

## In vivo Fluorescence Microscopy for Quantitative Analysis of the Hepatic Microcirculation in Hamsters and Rats

M.D. Menger<sup>a</sup>, I. Marzi<sup>b</sup>, K. Messmer<sup>a</sup>

<sup>a</sup> Institute for Surgical Research, University of Munich, FRG;

<sup>b</sup> Department of Trauma Surgery, University of Saarland, Homburg/Saar, FRG

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**Abstract.** Using intravital fluorescence microscopy and epi-illumination, the hepatic microcirculatory system of Syrian golden hamsters was analyzed, and the morphology and microhemodynamics were compared to those of rats. After contrast enhancement with 1 µmol/kg acridine orange i.v., the epi-illumination technique allows for visualization of capillary sinusoids and postsinusoidal venules, which are running in parallel with the liver surface, while afferent microvessels could be visualized in only few of the liver lobules investigated. In rat livers, the capillary sinusoids showed morphology similar to that of hamsters, however, postsinusoidal venules could frequently not be observed when applying epi-illumination, since these microvessels are piercing perpendicularly into the depth of the liver tissue. Microhemodynamic analysis, including the sinusoidal perfusion rate, sinusoidal red blood cell velocity and diameters, microvascular white blood cell (WBC) count and the phenomenon of WBC-endothelium interaction, as well as the hepatocellular uptake of the fluorescent compound acridine orange were found to be similar in hamsters as compared to rats. Although transillumination for *in vivo* microscopy may have the potential to visualize the complete hepatic microcirculatory system due to an increased focus depth, the epi-illumination technique has the advantage for quantitative assessment not only of the morphology of the hepatic microcirculatory system and microvascular blood perfusion, but also allows for evaluation of cellular phenomena within the hepatic microvessels, such as WBC accumulation, WBC-endothelium interaction, phagocytotic activity of Kupffer cells, and hepatocellular transport of fluorescent compounds. Hepatic microcircular disturbances, including accumulation of WBCs and WBC-endothelium interaction are causative in the development of organ failure in conditions such as hemorrhagic and septic shock, and, in particular, postischemic reperfusion injury following liver surgery and liver transplantation. Since accumulation of WBCs and their interaction with the microvascular endothelium are primarily found in postsinusoidal venules, *in vivo* microscopy of the hamster liver represents a favorable model for studies on cellular phenomena within the hepatic microcirculation.

## Introduction

Hepatic microcirculatory disorders play a major pathogenic role in the development of organ failure in a variety of pathophysiological conditions such as hemorrhagic and septic shock, and, in particular, during postischemic reperfusion following liver surgery and liver transplantation. However, only few reports are available, analyzing the pathogenic mechanisms within the hepatic microcirculation during hypovolemia [1, 2], sepsis [3], transplantation [4, 5] and ischemia-reperfusion [6–9]. This might be due to the lack of adequate experimental models allowing for *in vivo* observation of the hepatic microcirculation. Several techniques have been described for the assessment of the hepatic microvascular perfusion, such as the hydrogen gas clearance method [10, 11], or laser Doppler flowmetry [12] as indirect methods, and intravital microscopy as a direct approach [13]. The technique of intravital microscopy is superior as compared to the indirect techniques due to the visualization of the hepatic microvasculature. Apart from the assessment of microvascular perfusion, this technique has the potential to evaluate a variety of microvascular phenomena occurring within the hepatic microcirculation, such as changes of microvascular blood perfusion, hepatocellular transport, white blood cell (WBC) accumulation, WBC-endothelium interaction, as well as phagocytotic activity of the Kupffer cells.

In 1833 Kiernan [14] has been the first studying the morphology of the hepatic microvasculature. During the following decades a series of studies were published elucidating the microvascular structure of the liver using dye injection techniques, such as indigo carmine and India ink [15–19]. In the 20 s and 30 s of this century research of the

liver microcirculation was focussed on the nature of anastomoses between hepatic and portal circulation [20–22]. However, all these studies were limited due to the static analysis from histological preparations.

First dynamic investigations of the hepatic microcirculation were performed by Knisely [23] in 1936, describing a method for illuminating living structures for microscopic studies. Using the quartz rod method of transillumination, basic concepts of the living anatomy and certain aspects of physiology and pathology of the liver microcirculation were derived from investigations in poikilothermic [amphibians: frogs, 13, 24] and homeothermic, [mammals: rats, mice, guinea pigs, rabbits, monkeys, 24–26] animals; however, up today no information is available on the hepatic microcirculation of hamsters.

In recent years hepatic microvascular regulatory mechanisms, including adrenergic and cholinergic response have been investigated [27–32] using advanced techniques for intravital microscopy. In particular, the Allen video-enhanced contrast, differential interference contrast (AVEC-DIC) microscopy [33, 34] allows for quantitative assessment of hepatic microvascular perfusion. However, this technique has not the potential to study cellular phenomena occurring within the microvasculature, such as WBC accumulation and WBC-endothelium interaction. Since WBCs, in particular neutrophils, appear to play a major role in pathophysiological conditions, such as hypovolemia, sepsis and ischemia-reperfusion [7, 35–40], the purpose of this study was to establish a model yielding in visualization of hepatic microvascular perfusion in hamsters and rats, including flow behavior of WBCs, using an *in vivo* fluorescence microscopy technique with epi-illumination and contrast en-

hancement by acridine orange. Previous studies from our laboratory [4–8] revealed that postsinusoidal venules of the hepatic microcirculation in rats frequently cannot be visualized using the epi-illumination technique. Since it is well known that accumulation of WBCs and their interaction with the microvascular endothelium takes place preferentially in postcapillary venules [36, 38], we aimed at finding out whether the postsinusoidal venules can be visualized in the hamster liver. Therefore, the microangioarchitecture and microhemodynamics of the hepatic microcirculation of Syrian golden hamsters were compared to the hepatic microcirculation of rats.

## Material and Methods

### Animals

Our study was carried out in Syrian golden hamsters (12–14 weeks old, 120–150 g body weight), as well as Wistar, Lewis and Sprague-Dawley rats (12–18 weeks old, 250–350 g body weight). The animals were housed 1 per cage and had free access to tap water and standard pellet food.

### Experimental Design

The animals were anesthetized by intraperitoneal injection of sodium pentobarbital (Nembutal, Abbott, North Chicago, Ill.), using 50 mg/kg body weight for Syrian golden hamsters, and 30 mg/kg body weight for rats. Only in rats, a tracheotomy was performed to facilitate respiration (room air). Subsequently, indwelling catheters were placed into the carotid artery and jugular vein, allowing for assessment of macrohemodynamic parameters, as well as for the injection of fluorescent dyes.

A median laparotomy was performed and the falciform ligament was incised to partially free the liver from the diaphragm in order to facilitate the exteriorization of the liver for intravital microscopy, and to prevent movement of the liver induced by the respiratory system. Gauze packing was placed between the liver and the intestine to eliminate movements originating from the gastrointestinal tract. The animals

were positioned on their left side on a Plexiglas stage and the left liver lobe was exteriorized and placed on a mechanical stage, such that the lower surface of the liver lobe was situated horizontal to the microscope, which guaranteed adequate homogeneous focus level for the microscopic procedure on the area of liver surface under investigation. In addition, the adjustment of the mechanical stage allowed to avoid mechanical obstruction of the macrovessels feeding and draining the liver lobe. Intravital microscopy of the hepatic microcirculation was performed in Syrian golden hamsters ( $n = 5$ ), as well as in Wistar ( $n = 3$ ), Lewis ( $n = 4$ ), and Sprague-Dawley rats ( $n = 8$ ).

### Intravital Fluorescence Microscopy

For intravital microscopy, the lower surface of the left liver lobe was covered with saran wrap in order to prevent drying of the tissue and exposure to ambient air. The liver was attached to a modified Leitz-Orthoplan microscope (Leitz, Wetzlar, FRG), and 1  $\mu\text{mol}/\text{kg}$  acridine orange (Sigma, Chemical Co., St. Louis, Mo.) was injected intravenously, allowing fluorescent bright *in vivo* staining of the WBCs for the microscopic procedure [41].

For epi-illumination a 100-watt, HBO, mercury lamp attached to a Ploemo-Pak illuminator with an  $I_{2/3}$  blue filter block (excitation wavelength: 450–490 nm, emission wavelength: 515 nm, Leitz, Wetzlar, FRG) was used. The observations were recorded by means of a charge coupled device (CCD)-video camera (COHU FK 6990; Prospective Measurements, San Diego, Calif.) and transferred to a U-matic video system (VO-5800 PS; Sony, Munich, FRG) for off-line evaluation. In order to facilitate evaluation of dynamic video images, a time frame generator (UTG-33, FOR.A, Tokyo, Japan) was superimposed to the video screen. Using 12  $\times$  eyepieces and a long distance working objective (Fluotar 10 $\times$ /0.30, FAA 7,7; Leitz, Wetzlar, FRG), as well as a water immersion objective (W 25 $\times$ /0.60, Leitz, Wetzlar, FRG), magnifications of  $\times 280$  and  $\times 700$  were achieved on the video screen (PVM-1371 QM, diagonal: 330 mm; Sony, Munich, FRG).

Using this technique, *in vivo* analysis of the hepatic microvasculature was performed in 6–10 liver lobules from each animal. The microcirculation of each liver lobule was recorded for 30–60 s in order to avoid microcirculation disorders due to epi-illumination. The total *in vivo* microscopic procedure was performed during a 10-min period.

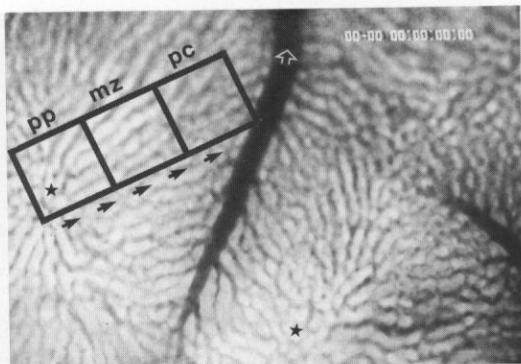
### *Quantitation of Microvascular Phenomena*

Quantitation of microhemodynamics of capillary sinusoids and postsinusoidal venules (central venules) was performed off-line by frame-by-frame analysis of videotaped images. In addition, capillary sinusoids were grouped in three zones, i.e. periportal, midzonal, and pericentral segment of the hepatic sinusoids, according to zonal labeling resulting from the endothelial-hepatocellular transport of fluorescent compounds as reported by Gumucio et al. [42]. Analyses included determination of microvascular blood perfusion, as well as assessment of the flow behavior of WBCs, including WBC accumulation and WBC-endothelium interaction.

Analyses in capillary sinusoids included the determination of the following parameters: sinusoidal perfusion rate (percentage of perfused sinusoids); sinusoidal diameters (analyzed by means of the Computer-Assisted Microcirculation Analysis System, CAMAS [43]; sinusoidal red blood cell (RBC) velocity [analyzed by means of the dual slit technique, 44]; accumulation of WBCs [WBC concentration within the complete sinusoidal network of a liver lobule, calculated from randomized frozen video images ( $n = 5$ ) during 10–20 s, including nonadhesive, rolling, temporary adhesive as well as permanently adhesive WBCs]; number of permanently adhesive WBCs (number of WBCs adhering to the endothelial wall over a time period of more than 20 s calculated for the complete sinusoidal network of a liver lobule); number of temporary adhesive WBCs (number of WBCs adhering to the endothelial wall over a time period of less than 20 s calculated for the complete sinusoidal network of a liver lobule; temporary adhesive WBCs are expressed as percentage of the total number of stained WBCs circulating within the sinusoidal network); and number of rolling WBCs (number of WBCs rolling along the endothelial lining of the sinusoids; rolling WBCs are calculated for the complete sinusoidal network of a liver lobule, and expressed as percentage of the total number of stained WBCs circulating within the sinusoidal network).

All microcirculatory parameters were determined within the complete sinusoidal network of the liver lobules, as well as within the sinusoidal subgroups, i.e. the periportal, midzonal, and pericentral sinusoidal segment, according to the zones 1, 2 and 3 described by Gumucio et al. [42].

Analyses in postsinusoidal venules included the determination of the following parameters: diameters



**Fig. 1.** Microvasculature of a hamster liver lobule. Note the stellate origin ( $\star$ ) of the periportal sinusoids (pp). Blood flow (arrows) continues to the midzonal (mz) and pericentral (pc) segment of the sinusoids, and is drained into the postsinusoidal venule (open arrow).

of postsinusoidal venules (analyzed by means of the Computer-Assisted Microcirculation Analysis System, CAMAS) [43]; RBC velocity in postsinusoidal venules [analyzed by means of the dual slit technique, 44]; accumulation of WBCs [WBC count within the postsinusoidal venules, calculated from randomized frozen video images ( $n = 5$ ) during 10–20 s, including nonadhesive, rolling, temporary adhesive as well as permanently adhesive WBCs; WBC count in postsinusoidal venules is expressed as number of WBC/mm<sup>3</sup> of blood within the postsinusoidal venules]; number of permanently adhesive WBCs (number of WBCs adhering to the endothelial wall of postsinusoidal venules over a time period of more than 20 s; permanently adhesive WBCs are expressed as number of WBC/mm<sup>2</sup> of microvascular endothelium); number of temporary adhesive WBCs (number of WBCs adhering to the endothelial wall of postsinusoidal venules over a time period of less than 20 s; temporary adhesive WBCs are expressed as percentage of the total number of stained WBCs circulating in the postsinusoidal venule under investigation); number of rolling WBCs (number of WBCs rolling along the endothelial lining of the postsinusoidal venules; rolling WBCs are expressed as percentage of the total number of stained WBCs circulating in the postsinusoidal venule under investigation).

Figure 1 shows the microvasculature of a hamster liver lobule with its sinusoids and postsinusoidal ve-

nules, including a superimposed schematic drawing, which indicates blood flow direction as well as the subgrouping of the sinusoids.

## Results

### *Morphology of the Hepatic Microvasculature of Hamsters and Rats*

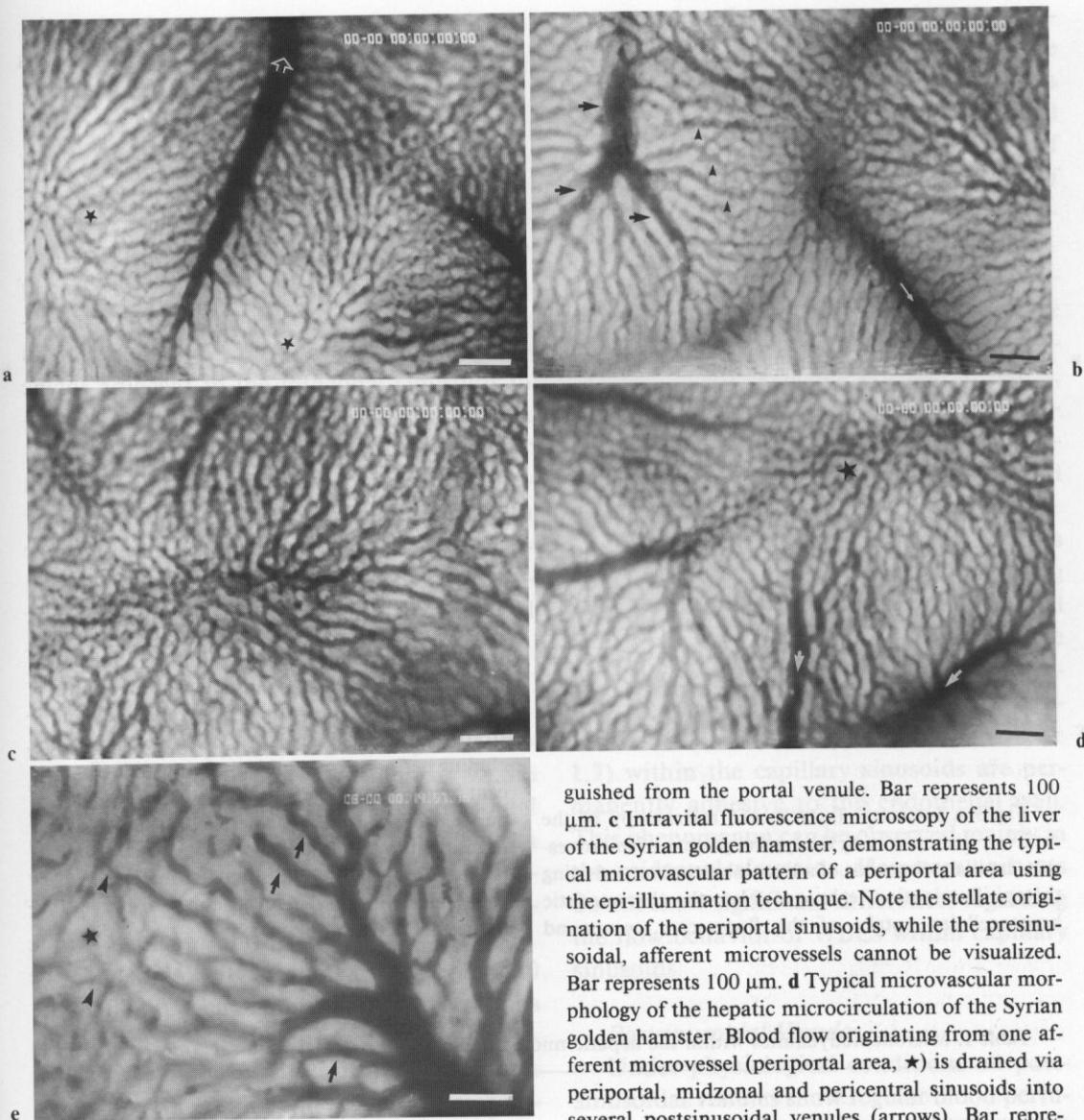
The hepatic microcirculatory unit consists of three microvascular components: the afferent vessels, represented by the portal venule and the hepatic arteriole, the capillary sinusoids, serving for the nutritional blood supply of the liver, and the postsinusoidal venule (terminal hepatic venule/central venule), draining the hepatic microcirculation. Intravital fluorescence microscopy of the hepatic microcirculatory system of the Syrian golden hamster allows to identify very clearly the network of capillary sinusoids and the postsinusoidal venules, which are running parallel to the liver surface (fig. 2a). However, in most of the liver lobules investigated, the afferent microvessels cannot be visualized. Only few of the liver lobules allow to visualize the morphology of all three components of the hepatic microcirculatory unit (fig. 2b), while regularly, the periportal area is recognized by a stellate origin of the periportal capillary sinusoids (fig. 2c). In addition, although the afferent microvessels are visualized in some of the liver lobules, dynamic fluorescence microscopy using epi-illumination has not the potential to discriminate between blood flow from the portal venule and the hepatic arteriole, respectively.

Microvascular blood flow originating from one afferent vessel is drained through the capillary sinusoids into several postsinusoidal venules (fig. 2d), and, similarly, one

particular terminal venule serves as venular drainage for several afferent microvessels (fig. 2a). In the hamster, the image quality achieved by *in vivo* microscopy, even at higher magnifications (fig. 2e), allows for quantitative analysis of microhemodynamic parameters within the capillary sinusoids and within the postsinusoidal venules.

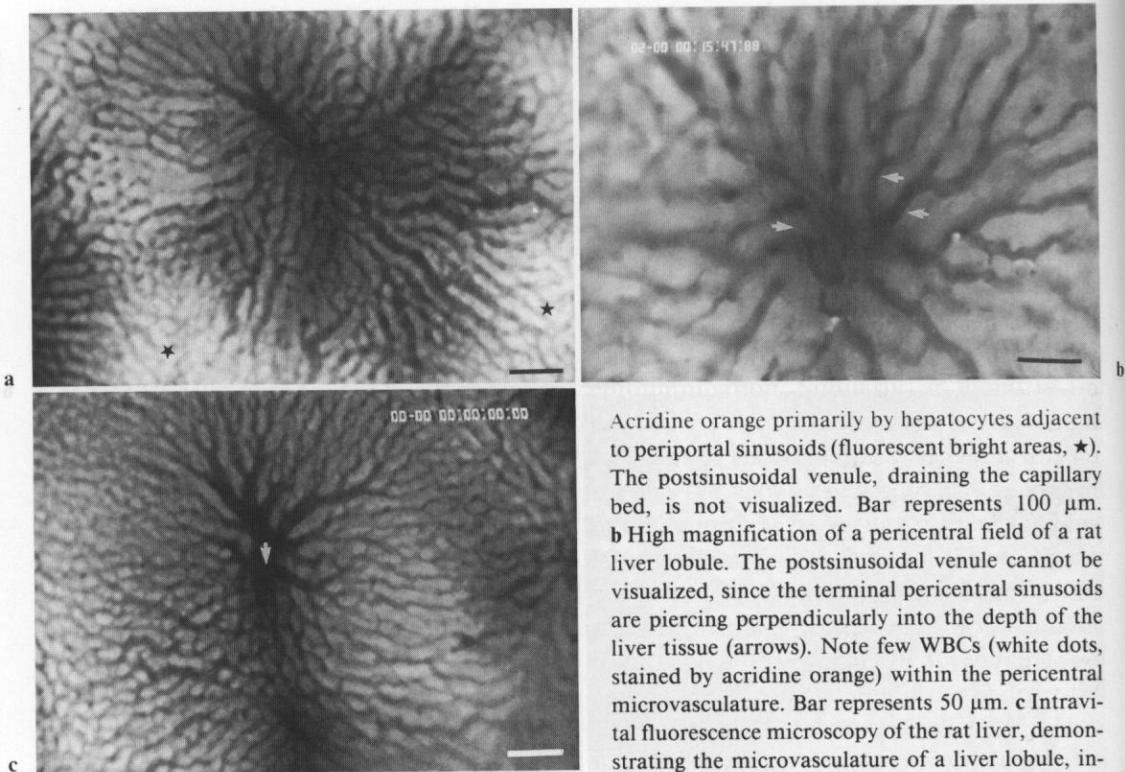
Figure 3a shows a typical video image of the hepatic microvasculature of the rat, using intravital fluorescence microscopy and epi-illumination. Similarly, as found in the liver of the hamsters, the afferent microvessels cannot be visualized. However, in contrast to the hepatic microcirculatory system of the hamsters, more than 50% of the liver lobules do not allow visualization of the postsinusoidal venules in the rat (fig. 3b), since the capillary sinusoids vanish from the microscopic focus level due to piercing perpendicularly into the depth of the liver tissue. By scanning the liver surface the microscopic technique reveals postsinusoidal venules partially, as demonstrated in figure 3c, and only in a few lobules postsinusoidal venules can be analyzed to the same extend as in Syrian golden hamsters (fig. 2a). The image quality achieved by *in vivo* microscopy of the rat liver is inferior to video images of the hamster liver, nevertheless, quantitative

**Fig. 2. a** Intravital fluorescence microscopy of the liver of the Syrian golden hamster, demonstrating the capillary sinusoids of two liver lobules draining into one postsinusoidal venule (open arrow), which is visualized over a length of approximately 650 µm. Note the characteristic uptake of the fluorescent compound acridine orange (1 µmol/kg body weight i.v.) by hepatocytes adjacent to the periportal, as well as to the midzonal sinusoids (fluorescent bright areas, ★). Bar represents 100 µm. **b** Intravital fluorescence mi-



croscopy of the liver of the Syrian golden hamster, demonstrating all three components of the hepatic microcirculatory system, including the afferent microvessels (arrows) surrounded by bright hepatocytes due to their uptake of acridine orange, the capillary sinusoids (arrowheads), and a postsinusoidal venule (white arrow). Although the afferent microvessels can be visualized, the hepatic arteriole cannot be distin-

guished from the portal venule. Bar represents 100  $\mu\text{m}$ . c Intravital fluorescence microscopy of the liver of the Syrian golden hamster, demonstrating the typical microvascular pattern of a periportal area using the epi-illumination technique. Note the stellate origination of the periportal sinusoids, while the presinusoidal, afferent microvessels cannot be visualized. Bar represents 100  $\mu\text{m}$ . d Typical microvascular morphology of the hepatic microcirculation of the Syrian golden hamster. Blood flow originating from one afferent microvessel (periportal area, ★) is drained via periportal, midzonal and pericentral sinusoids into several postsinusoidal venules (arrows). Bar represents 100  $\mu\text{m}$ . e Intravital fluorescence microscopy of the liver of the Syrian golden hamster. Higher magnification reveals the increase of sinusoidal diameters in pericentral (arrows) vs. periportal (arrowheads) sinusoids, as well as the characteristic uptake of the fluorescent compound acridine orange (1  $\mu\text{mol/kg}$  body weight i.v.) by hepatocytes (★) adjacent to periportal and midzonal, but not pericentral sinusoids (fluorescent bright areas). Bar represents 50  $\mu\text{m}$ .



**Fig. 3.** **a** Intravital fluorescence microscopy of the rat liver, demonstrating the typical pattern of microvessels (capillary sinusoids of a liver lobule) observed using the epi-illumination technique. Note the characteristic hepatocellular uptake of the fluorescent compound

Acridine orange primarily by hepatocytes adjacent to periportal sinusoids (fluorescent bright areas, ★). The postsinusoidal venule, draining the capillary bed, is not visualized. Bar represents 100 µm. **b** High magnification of a pericentral field of a rat liver lobule. The postsinusoidal venule cannot be visualized, since the terminal pericentral sinusoids are piercing perpendicularly into the depth of the liver tissue (arrows). Note few WBCs (white dots, stained by acridine orange) within the pericentral microvasculature. Bar represents 50 µm. **c** Intravital fluorescence microscopy of the rat liver, demonstrating the microvasculature of a liver lobule, including periportal and midzonal sinusoids (fluorescent bright staining of the liver tissue), as well as pericentral sinusoids. The pericentral sinusoids are draining into the postsinusoidal venule, which can be visualized partially (arrow) before piercing perpendicularly into the depth of the liver tissue. Bar represents 100 µm.

**Table 1.** Microhemodynamics within the hepatic microvasculature of hamsters and rats (mean ± SD)

	Hamsters	Rats
Mean arterial pressure, mm Hg	115 ± 10.6 (5)	135 ± 8.5 (8)
Heart rate, min <sup>-1</sup>	378 ± 34.2 (5)	359 ± 23.5 (8)
Sinusoidal RBC velocity, mm/s	0.51 ± 0.13 (87)	0.53 ± 0.12 (56)
Diameters of periportal sinusoids, µm	12.2 ± 3.7 (87)	9.7 ± 1.6 (56)
Diameters of pericentral sinusoids, µm	15.9 ± 4.6 (87)	14.2 ± 4.0 (56)
RBC velocity of postsinusoidal venules, mm/s	>1.00 (87)	>1.00 (56)

Figures in parentheses represent number of animals and microvessels, respectively.

analysis of microhemodynamic parameters within the capillary sinusoids and postsinusoidal venules is feasible.

#### *Hemodynamics within the Hepatic Microcirculation of Hamsters and Rats*

Intravenous application of acridine orange yields information on hepatocellular uptake of the fluorescent compound into the hepatocytes adjacent to the periportal sinusoids, and to a minor degree into hepatocytes adjacent to the midzonal sinusoids in hamsters (fig. 2) and rats (fig. 3). Contrast enhancement by acridine orange allows for microcirculatory analyses in capillary sinusoids and in postsinusoidal venules in both species studied, while analysis within the afferent microvessels cannot be performed regularly, since these vessels can be visualized only on rare occasions.

##### *Capillary Sinusoids*

Sinusoidal perfusion rate (e.g. simultaneous perfusion of the sinusoids) is 98.6% (140/142) within the complete sinusoidal network, and no difference is found between periportal, midzonal, and pericentral sinusoids. The sinusoidal perfusion rate is similar in hamsters and in rats.

Sinusoidal RBC velocity ranges from 0.20 to 0.80 mm/s, characterized by an enormous heterogeneity within sinusoids of the identical liver lobule. In addition, RBC velocities show a gradient along the sinusoid, presenting with lower values in periportal sinusoids as compared to midzonal sinusoids, while the highest values can be observed in pericentral sinusoids. There are no significant differences in sinusoidal RBC velocity between hamsters and rats (table 1).

In addition, pericentral sinusoids reveal larger diameters as compared to periportal

sinusoids (see fig. 2, 3). In hamsters pericentral diameters at the region of inflow into the terminal venule range from 10 to 30  $\mu\text{m}$  as compared to from 7 to 20  $\mu\text{m}$  in periportal sinusoids. Similarly, in rats diameters of pericentral sinusoids are found in a range of 9–26  $\mu\text{m}$  as compared to 7–13  $\mu\text{m}$  in periportal sinusoids (table 1).

Mean WBC concentration within the capillary sinusoids of a complete liver lobule amounts to  $5.4 \pm 1.8$ , and no significant differences are observed comparing periportal, midzonal and pericentral sinusoids. In addition, no differences in sinusoidal WBC concentration are found between hamsters and rats.

Analysis of the flow behavior of WBCs reveals that  $1.2 \pm 0.4\%$  of the circulating cells temporarily adhere to the endothelial lining, while  $98.8 \pm 0.4\%$  are nonadhesive cells. WBCs rolling along the endothelial lining are not found within the capillary sinusoids. In addition, only few WBCs ( $2.1 \pm 1.7$ ) within the capillary sinusoids are permanently adhesive to the endothelial wall. This phenomenon can be observed mainly in the midzonal sinusoids. Hamsters and rats do not show significant differences regarding the flow behavior of WBCs within capillary sinusoids.

##### *Postsinusoidal Venules*

Under physiological conditions all postsinusoidal venules show regular blood perfusion. At the origin of the venules the diameters are  $\sim 30 \mu\text{m}$ , and increase continuously according to the number of pericentral sinusoids draining into the venule (fig. 2a). The venules visualized in rats reveal an identical morphological pattern as compared to those in hamsters. In both species, RBC velocity within these microvessels is  $> 1.00 \text{ mm/s}$ ,

and exceeds frequently 1.50 mm/s, values which are out of the detection limit of the dual slit technique.

The WBC count analyzed from postsinusoidal venules ranges from 5,000 to 12,000 cells/mm<sup>3</sup>, which corresponds to the systemic WBC count. WBC-endothelium interaction can rarely be observed; however, in contrast to capillary sinusoids, only a few WBCs are rolling along the endothelial lining of the postsinusoidal venules. The overall flow behavior of WBCs within the terminal venules does not reveal a significant difference between hamsters and rats. In addition, microcirculatory analysis in Wistar, Lewis and Sprague-Dawley rats reveals no significant differences.

## Discussion

### Model

In contrast to indirect techniques for analysis of the hepatic microcirculation, *in vivo* microscopy has the potential to visualize the microvessels, and allows for quantitative assessment of dynamic microvascular phenomena. Intravital microscopy of the hepatic microcirculatory system was described first by Knisely [24] in 1939. During the following years, several investigators have used this technique for *in vivo* analyses of the morphology of the hepatic microvasculature [13, 26], as well as for their response to different physiological stimuli [27–29, 31, 32]. The technique requires transillumination of the liver tissue and does not allow for analysis of dynamic cellular phenomena, such as WBC accumulation and WBC-endothelium interaction.

Since white blood cells and their behavior within the microcirculation are of crucial

importance in a variety of pathophysiological conditions, ischemia-reperfusion in particular [7, 35–40], we have focussed our interest on the WBCs using acridine orange and the epi-illumination technique for intravital fluorescence microscopy of the hepatic microcirculation. Few authors have already used epi-illumination for the study of hepatocellular transport of fluorescent compounds [42, 45]; however, the dynamics of intrahepatic traffic and behavior of WBCs have not been described.

Using the epi-illumination technique with fluorescent molecules for *in vivo* assessment of the particular role the WBCs play in ischemia-reperfusion [46], hemorrhagic shock [2] and liver transplantation [4–8], we have demonstrated that the technique can be standardized and yields quantitative data on various parameters derived from the hepatic microvasculature in rats. In addition, in this study we have established for the first time standardized and quantitative analysis of the hepatic microvasculature of Syrian golden hamsters.

The technique fails to visualize the afferent microvessels of the hepatic microcirculation. However, it is well known that accumulation of WBCs and WBC-endothelium interaction takes place preferentially in postcapillary venules [36, 38]. Our technique is sufficient to analyze flow behavior of WBCs in postsinusoidal venules, as recently demonstrated for the case of ischemia-reperfusion in rats [46].

In addition, the technique has the potential to simultaneously investigate phagocytic activity of Kupffer cells [3], and liver functions, such as hepatocellular transport [42, 45] using fluorescent-labeled latex beads and fluorescent molecules activated at different wavelength of fluorescent light.

### Morphology and Microhemodynamics

The present study has elucidated the similarity of phenomena in the hepatic microcirculation of hamsters and rats, including the morphology of the sinusoids, the hepatocellular transport of the fluorescent compound acridine orange, and microhemodynamic parameters, such as sinusoidal RBC velocity and sinusoidal diameters. In addition, under physiological conditions intrahepatic microvascular WBC concentration was similar in both species and spontaneous WBC-endothelium interaction was rarely encountered.

However, analysis of the morphology of the hepatic microvasculature revealed also some distinct differences between hamsters and rats. Few liver lobules from Syrian golden hamsters allowed to visualize the afferent microvessels of the microcirculatory system, while those microvessels could never be demonstrated in rats. In addition, in hamsters the postsinusoidal venules could regularly be visualized by intravital fluorescence microscopy, since these vessels are aligned in parallel to the liver surface. In contrast, in rats postsinusoidal venules could frequently not be visualized, since these vessels are piercing perpendicularly into the depth of the liver tissue. Therefore, analysis within postsinusoidal venules in rats requires thorough scanning of the liver lobe in order to identify terminal venules accessible for investigation.

The reason for this particular failure (visualization of postsinusoidal venules in rat livers) might be due to the reduced focus depth when using epi-illumination for intravital microscopy. This may also explain the superior visualization of the hepatic microcirculatory system in hamsters, since the organ and, similarly, the liver lobules are smaller in size compared to those of rats (see

fig. 2a vs. 3a), and the liver capsule may be more translucent. The transillumination technique allows for analysis of the complete hepatic microvascular system [26] based on increased focus depth as compared to the epi-illumination technique. However, using transillumination only the edge of the liver lobe can be investigated, and cellular phenomena, including WBCs, Kupffer cells, as well as hepatocellular transport cannot be visualized. Using epi-illumination and exposure to fluorescent molecules our technique allows to investigate the total surface of the liver lobe, and to quantify cellular phenomena, such as flow behavior of white blood cells and phagocytosis of the Kupffer cells.

*In conclusion*, the advantage of using Syrian golden hamsters for *in vivo* studies of the hepatic microcirculation consists of the better image quality for intravital fluorescence microscopy. In addition, postsinusoidal venules can be investigated regularly, which may be of particular importance analyzing microvascular phenomena following events of inflammation and reperfusion. The advantages using rats for *in vivo* microscopic studies of the hepatic microcirculation are primarily the size of the animal (allows for liver transplantation), the genetics, which are well defined (analysis of immunologic response), and the availability of antibodies directed against inflammatory mediators, such as anti-TNF or anti-IL-1.

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Dr. med. Michael D. Menger  
Institut für Chirurgische Forschung  
Klinikum Grosshadern  
Ludwig-Maximilians-Universität  
Marchioninistrasse 15  
D-W-8000 München 70 (FRG)