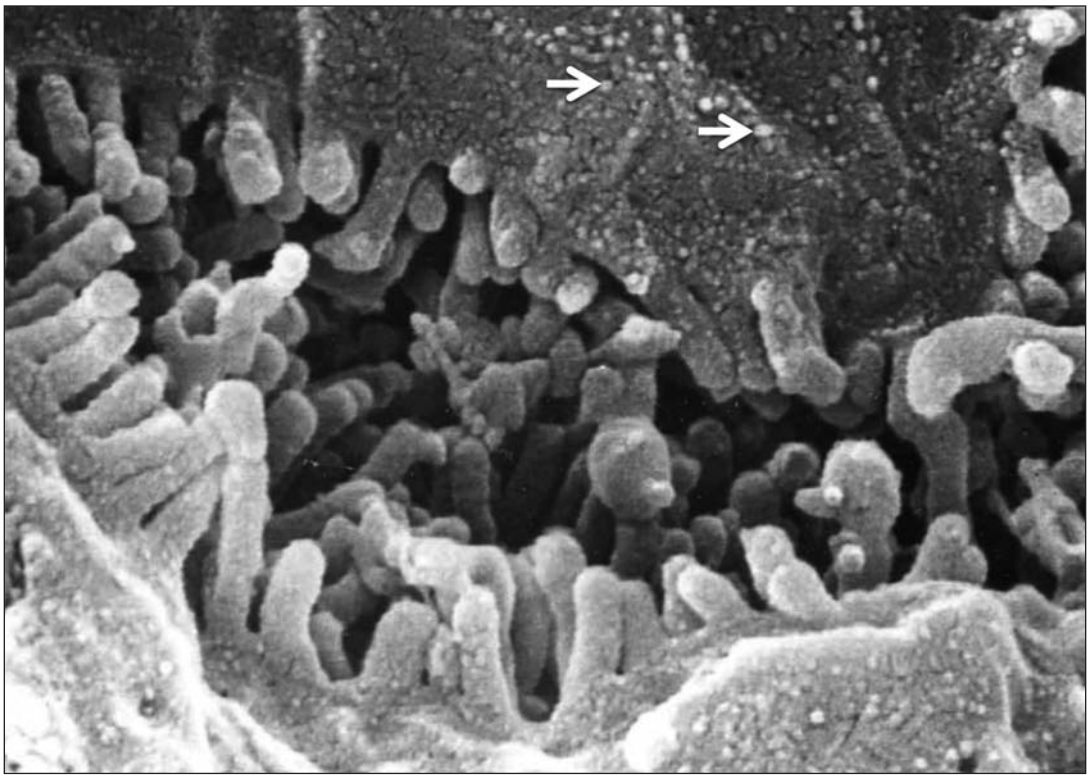

Figure 9C. At high power (15,000X), our method allows the ultrastructural study of biliary cell villi. The granular structure observed at the bottom of the figure (arrows) putatively represents the components of the tight junctions of bile canaliculi.

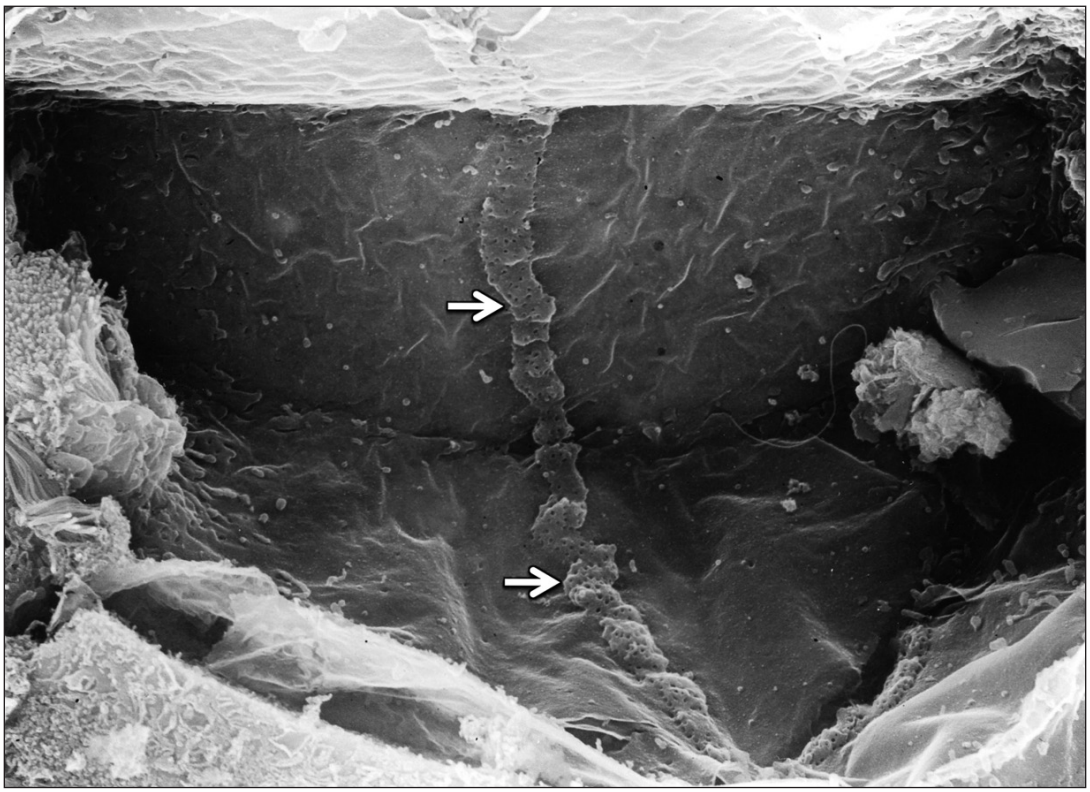

Figure 10A. After removing the entire cytoplasm of hepatocytes, by our method it is possible to analyze the hepatocyte cell membrane from its interior side, following the extension of bile canaliculi (arrows) (3,500X).

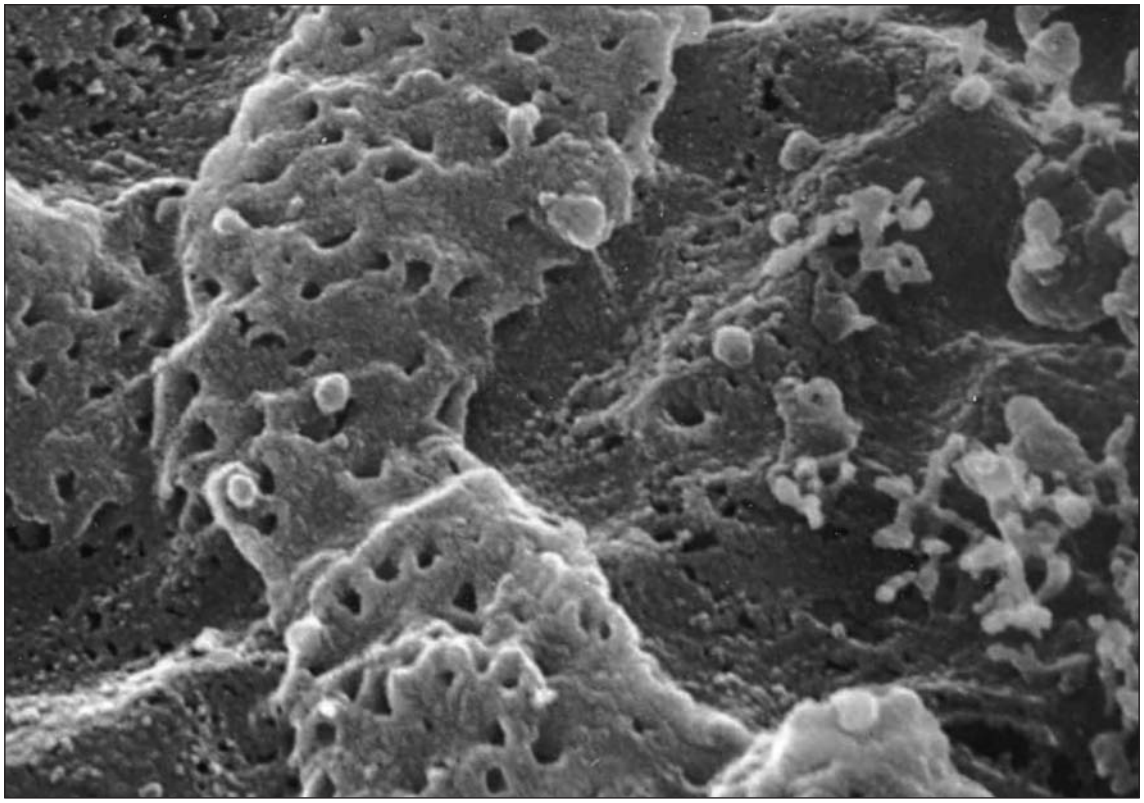

Figure 10B. At higher power, bile canaliculi seen from the interior of hepatocytes appear as sponge-like structures, each hole corresponding to the origin of a single villus.

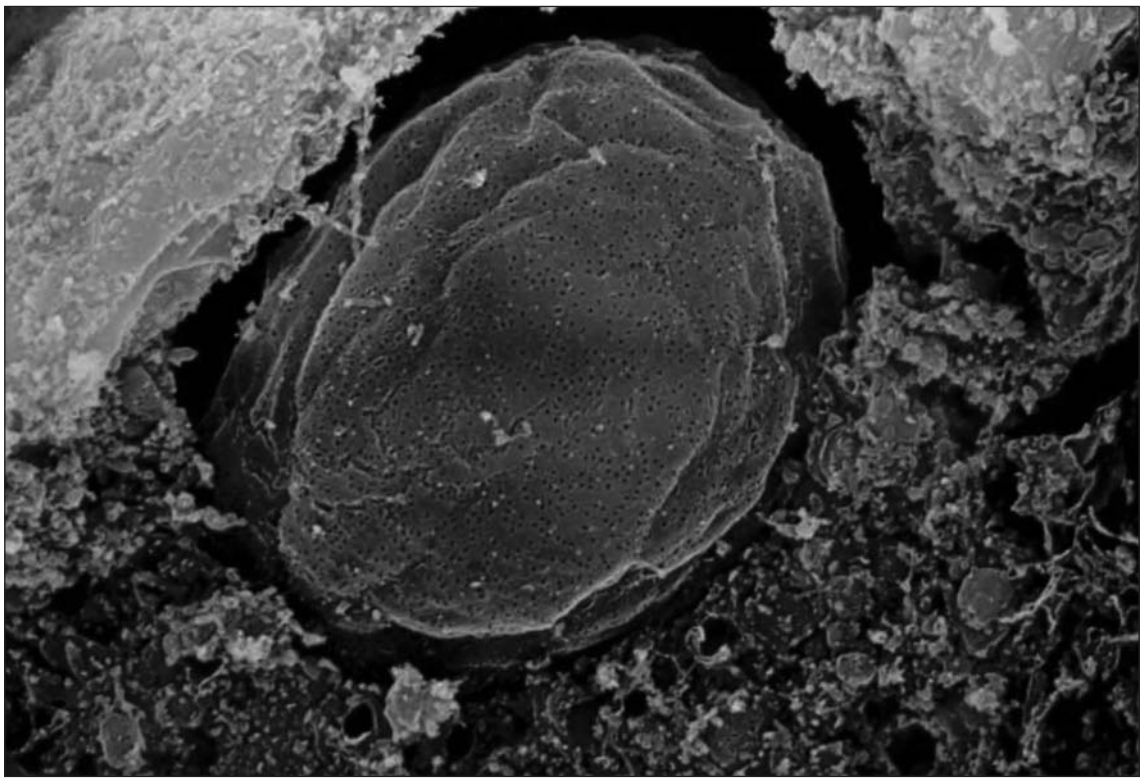

Figure 11A. The exterior side of the nuclear membrane shows marked irregularities and infoldings of the nuclear membrane, that is characterized by the presence of multiple holes representing the nuclear pores.

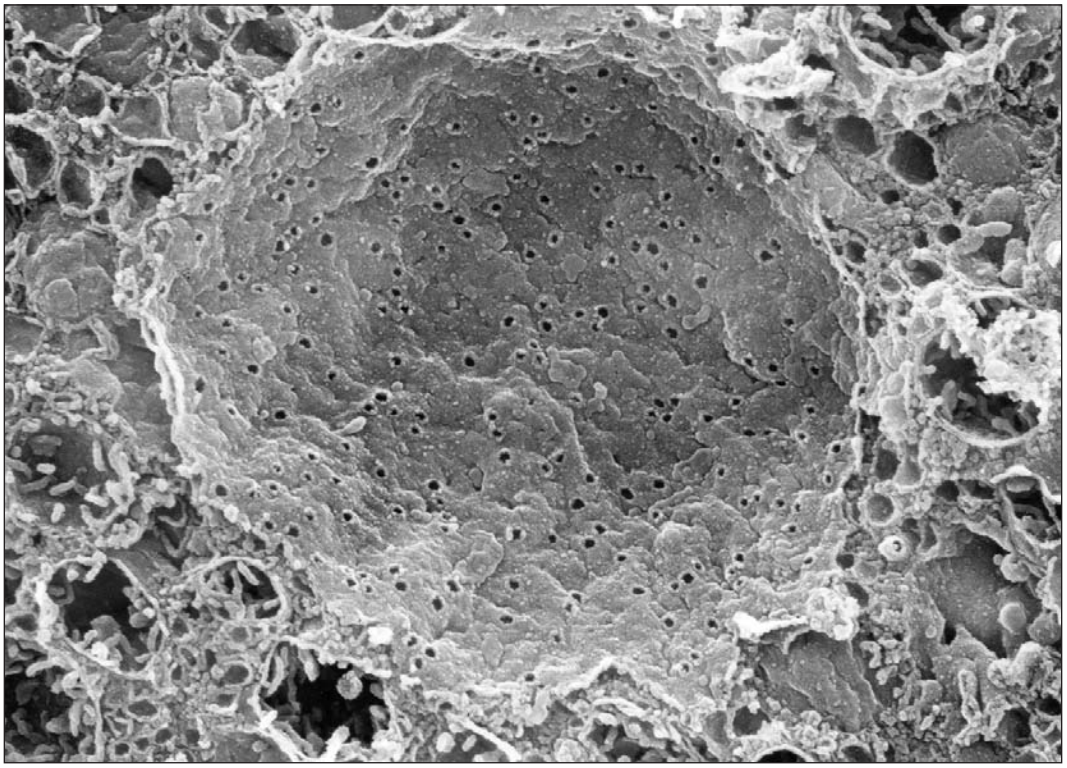

Figure 11B. When nuclei are cut and chromatin removed, it is possible to observe the interior side of the nuclear envelope. Nuclear pores appear to be unevenly distributed throughout the nuclear membrane, also showing marked differences regarding their diameter (10,000X).

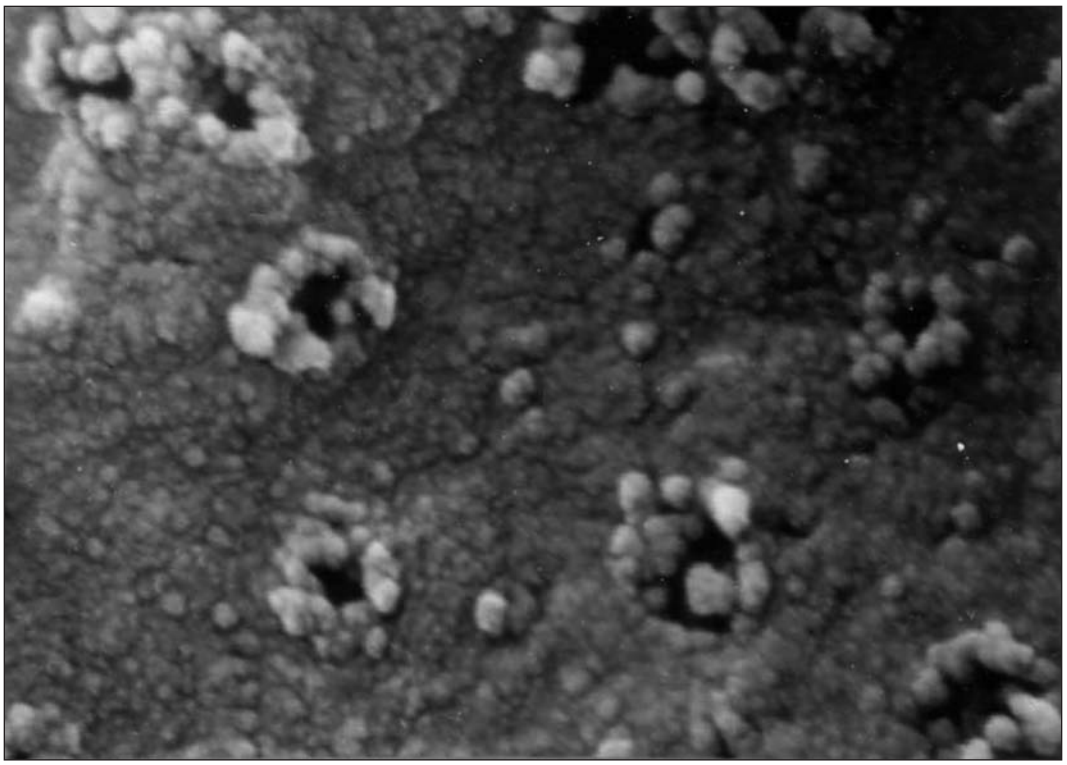

Figure 11C. At high power, the complex structure of nuclear pores is better revealed: each pore is surrounded by numerous globular subunits, different in shape and dimensions, introducing a previously unrecognized variability in the structure of nuclear pores.

merging from the complex glomerular-like structure of the mitochondrion (see **Fig. 6A**). The study of mitochondrial cristae, their distribution throughout the mitochondrial membrane and their reciprocal relationships were well evident by SEM after maceration, allowing a better comprehension of the organization of the interior of mitochondria (see **Fig. 6B**). The 3D appearance of the trans-Golgi-network (see **Fig. 7**) underlines the validity of our method, revealing a complex sponge-like structure, previously unreported by TEM studies. Moreover, the 3D study of the Golgi apparatus might allow the complex relationships between the main Golgi bodies and the vesicles merging and surrounding it, giving a detailed image of the complexity of its function, both in health and disease. The application of the osmic maceration method to the analysis of the intrahepatic biliary tract might represent a breakthrough in research studies on the biliary tree physiology and in bile duct diseases particularly in pediatric patients. As shown in **Figures 9-10**, this technique allows to go deep in the analysis of the structure of biliary canaliculi, a structure whose pathological changes are rarely evidenced by histology and by TEM. Here we show that by the maceration method it is possible to analyze the lumen of bile canaliculi, to observe the number and height of villi, to observe the canaliculi from the inner side of the cell membrane, and to study the continuity of these biliary structures from one hepatocyte to the next. All these data taken together, associated with histological and TEM data obtained from the same liver biopsy, could allow a better analysis of the biliary tree and, probably, a better knowledge of the degree of their damage in the complex field of vanishing intrahepatic bile disorders. The ability of our method to analyze in deep the nuclear envelope deserves some consideration. Nuclear pores are an important structure for the relationship between chromatin and cytosol. At histology, nuclear pores are not identifiable, whereas at TEM they are seen only at higher power and their study is always insufficient. Here we show that the osmic maceration methods allows a study of the nuclear pores number, density and diameter. Moreover, at high power, we have shown the ability of our technique to analyze the number and size of the subunits that define each nuclear pore. These data open, in our opinion, a new field in the research of nuclear pathology, with possible intriguing data on pathological nuclear pore changes in the setting of different liver diseases.

Conclusions

Data here reported clearly evidence that the application of the OsO_4 maceration method to the study of liver biopsies should deserve attention from pathologists, given the amount of useful data that may be obtained by this technique. Further studies are needed in pathological specimens, in order to better define the role of SEM after OsO_4 maceration in the routine pathology practice. In particular, our preliminary data suggest that the field of intrahepatic biliary disorders might receive major advantages by the application of our method, given its peculiar and unique ability to allow the analysis in deep of the finest biliary structures, i.e. bile canaliculi.

Declaration of interest

The Authors declare that there is no conflict of interest.

References

1. Daugherty CC, Vogler C, Buchino JJ. Electron microscopy in the diagnosis of pediatric disease. Pediatr Clin North Am. 1989;36:199-226.
2. Cornelis A, Van Meerbeek D, Nyssen M, Roels F. Specific cytochemical stains in the image analysis of subcellular organelles. Anal Quant Cytol Histol. 1985;7:256-66.
3. Itoshima T, Kiyotoshi S, Kawaguchi K, Ogawa H, Ohta W, Ito T, Hirakawa H, Shimada Y, Nagashima H. Kupffer cell hyperplasia in liver diseases. Demonstration by scanning electron microscopy of biopsy samples. Gastroenterol Jpn. 1981;16:223-31.
4. Tanaka K, Mitsushima A. A preparation method for observing intracellular structures by scanning electron microscopi. J Microsc. 1984;133:213-22.
5. Riva A, Congiu T, Faa G. The application of the OsO4 maceration method to the study of human bioptic material. A procedure avoiding freeze-fracture. Microsc Res Tech. 1993;26:526-7.
6. Faa G, Ambu R, Congiu T, Mocci C, Parodo G, Pilleri G, Farci AM, Vallebona E, Costa V, Riva A. Scanning electron microscopy of chronic hepatitis C. An OsO4 maceration study on human biopsies. J Submicrosc Cytol Pathol. 1994;26:415-23.
7. Faa G, Iannelli S, Congiu T, Mocci C, Ambu R, Parodo G, Sechi P, Loffredo F, Riva A. A 3D study of intra and extracellular structures of liver in beta-thalassemia intermedia: an OsO4 maceration analysis. J Submicrosc Cytol Pathol. 1997;29:73-8.
8. Ohtani O, Ushiki T, Taguchi T, Kikuta A. Collagen fibrillar networks as skeletal frameworks: a demonstration by cell-maceration/scanning electron microscope method. Arch Histol Cytol. 1988;51:249-61.