catheter (inlet) is inserted into the portal vein and secured by ligatures, it is possible to insert the plastic tube (outlet) further into the portal vein, without rupture of the vessel. An additional benefit is that the atraumatic outlet of the catheter has a round shape, which results in more efficient perfusion when compared to the pointed-end needles. Accidental puncture of the wall of the portal vein at the start of perfusion is a major source of perfusion failure. In case of perfusion failure, an emergency solution is offered by the technique of injection fixation. In that case, the steps of protocol 2 should be followed. This might save losing an animal from the experimental setup.

A point of interest in choosing the right diameter of needle lies also in the effect of "jet streaming" that is caused by a too-small needle orifice. With an easy, moderate flow, the fluids probably distribute evenly through the branches of the portal vein, whereas a jet-stream might, simply because of its strength and direction, blow the fluids into one branch of the portal vein, thus causing uneven distribution and pressure of fixing fluids in the different liver lobes.

When injecting glutaraldehyde into a wedge biopsy, one should take care to introduce a fluid-filled needle into the tissue. When the needle cuts the tissue as it does in the case of a needle biopsy, the sampled tissue comes out at the moment the operator starts injecting the fixative. Instead, if the operator fills the needle with fluid and cuts through the tissue without allowing the tissue to enter the lumen of the needle, this effect can be avoided. Moreover, when the fluid-filled needle is retracted slightly after reaching the maximum track for injection (evidently before starting perfusion), a space will be created within the tissue, from where fluids will distribute easily in all directions as soon as the injection of the fixative solution starts.

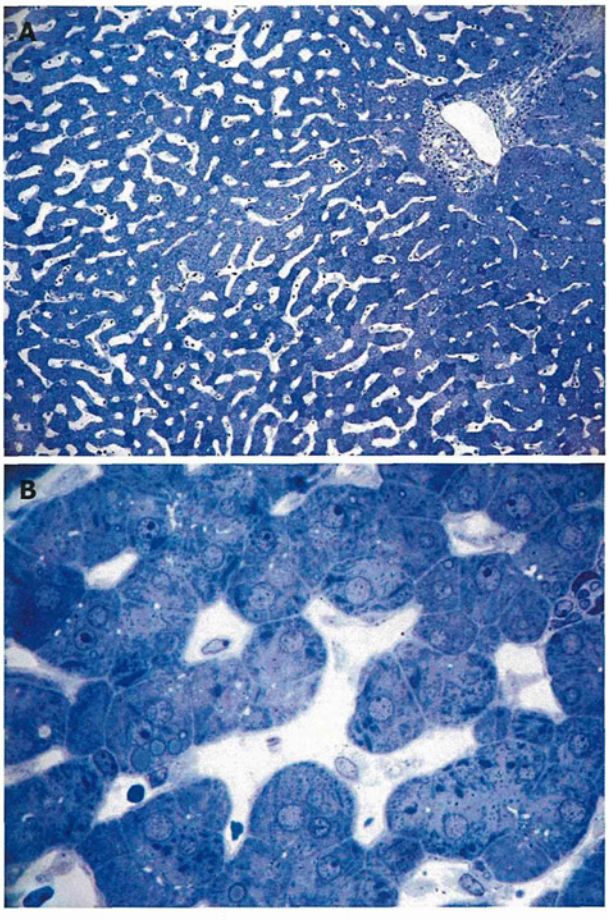

Figure 6 Light micrograph of a wedge biopsy of human liver, injected with glutaraldehyde, plastic section stained with Toluidine Blue. A: Original magnification 10 × objective lens. Note that, as a result of successful perfusion, sinusoids are open, and only a few red blood cells are present. A portal tract is present in the upper right corner; B: Original magnification 63 × oil-immersion lens. Details such as giant mitochondria are visible in the parenchymal cells, amongst others such as small mitochondria, lipid inclusions and bile canaliculi. Sinusoids are patent and show the presence of sinusoidal cells.

RESULTS OF THE DIFFERENT METHODS OF FIXATION

When LM sections, SEM preparations and ultrathin sections of liver tissue or cell cultures are studied, these three approaches provide information to the investigator, each in its own way.

Toluidine-Blue-stained 1-μm sections provide information at magnifications from 5 × to 1000 × over wide areas as compared to TEM sections (Figure 6A and B). These sections already show the criteria for good or bad fixation (see discussion), such as open sinusoids (Figure 7A and B), absence of blood cells, equal density of similar cells, and open bile canaliculi (particularly in the periportal area). Recognition of typical histological details such as the central vein, bile ducts and portal vein with its accessory structures and many cells, such as parenchymal, sinusoidal and visiting cells, together with conditions such as steatosis (Figure 8A and B), fibrosis and inflammation, can be observed at an estimated system resolution of about 0.2 μm. Crucial details such as endothelial fenestrae (measuring 140 nm in mice and rats, and 105 nm in humans and some rabbit strains) (Figures 9-12), although present, therefor escape observation by LM. Perfusion fixation and plastic embedding also add details to an LM study. In the case of immersion fixation, paraffin embedding and hematoxylin and eosin staining, observations are mainly restricted to the size, shape and position of entire cells and their nuclei. In the case of perfusion fixation and plastic embedding, intracellular details can be observed in the LM in greater detail, with the knowledge that the same structures of the same preparation can also be seen in maximal detail by TEM. For example, the typical giant mitochondria that occur in the human liver parenchymal cells (Figures 13 and 14) can easily be observed in plastic sections by LM, and can be seen in greater detail by TEM of the same specimen. Therefore, the Toluidine-Blue-stained plastic section is a useful histological introduction to further detailed study by TEM.

Perfusion-fixed total organs or injection-perfused wedge biopsies also make ideal preparations for SEM study. When tissue strips of 1 mm × 1 mm × 5 mm in 100% ethanol are frozen and fractured (before CPD) at the temperature of liquid nitrogen, the fracture surface is

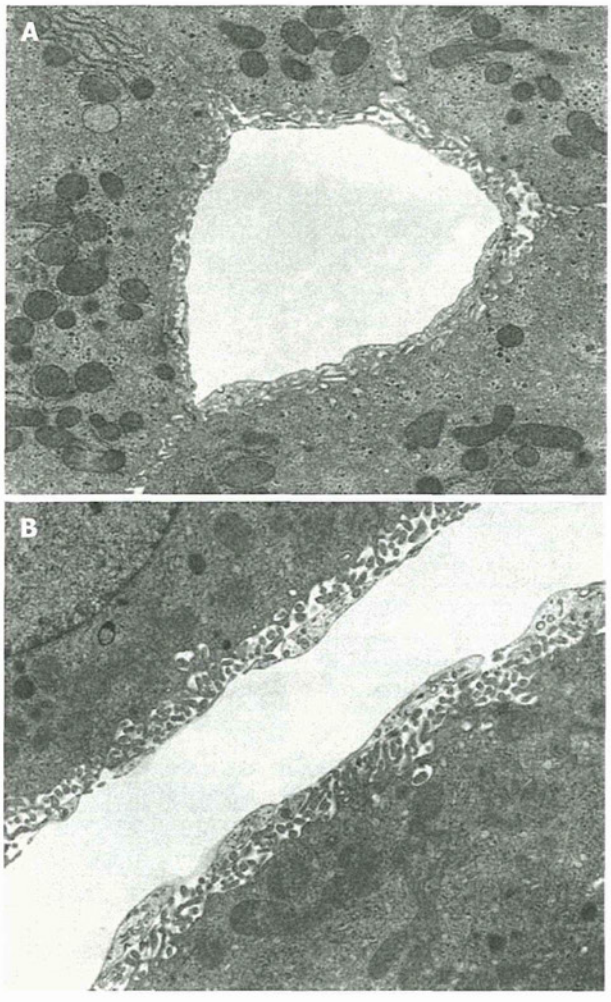

Figure 7 TEM micrograph of a transversely cut sinusoid of rabbit liver (A) and longitudinally cut sinusoid of mouse liver (B), fixed by perfusion through the portal vein. A: The Space of Disse shows the presence of microvilli extending from the parenchymal cells. Within the cytoplasm of the parenchymal cells, mitochondria, rough endoplasmic reticulum and glycogen are recognizable. Original magnification 6600 ×; B: Underneath the thin layer of fenestrated endothelium, bordering the sinusoidal lumen, the Space of Disse shows the presence of microvilli extending from the parenchymal cell surface. Within the cytoplasm of the parenchymal cells, mitochondria are recognizable. Original magnification 8900 ×.

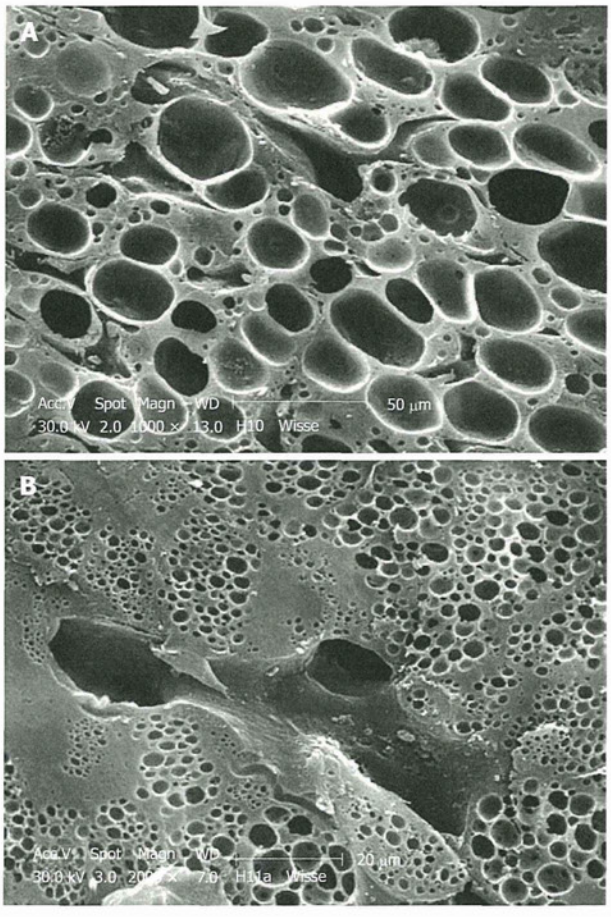

Figure 8 SEM micrograph of an injection-fixed human wedge biopsy showing macrovesicular steatosis (A) and microvesicular steatosis (B). A: Fat droplets are of such dimensions that one appears to fill the cytoplasm of an entire parenchymal cell; B: Fat droplets are smaller than those in Figure 8A and are spread within the cytoplasm of parenchymal cells.

more or less flat and easily correlates with a 2D histological section. Unfortunately, intracellular details are rare, but some cell surface details are abundant. SEM offers details about the endothelial lining of the sinusoids, easily shows the presence of fenestrae (Figures 11 and 12), reveals the presence of endothelial gaps (Figures 15 and 16), shows some details about the space of Disse, and shows the central and portal veins and the bile duct with its typical epithelial cells with one cilium each (Figure 17). Alternatively, fracturing the tissue in the already dry state after CPD (Figure 17) reveals the lateral cell membranes of parenchymal cells together with the 3D network of bile canaliculi, strictly separated from the network of sinusoids. SEM preparations are very useful in judging conditions such as sinusoidal dilatation (Figure 18) or sinusoidal obstruction syndrome[31]. Sinusoidal dilatation occurs in regions apparently different form normal, where the average sinusoidal diameter is measured as 4-6 µm by SEM. In SEM preparations, the dimensions of many structures are reduced by an average 30% shrinkage as a result of the drying procedure[23,24]. Therefore, the use of SEM preparations to measure or judge the size of any detail in the preparation should be avoided.

TEM of perfusion or injection fixed liver tissue reveals the fine structure of all the different cell types in the tissue. It is assumed that the histological topography and the shape of the cells are optimally preserved due to hardening of the intact tissue by glutaraldehyde perfusion. This initial hardening process needs only a few minutes for the entire organ. Important to note is the fact that this condition can be obtained without touching or deforming any part of the organ or tissue. It is assumed that the fluids themselves do not cause distortion as reported for immersion fixation[19].

Inherent to the observation with TEM, abundant details can be observed in all different cells and organelles at magnifications in the range of 50-1 000 000 ×, with a resolution in the order of about 5 nm. A low magnification survey of sections at 50-600 × (by using a different

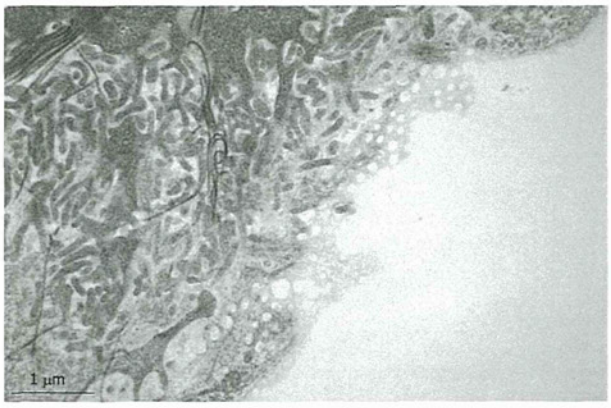

Figure 9 TEM micrograph of the tangentially cut endothelium of a human liver sinusoid, fixed by injection of glutaraldehyde fixative into a wedge biopsy. Underneath the thin layer of fenestrated endothelium, the Space of Disse shows the presence of microvilli, thin fibers of reticulin, and processes of fat-storing cells. Fenestrae in such preparations have an average diameter of about 105 nm. Original magnification 19 000 ×.

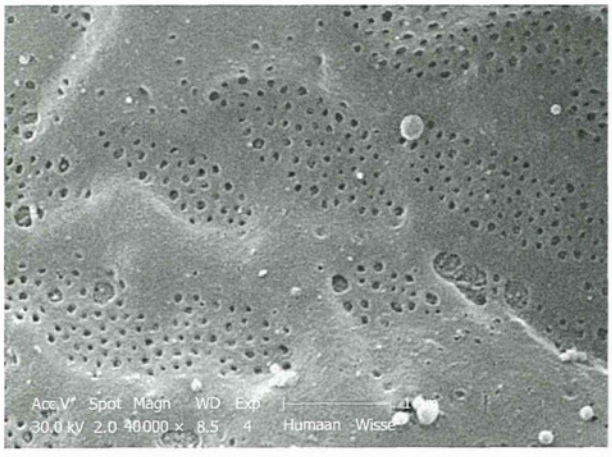

Figure 11 SEM micrograph of the fenestrated endothelial lining of a human injection-fixed wedge biopsy. Note the grouping of fenestrae in sieve plates. Compare this figure with Figure 12, which shows an SEM micrograph of a mouse sinusoid at the same magnification. Fenestrae are apparently sensitive to fixation and are lost during immersion fixation. Their presence can therefore be used as a criterion of good fixation.

combination of TEM lenses) (Figure 19), provides information about the quality and content of the sections, and allows the preselection of interesting areas in the preparation. This low magnification TEM is also recommended as a bridge to the study of Toluidine-Blue-stained sections for LM. TEM observations also reveal the difference between sinusoids and capillaries (Figures 20 and 21). Perfusion fixation also reveals extraordinary structures such as pored domes[32] or defenestration centers (Figure 22)[33]. Higher magnifications provide supramolecular information about the cytoskeleton, glycogen rosettes, ribosomes, mitochondrial cristae, chromatin fine structure, nucleolar composition, unit membrane structure, Golgi apparatus, the presence of iron storage proteins, and lysosomal contents.

Results obtained with cell cultures follow the general pattern described for the three kinds of specimen already

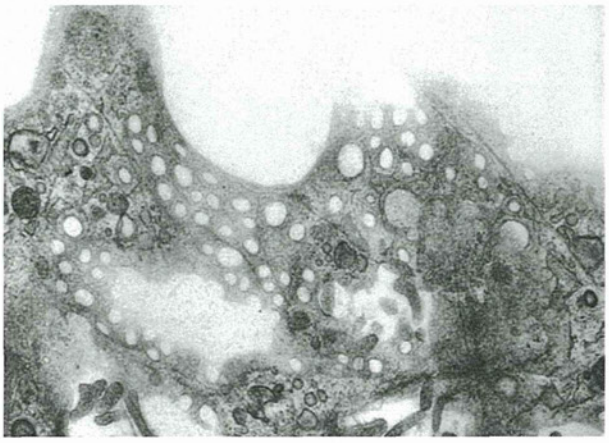

Figure 10 TEM micrograph of tangentially cut sinusoidal endothelium of a rabbit liver, fixed by perfusion through the portal vein. The fenestrae are grouped in sieve plates, but are intermingled with elements of the cytoskeleton, i.e. microtubules and microfilaments. Within the electron-dense sieve plate cytoplasm, saccular interconnecting cisternae are seen. These structures are fixation dependent and are only found in rabbit liver. Original magnification 21 000 ×.

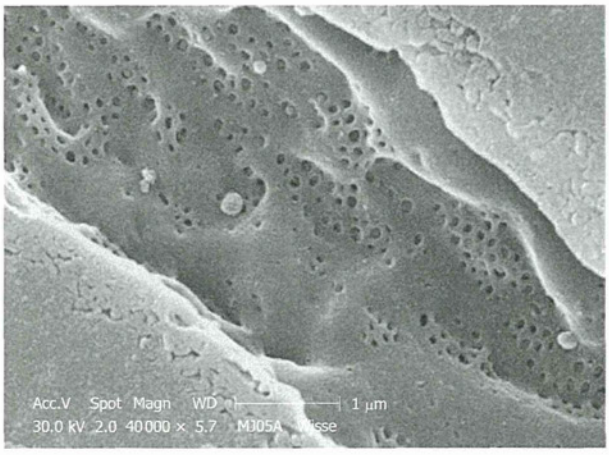

Figure 12 SEM micrograph of the fenestrated endothelial lining of a mouse portal-vein-perfused liver. Compare this figure with Figure 11, taken under exactly the same conditions. Comparison with TEM of plastic sections shows that mouse (and rat) fenestrae are bigger (140 nm) than human (and rabbit) fenestrae (105 nm). Measurements on this type of SEM preparation should be avoided, because the drying procedure results in about 30% shrinkage and incorrect measurement of all components in the tissue.

discussed. In the case of cultured cells, fixation at the cellular level is quite easy. A big difference is that fluids have direct access to the cells. Therefore, precautions should be taken to avoid over-fixation and extraction, which can result in damaged membranes and extracted cells.

GENERAL DISCUSSION

A detailed literature survey has revealed that there are three commonly used fixation methods for EM studies of liver tissue. For a review see Hayat[21]. Since Fahimi[5] and Wisse[6] have demonstrated that perfusion fixation, rather than immersion fixation, is essential in preserving the fine structure and architecture of the liver, perfusion-fixation has been the gold standard ever since, especially

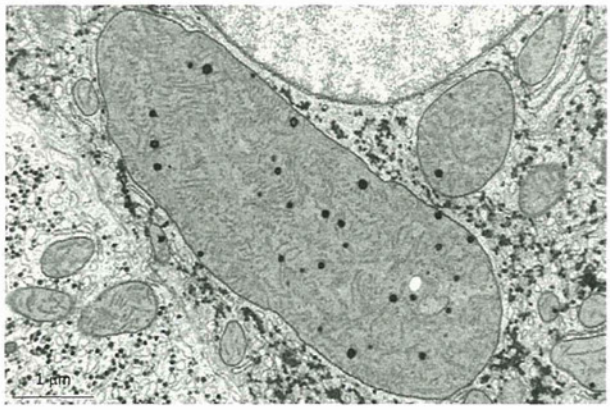

Figure 13 Typical structures that are often encountered in human liver parenchymal cells are the giant mitochondria. This wedge biopsy was fixed by injection, but during the osmium postfixation, ferrocyanide has been added. This compound enhances the contrast of membranes, including the cristae. Giant mitochondria also contain electron-dense granules, as normally seen in small mitochondria (compare Figure 14). Giant mitochondria can also be recognized in plastic sections observed by LM (see Figure 6B). Magnification 19 000 ×.

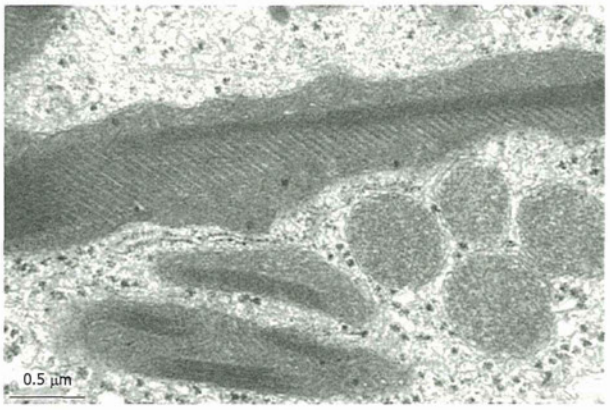

Figure 14 Giant mitochondria in a wedge biopsy fixed by injection. No ferrocyanide has been added during the osmium postfixation, therefore, membrane contrast is low. Within the giant mitochondria, the occurrence of typical crystals can be observed, together with a highly rhythmical pattern of the cristae. Magnification 34 000 ×.

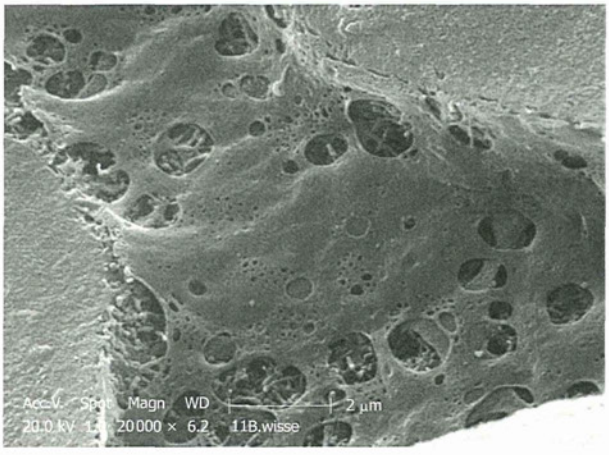

Figure 15 A major problem in EM preparations of liver tissue is the occurrence of gaps in the endothelial lining. It is assumed that they appear during fixation due to instability of the cytoskeleton, which allows the fusion of fenestrae or the *de novo* formation of gaps. This SEM picture is from a rabbit liver fixed by portal perfusion.

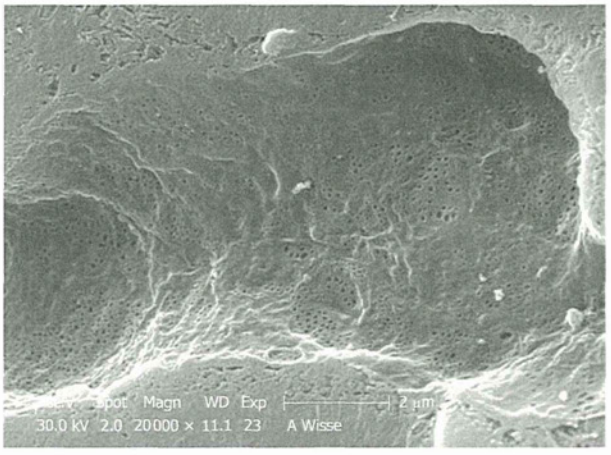

Figure 16 SEM micrograph of another portal-vein-perfused rabbit liver, which shows a complete intact sinusoid, and apparently no gaps have been formed. Fenestrae occur in sieve plates (groups), the space of Disse seems to be filled by microvilli of the parenchymal cells.

for the study of the sinusoids and sinusoidal cells in experimental animals[6].

Irrespective of the numerous methods available to prepare liver tissue for EM studies, perfusion-fixation *via* the portal vein is decidedly superior and more reproducible compared to other methods of fixation, in particular immersion fixation. Injection of the fixative into wedge biopsies, however, comes close and when Toluidine-Blue-stained LM sections are used to select tissue for the quality of fixation, this type of fixation can replace that through the portal vein. Perfusion-fixation *via* the portal vein and immediate arrest of the liver circulation (including the hepatic artery), meets the conditions of instantaneous and simultaneous fixation of all cells in the organ and restricts gradients of fixation to the cellular level. Fahimi[5] and Wisse *et al*[22] have reported the success of portal vein perfusion, followed by retrograde perfusion with fixative through the hepatic vein, which results in more evenly fixed tissue. By staining with Schiff's reagent, we could demonstrate that within 5-7 min, liver tissue stains equally well after such "push-pull" fixation[22], which indicates that saturation of the tissue with glutaraldehyde is obtained over this short time interval. Perfusion through the aorta[28] or any other indirect route may be successful, but seems unnecessary when the portal vein is available. We can summarize the advantages of perfusion fixation as follows: (1) instantaneous and simultaneous fixation of all cells in the organ; (2) avoidance of hypoxia and autolysis; (3) fewer problems resulting from diffusion and exhaustion of the fixative during immersion; (4) use of lower concentrations of fixing chemicals is possible; (5) glutaraldehyde perfusion hardens the tissue, which allows easier cutting; (6) less mechanical damage, deformation or distortion; (7) empty vessels and sinusoids allow better penetration of

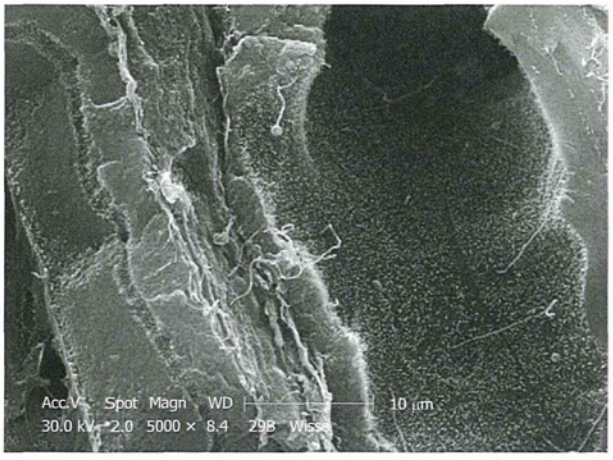

Figure 17 SEM micrograph of rabbit liver, fractured after CPD. In such preparations, the fracture plane separates the intact lateral cell membranes of parenchymal cells, which exposes the bile canaliculi, which are strictly separated from the sinusoids (to the extreme left). The right hand side of the picture shows a bile duct. The luminal surface of the bile duct epithelial cells is covered with numerous small microvilli and single cilia protruding into the lumen of the bile duct.

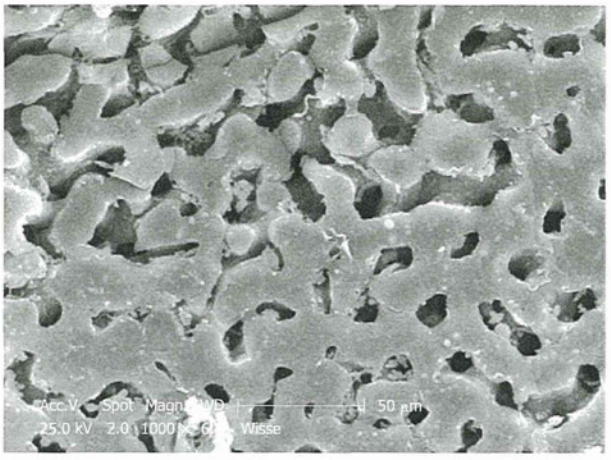

Figure 18 SEM micrograph of a human injection-fixed wedge biopsy that shows two populations of sinusoids, i.e. normal size (lower half of the picture), and those in the upper half of the picture show sinusoidal dilatation.

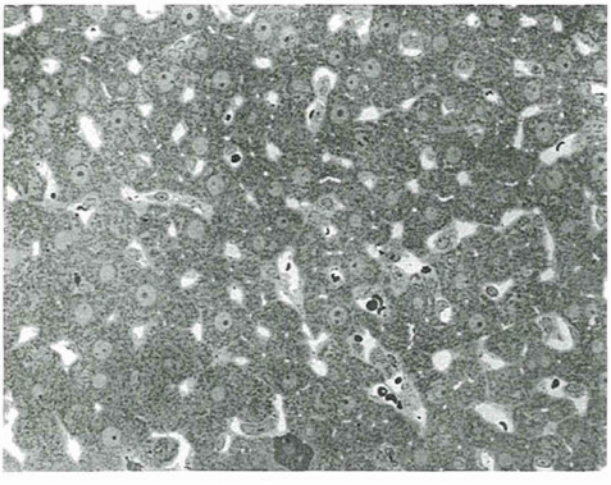

Figure 19 TEM image after perfusion fixation of rat liver through the portal vein, which results in an image of parenchymal cells of equal density (with one exception in the low middle part), open sinusoids and open bile capillaries. Original magnification 720 ×.

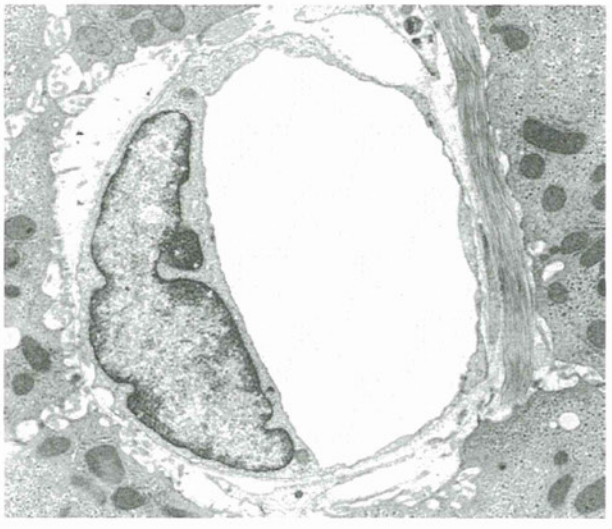

Figure 20 TEM micrograph of a capillary found in diseased human liver, fixed by injection of fixative into a wedge biopsy. Capillary endothelial cells are different from normal sinusoidal endothelial cells; their vacuolar apparatus (pinocytotic vesicles, endosomes, lysosomes, Golgi apparatus) is not developed. The thin cytoplasm contains fenestrae and the capillary is surrounded by a thin, continuous basal lamina that is not present in normal sinusoids. Magnification 6600 ×.

washing fluids, osmium and ethanol; (8) exact timing and shorter fixation times are possible; (9) more and better preservation of histological relationships and fine structural details; (10) better preservation of cytochemical reactive sites; (11) fewer artifacts; (12) perfusion can be extended to a program of reagents, fixatives and buffers without further manipulation; (13) less accessible organs and tissues may be fixed before excision; and (14) easy to directly judge good perfusion and fixation by change in color and hardening of the organ.

An additional advantage of the method is that perfusion-fixed liver tissue is firm enough to be cut into finer pieces without deformation, supposedly leaving cells in their right histological arrangement. Hand cutting by a sharp, thin razor blade allows the cutting of glutaraldehyde fixed tissue into tissue blocks of 1 mm × 1 mm × 1 mm (for TEM) or slices (1 mm × 10 mm × 10 mm) for flat embedding and large sections for LM, and strips of 1 mm × 1 mm × 5 mm for further preparation for SEM. It is assumed that the open vessels and sinusoids allow the penetration of osmium and other fluids in a much better way as compared to immersion fixation, for which a wall of fixed components at the outer rim of the tissue stands in the way of and will still react with the penetrating fluid.

Researchers may choose from two perfusion methods that both result in excellent preservation of hepatic tissue. One uses gravity to perfuse the vascular bed at 12 cm water pressure, a value that corresponds to the physiological portal pressure (Figure 1A); whereas the other method actively pumps solutions through the portal vein with the

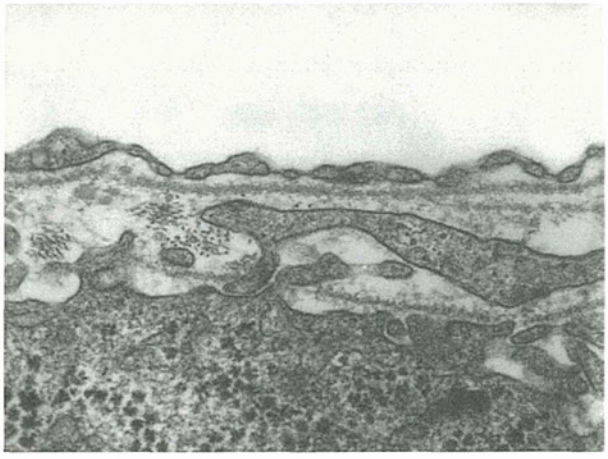

Figure 21 TEM micrograph of a capillary found in diseased human liver, fixed by injection of fixative into a wedge biopsy. The thin endothelial lining contains fenestrae, closed by a diaphragm. Underneath the endothelium, one can observe a continuous basal lamina. Fenestrae in this type of capillary are about 60 nm in diameter. It is supposed that transport from this capillary to the tissue is hampered by the structures mentioned. Magnification 28 500 ×.

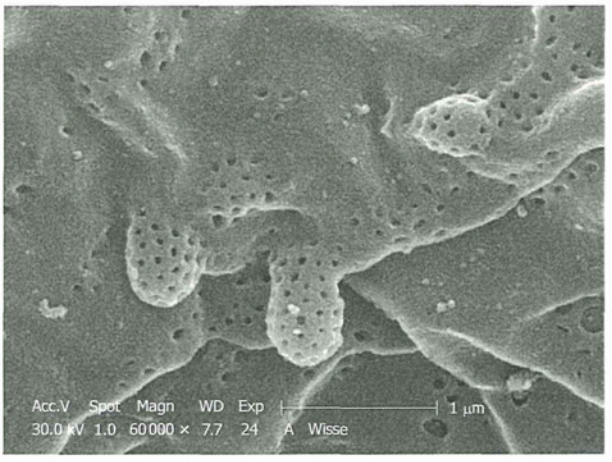

Figure 22 SEM micrograph of a portal-vein-perfused rabbit liver. Due to the quality of fixation, fenestrae are preserved, including a surprising detail, named pored domes[32], which are probably the *in vivo* equivalent of the defenestration center observed in endothelial cell cultures[33]. This structure was not seen in human, mouse, rat or pig liver.

aid of a low-speed peristaltic pump. In both cases the typical rate of 1 mL/min per g liver weight (Figure 1B) should be achieved.

Perfusion fixation seems to be difficult when a human needle biopsy is to be investigated by EM. Attempts have been made by Bioulac-Sage *et al*[14] and Balabaud *et al*[8] to flush fluids through the tissue of a liver needle biopsy arrested at one end of a narrowing Pasteur pipette (holding the tissue) with a slightly elevated pressure, while aspirating fluids at the other end with slightly negative pressure. Muto *et al*[10], Vonnahme[12] and De Wilde *et al*[15] have obtained good fixation results on human or rat liver needle biopsies by injecting glutaraldehyde with tiny glass or metal needles at intervals into the cylindrical tissue. De Wilde *et al*[15] have used low-melting-point agar to arrest and handle the tissue. Surprisingly, and in spite of good results, this technique has not found its way into clinical practice, and it is not mentioned in the needle biopsy review of Rockey *et al*[3].

It is evident that immersion-fixed needle biopsies have provided a wealth of information about the fine structure and deviations of parenchymal cells. David *et al*[34] have demonstrated by morphometric methods that there are only slight differences between parenchymal cells from immersion- or perfusion-fixed tissue. It is recommended to limit the study of immersion-fixed needle biopsies to the outer and middle zone of cells that are well fixed. They can easily be found when material is preselected by Toluidine-Blue-stained LM sections. Apparently, the structure of sinusoids and sinusoidal cells, as damaged as they are after immersion fixation, has not until now played a significant role in clinical practice.

With regard to artifacts, we remember a citation from one of the founding fathers of EM, George Palade, who said that one of the major difficulties in EM is to distinguish between genuine structures and artifacts. Some even say that EM is "an art of artifacts". It takes indeed a lot of experience and insight to distinguish between real structures and artifacts. Alternative methods are scarce, but sometimes it helps to compare results of chemical fixation (as summarized in this review) with alternative techniques such as freeze fracture of fresh tissue[6]. In equilibrium with the fact that artifacts are not easy to define and to prove, it is also difficult to indicate the true nature of real structures and their function. Artifacts in a specimen can be caused during one or more of the many steps of sample preparation, and can be caused by anesthesia, osmotic pressure, quality of the reagents, inadequate operation, pH, temperature, postmortem changes or autolysis, slow or incomplete fixation, cutting of the tissue, postfixation, dehydration, distortion, embedding, sectioning, contrast staining, microscope conditions, (digital) photography, image storage, and handling. Examples of possible artifacts are the occurrence of endothelial gaps, shrunken cells, light cells next to dark cells (Figure 23), widening of the Space of Disse, changes in cell shape, changes in the rounded shape of the nucleus of the parenchymal cells, condensation of chromatin[19], relocation or disappearance of subcellular components (such as endothelial fenestrae[6]), swollen mitochondria, collapsing and blebbing (Figure 24) of cell membranes and vesicle formation in intercellular spaces.

With regard to the occurrence of gaps larger than fenestrae in the endothelial lining (Figure 15) a lot of discussion has taken place[22,23]. Indirect proof of their artifactual nature is given by the fact that, in intact animals, endothelial fenestrae have an apparent sieving effect on, for example, adenoviral vectors of 93 nm used for gene transfer[35,36]. The appearance of large gaps in the sinusoidal lining can result from osmolarity problems, from destabilization of the cytoskeleton or from the use of a high perfusion pressure during the washing and subsequent glutaraldehyde fixation during the perfusion-fixation procedures[28,34].

Other subtle artifacts are caused by a process often

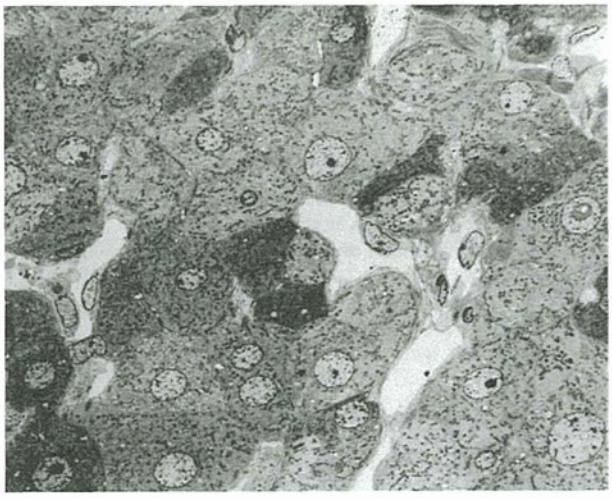

Figure 23 TEM micrograph of an injection-fixed human liver wedge biopsy at 700 × magnification, which shows the presence of dark and light cells next to each other. It is assumed that the differences in density reflect differences in fixation, which often occur in bad fixation.

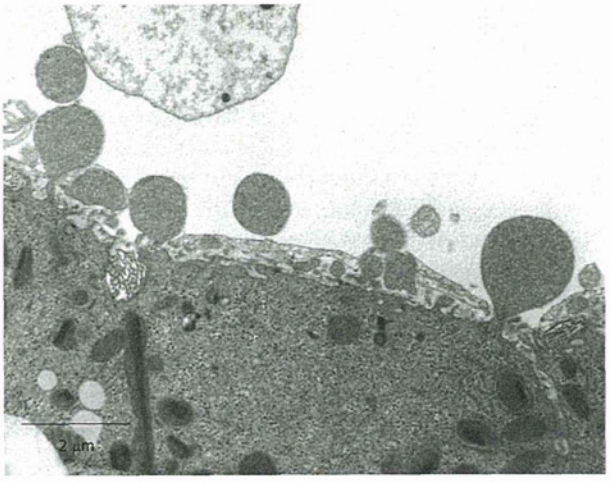

Figure 24 TEM micrograph of an injection-fixed human liver wedge biopsy, which shows the presence of blebbing, which occurs when cells survive in bad conditions. Incomplete fixation is thought to be the cause. Blebs are either swollen microvilli or originate from the peripheral cytoplasm of parenchymal cells. Original magnification 7900 ×.

referred to as over-fixation or "weekend fixation". People sometimes store their precious samples for hours, days or even weeks in glutaraldehyde fixative. Typical for those preparations is that they are rich in free vesicles that seem to form from loose phospholipids derived from the cell membrane. Conversely, blebbing of living parenchymal cells occurs at low oxygen and glutaraldehyde concentrations (Figure 24). Related to this is the clumping of individual glycogen inclusions, poorly staining cell membranes, and failure to achieve staining of glycogen in parenchymal cells. With regard to the transport of material between laboratories, we have good experience of sending samples in 70% ethanol by fast courier services, which are able to deliver between different continents within 1 or 2 d.

How does one assess the outcome of properly fixed liver tissue? In order to distinguish good quality of fixation of the liver, we recommend the following criteria: (1) sinusoids should be open; (2) plasma and blood cells should be practically absent in sinusoids; (3) similar cell types should show similar images; (4) endothelial fenestrae should be present; (5) bile canaliculi (especially in the periportal area) should be open[6,8,22,23]; and (6) nuclei of parenchymal cells should be round (When nuclei of parenchymal cells are elliptic, the nuclei are compressed in the direction of cutting in the ultramicrotome).

The following criteria can be use to distinguish bad fixation: (1) sinusoids are collapsed or largely destructed and still filled with plasma and blood cells; (2) dark and light cells occur next to each other; (3) different images of similar cells; (4) endothelial fenestrae are absent; (5) bile canaliculi are collapsed; and (6) irregularly shaped nuclei of parenchymal cells.

Fahimi[5] has immersed thin slices of perfusion-fixed liver tissue into distilled water. When they change to a white color, the fixation is insufficient. This is a simple, rapid and objective test for good fixation.

Although this paper focuses mainly on the study of ultrathin liver sections with TEM, SEM is also a powerful tool to assess the quality of perfusion-fixed liver tissue. The abundant presence of red blood cells within sinusoids is an indicator of improper perfusion. Sometimes investigators unwarily apply unrelated or unsuitable perfusion or fixation procedures that are fine-tuned for specific applications, tissues, species or even are not applicable for animal tissue material, such as microbial and plant sample preparation protocols. Animal tissue and their cell components have a typical osmotic pressure of 320 mOsmol. Since the osmolarity of the glutaraldehyde component of the fixative seems to be less important[19,37], the osmolarity of the buffers should match the osmotic values of the tissue. Isotonic solutions that include the osmotic pressure of glutaraldehyde have a swelling effect on cells[37]. The protocols described in this review have contributed to the success of EM studies in several groups. The composition of the fluids applied reports the use of sucrose, which is added to a 0.1 mol/L sodium cacodylate buffer to correct its osmotic value to 320 mOsmol.

In vitro perfusion of whole livers has been used often to study preservation conditions of livers for transplantation, or metabolic and physiological processes. It is important to note that perfusion fixation fits well into such schemes and provides essential information about the condition of those livers at the end of an experiment. In addition, perfusion can also be used to incubate the tissue for cytochemical reactions of enzymes, epitopes, and all other reactive elements within the tissue.

CONCLUSION

In a clinical environment, the immersion-fixed needle biopsy is an important standard. The role of needle biopsies in diagnosis, prognosis and management of patients with liver disease[3] makes the needle biopsy a gold standard,

notwithstanding immersion fixation, paraffin embedding, thick 5-μm sections, and the use of low-magnification LM. Together with immunological techniques, specific staining and the professional training of pathologists, needle biopsies fulfill clinical needs. Pathological diagnosis[38] uses pattern recognition in tissues that reveal conditions such as steatosis, inflammation, hyperplasia and neoplasia. EM seems to be of limited or no use in diagnosis[3], in contrast to its use in research. However, EM still has a potential value in a clinical environment.

However, at the other end of the spectrum, there is a group of scientists that knows that perfusion fixation, plastic embedding and the use of EM provides details about cells and tissues down to the nanometer level. Surprisingly, EM does not seem to succeed in helping pathologists to improve further their diagnosis with data derived at this supramolecular level. One of the key elements in this process is, according to the present authors, the lack of use of perfusion fixation for the study of liver tissue from patients. Indeed, we suppose that applying this type of fixation will provide evidence that might be used to the advantage of patients, for understanding the pathogenesis of certain diseases. This was the motivation for writing this review; to summarize the techniques and bring them to the attention of those involved in the process of using microscopic observations to support diagnosis and prognosis of liver diseases. There seems to be a gap between the use of low-resolution LM observations on immersion-fixed, paraffin-embedded, 5-μm sections and high-resolution microscopy of perfusion-fixed tissue. We hope that this review might help to bridge that gap. Essential to this approach is the recognition of the fact that liver tissue should be fixed at the cellular level with fluids that are as close to physiological and physical conditions as possible.

A good fundamental knowledge of the fine structure and histology of the normal liver is needed because some experimental conditions, pathological processes and severe liver diseases result in outcomes comparable to bad fixation. Although it is difficult to decide between genuine and artifactual structures in normal liver, the decision becomes even more difficult when disease conditions are added to the process.

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Effect of Hepatitis C Virus Infection on the mRNA Expression of Drug Transporters and Cytochrome P450 Enzymes in Chimeric Mice with Humanized Liver[S]

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ABSTRACT:

The expression of drug transporters and metabolizing enzymes is a primary determinant of drug disposition. Chimeric mice with humanized liver, including PXB mice, are an available model that is permissive to the in vivo infection of hepatitis C virus (HCV), thus being a promising tool for investigational studies in development of new antiviral molecules. To investigate the potential of HCV infection to alter the pharmacokinetics of small molecule antiviral therapeutic agents in PXB mice, we have comprehensively determined the mRNA expression profiles of human ATP-binding cassette (ABC) transporters, solute carrier (SLC) transporters, and cytochrome P450 (P450) enzymes in the livers of these mice under noninfected and HCV-infected conditions. Infection of PXB mice with HCV resulted in an increase in the mRNA expression levels of a series of interferon-stimulated genes in the liver. For the majority of genes involved in drug disposition, minor differences in the mRNA expression of ABC and SLC transporters as well as P450s between the noninfected and HCV-infected groups were observed. The exceptions were statistically significantly higher expression of multidrug resistance-associated protein 4 and organic anion-transporting polypeptide 2B1 and lower expression of organic cation transporter 1 and CYP2D6 in HCV-infected mice. Furthermore, the enzymatic activities of the major human P450s were, in general, comparable in the two experimental groups. These data suggest that the pharmacokinetic properties of small molecule antiviral therapies in HCV-infected PXB mice are likely to be similar to those in noninfected PXB mice. However, caution is needed in the translation of this relationship to HCV-infected patients as the PXB mouse model does not accurately reflect the pathology of patients with chronic HCV infection.

Introduction

Elimination of endogenous and exogenous substances is one of the most important physiological functions of the liver, which comprises the sinusoidal uptake from the blood circulation, intracellular phase I and phase II metabolism, and canalicular efflux of parent compound and/or metabolites into bile. Cumulative evidence suggests that members of the solute carrier (SLC) and ATP-binding cassette (ABC) transporters are expressed on either sinusoidal or canalicular membrane of the hepatocytes where they are responsible for the sinusoidal uptake and bile canalicular efflux of a diverse set of compounds (Chandra and Brouwer, 2004; Shitara et al., 2006; Dobson and Kell, 2008). On the other hand, cytochrome P450 enzymes are localized to the endoplasmic reticulum of hepatocytes and are the major enzymes involved in phase I drug metabolism and bioactivation, accounting for approximately 75% of the oxidative metabolism of marketed drugs (Gonzalez, 1990; Rendic and Di Carlo, 1997). Other enzymes such as glutathione transferase, UDP-glucuronosyltransferase, and sulfotransferase are involved in the conjugation of xenobiotics in phase II metabolism (Meyer, 1996; Williams et al., 2004). The expression and function of these transporters and enzymes are important determinants of the physiological turnover of endogenous compounds and clearance of exogenous substances including clinically used drugs.

Hepatitis C virus (HCV) infects an estimated 170 million people worldwide, and its infection is a leading cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (World Health Organization, 1999). Currently, the combination therapy of pegylated interferon (IFN) and ribavirin is the only approved treatment for HCV infection. However, this treatment regimen is only effective in ap-

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ABBREVIATIONS: SLC, solute carrier; ABC, ATP-binding cassette; HCV, hepatitis C virus; IFN, interferon; uPA/SCID, urokinase plasminogen activator-transgenic severe combined immunodeficiency disorder; PCR, polymerase chain reaction; ISG, interferon-stimulated gene; hGAPDH, human glyceraldehyde-3-phosphate dehydrogenase; P450, cytochrome P450; LC, liquid chromatography; MS/MS, tandem mass spectrometry; MRP, multidrug resistance-associated protein; OATP, organic anion-transporting polypeptide; OCT, organic cation transporter; P-gp, P-glycoprotein; MDR, multidrug resistance; BSEP, bile salt export pump; NTCP, Na^+-taurocholate cotransporting polypeptide; OAT, organic ion transporter; C_t, cycle threshold.