Res Exp Med (Berl) 177, 125–134 (1980)

Research in
Experimental Medicine

Technical Report—A New Chamber Technique for Microvascular Studies in Unanesthetized Hamsters*

Bernhard Endrich, Kazuaki Asaishi, Alwin Götz, and Konrad Meßmer

Institute for Surgical Research, Klinikum Großhadern, University of Munich, Marchioninistr. 15, D-8000 Munich 70, Federal Republic of Germany

Summary. An experimental model was designed for direct, quantitative studies of hemodynamic and morphologic parameters in the microcirculation. It consists of implanting a modified Algire chamber in the dorsal skin flap of hamsters and the implementation of two permanent catheters in jugular vein and carotid artery. The microcirculation was studied using intravital microscopy and television techniques for in situ measurements of blood cell velocity and vascular diameters.

Due to the poor contrast between blood cells, blood capillaries and surrounding s.c. tissue, microvascular beds were visualized using fluorescent microscopy after i.v. injection of 0.2 ml of 5% FITC-Dextran 150. The combination of optical elements and low amounts of FITC-Dextran improved the contrast of the televised image without changing macro- and microhemodynamic parameters, and blood plasma was delineated as bright structure against the substantially darker background of red blood cells and surrounding tissue. This permitted the quantitative study of practically all blood vessels within a given field of s.c. tissue in unanesthetized animals. Blood cell velocity in arterioles was 0.7–1.1 mm/s, 0.2–0.7 mm/s in midcapillaries and reached 0.6 mm/s in collecting venules. Since i.v. injection of drugs and systemic pressure measurements are possible in this model, it provides a unique means for studying the reactivity of the microcirculation over a prolonged period.

Key words: Microcirculation – Microhemodynamics – Inflammatory response

Transparent chambers for microscopic and microcirculatory observation have been used for more than five decades [1, 2, 11, 16]. These experiments have established the growth characteristics of sprouting blood vessels after trans-

* Supported by DFG grant EN 114/2
Offprint requests to: Dr. B. Endrich (address see above)

0300-9130/80/0177/0125/$ 2.00

plantation of homologous and heterologous tissue [11, 22], the functional changes on individual blood cells during the passage of capillaries [2], and the anatomical pattern of the microvasculature in systems undergoing enhanced cellular replication [11]. However, with a few notable exceptions [2], these studies have been carried out in small animals where microvascular data could not be related to alterations of central hemodynamic parameters.

To provide this information, we have established the implanation of two permanent catheters and a transparent skin flap chamber in Syrian Gold hamsters. As a result, this design allows for high resolution photography, videorecording of microcirculatory events, and hemodynamic measurements in conscious animals over a prolonged time period.

Material and Methods

Operative Technique

Syrian Gold hamsters weighing between 95 and 110 g were anesthetized by an i.p. injection of pentobarbital[1] (60–70 mg/kg body weight). Upon surgical anesthesia, the entire back of the hamster was shaven and a depilatory used to remove any hair remaining. Two catheters were passed s.c. along the back of the animal almost to the base of the skull, then treated along the midclavicular line and brought exterior at the anterior neck near the right jugular vein.

The technique of implanting an Aluminium access chamber in the dorsal skin flap of rats—originally described by Papenfuss et al. [17]—was modified and adapted to meet carefully established criteria of stability (thin, translucent skin) in hamsters. Therefore, spacers made of stainless steel were used yielding in a frame to frame distance of 400–450 µm while the chamber was affixed in a position perpendicular to the animal's back. The thickness of the preparation and thus the distance between both frames were chosen on the basis of optical translucency needed for proper visualization of microscopic blood channels without compressing larger blood vessels.

To smoothen edges and the coarse surface, the Aluminium frames were covered with a thin layer (20–30 µm) of Teflon S at a temperature of 400° C. This measure also served to reduce thermal conductivity and to guarantee biological inertness. This chamber was very well tolerated by all animals.

The surgical procedure was done by placing the animal on a specially designed surgical stage. A midpoint line was drawn along the back and a dorsal skin fold was affixed to the surgical stage by means of silk sutures (5-0) to stabilize the preparation. One part of the chamber frame consisting of the frame, two lower bolts and spacers was slightly pushed under the skin-fold causing the skin to protrude. Two holes were cut carefully through the skin, this part of the chamber introduced through the holes and fixed using two baby mosquito hemostats.

A template, equivalent to the outer diameter of the chamber's collar served to mark a circle outlining the subsequent incision. A crisscross cut resulting in four skin flaps was made, and each of the four flaps was removed with a fine, curved scissor tracing precisely the perimeter of the outline with an effort to follow the hypodermis. The area exposed was freed from overlaying fascia utilizing an operating microscope[2] leaving one layer of s.c. tissue at the opposite side of the epidermis intact. During this surgical procedure, the area was kept moist by allowing drops of warmed normal saline to irrigate the preparation that was limited to *one* side of the skin-fold only. After trimming the area under observation, the matching side of the chamber was inserted. Mechanical connection of the two chamber frames was accomplished using metal nuts for top and lower bolts. 5-0 sutures[3] were fixed at seven points around the chamber to support the position of the skin flap.

1 Nembutal, Abbott GmbH, Ingelheim, FRG

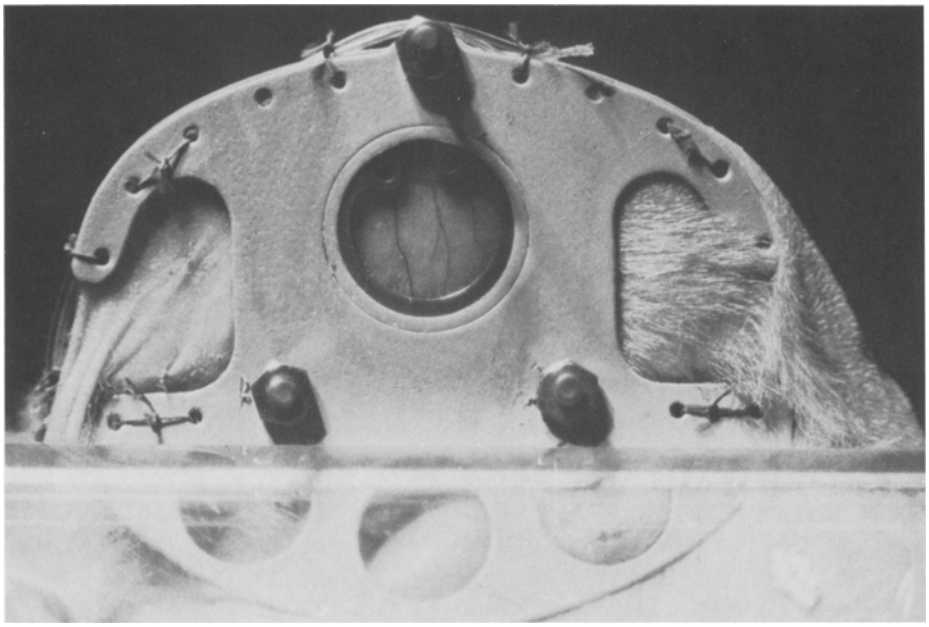

Fig. 1. Dorsal skin flap chamber in hamsters. For photographic documentation, the animal was taught to crawl into a plastic tube

After completing the implantation of the chamber, the animal was placed in supine position and the right carotid artery and right jugular vein were surgically isolated. Fine catheters (PE 10, I.D. 0.28 mm (0.11″), O.D. 0.61 mm (.0.24″))[4] filled with heparinized saline solution (50 U.S.P. in 1 ml) were introduced into the vessel and advanced to the ventricle. The correct position of the catheter was indicated by pressure recordings and pulsatile movements of the catheter. The position of both catheters was later checked by angiography or, in most animals, at autopsy. Both catheters were affixed to the entry points of the vessel cannulated (silk suture 5-0), to the tissue hole proximally and distally as well as to adjacent muscle and s.c. tissue. The anterior incision was then closed with 5-0 Ethibond suture and both catheters were positioned between the frame of the chamber (Fig. 1).

For the purpose of direct observation, as well as video- and photographic recordings, the hamster was later taught to crawl into a transparent plastic tube of approximately the same diameter as the animal in its crouched position. The tube has a narrow slot running lengthwise which allowed just the skin flap and the chamber to be outside the tube, while providing rigid support sufficient for placement on the microscope stage. Special care was taken to avoid any artificial constraint of the preparation.

After 24–48 h were allowed for recovery, measurements of systemic arterial and venous pressure were taken with the animal unanesthetized. The catheters were subsequently flushed with diluted heparin and closed with a blunted wire plug.

Microscopic Observation

Black and white overview photographs were taken of the preparation every day using a 35 mm camera equipped with a 100 mm lens[5]. A medium speed Ilford FP 4 (ASA 125) was chosen to improve the contrast between blood vessels and surroundings, and light was provided by an

2 Carl Zeiss, Oberkochen, FRG
3 Ethibond 6931 H, Ethicon GmbH, Norderstedt, FRG
4 Clay Adams Co., Parsippany, NJ, USA

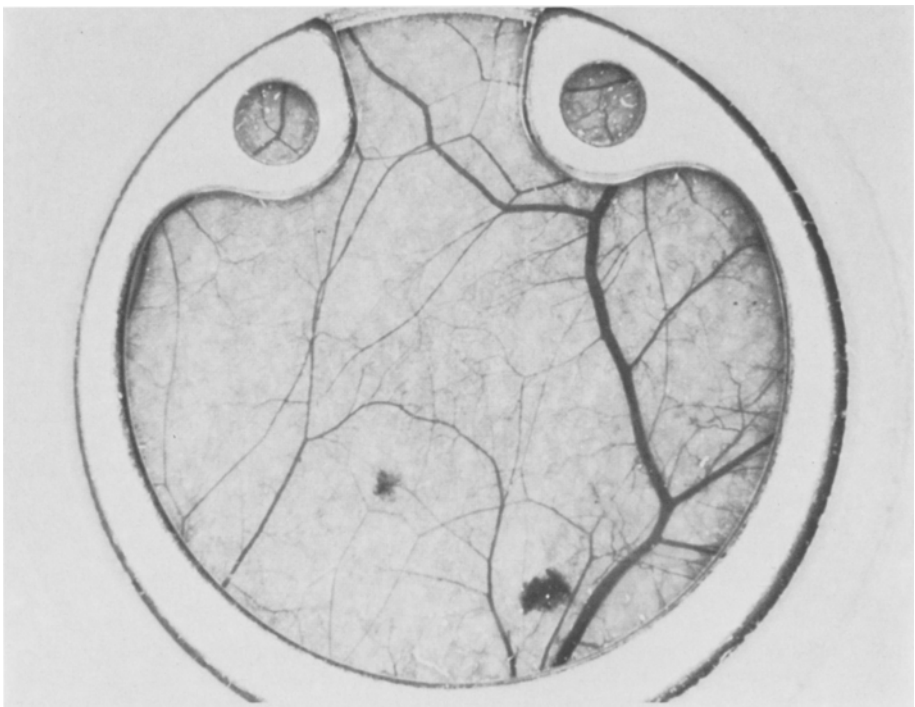

Fig. 2. Overview photography of a typical preparation (magnification 9×)

electronic ring flash incorporated in the lens. By employing an additional close-up lens, the magnification was increased up to 2× at the negative from which a photographic enlargement of 5–40× was possible (Fig. 2). These pictures served as a reference for areas under microscopic observation and microvascular analysis.

Intravital microscopy was done using a modified Leitz Orthoplan focussing unit consisting of a Fluo-opak illuminator, a FSA Tubus GW and a Vario-Tubus (enlargement factor 1–3.2×). For recording microvascular events, the preparation was transluminated by a flexible halogen fiber optic system and a 10× long working distance objective (0.22) with a Periplan Ocular (GW 4×/28) was used to yield a magnification of 50–160×. This image was then televised and recorded on video tape at a rate of 50 frames per second. The enlargement due to the television system implemented[6] and the TV-Monitor[7] increased the magnification by a factor of 10.

Epiillumination was achieved with a 75 W, dc, xenon lamp attached to the Fluo-opak illuminator. For incident fluorescent microscopy, a Ploemopak illuminator[8] with a I_2 blue filter block was used after an injection of 0.2 ml of a 5% FITC-Dextran 150[9] solution.

Measurements of Blood Cell Velocity and Vessel Diameter

The analysis of blood cell velocity and blood vessel diameter was carried out as indicated in Fig. 3. Briefly, the technique consists of recording on video tape the microvascular scenes of

5 Yashica Medical 100 DX, Yashica Europe, Hamburg, FRG
6 Camera COHU 4400, Prospective Measurements, San Diego, USA
7 Barco, Interberg Electronics, Munich, FRG
8 Leitz GmbH, Wetzlar, FRG
9 Fluorescein isothiocyanate covalently bound to dextran, Pharmacia AB, Uppsala, Sweden
10 Typ DU 3S, Ellab Instruments, Copenhagen, Denmark

Table 1. Mean arterial pressure (MAP), central venous pressure (CVP) and heart rate (HR) as well as vessel diameter and blood cell velocity for different microvascular segments [23] in s.c. tissue of unanesthetized hamsters (mean value ± SEM, n_a = number of animals, n = number of determinations)

MAP mm Hg $n_a = 8$		92.8 ± 9.3
CVP mm Hg $n_a = 8$		0.9 ± 1.2
HR min^{-1} $n_a = 8$		353.0 ± 30.0
	Diameter µm	BC-velocity mm/s
Arterioles $n = 13$	17–42	1.0 ± 0.19
Precapillaries $n = 16$	9–19	0.61 ± 0.21
Capillaries $n = 63$	5–10	0.35 ± 0.07
Postcapillaries $n = 63$	8–20	0.39 ± 0.07
Collecting venules $n = 69$	19–30	0.51 ± 0.09

interest, and subsequently isolating from the composite video signal certain areas. Specialized video processors (video photometric analyzer + cross correlation computation) provide directly, and on-line [12], analogous data proportional to the intensity of light in a selected area whose position and size can be controlled manually within a televised scene. Positioning two of these windows along a given capillary provides a means of implementing the so-called dual slit technique [12, 14] with the further advantage that it becomes possible to study simultaneously every blood vessel seen within a given field when the data is recorded on video tape.

At present, the velocities that can be reliably measured by this technique range from 0 to 2.5 mm/s for European TV-standards. Combining velocity data obtained from video tape and luminal data established simultaneously either directly by means of a video image shearing design [13] or from the microphotographs of the preparation under study will provide us with quantitative blood flow information and its distribution.

Control of Temperature

Rectal and local s.c. temperature can be measured using small animal temperature probes[10]. In a separate set of experiments, it was found that both parameters remained constant in unanesthetized animals for a period of a least 90 min (mean value rectal temperature 34,1° C; mean value local s.c. temperature 30,4° C). However, it should be noted that access to the preparation is essential for correct measurements of local temperature in the s.c. tissue under observation. Thus, removal and reinsertion of the chamber's coverslide may traumatize particularly venular vessels resulting in petechial bleeding. As a consequence, the control of local temperature on a routine basis was omitted even though apparent tissue trauma, as suggested by a sudden venular dilation with subsequent outpouring of blood cells, was observed in less than 10% of the animals studied.

Results and Discussion

The rationale for directly observing microcirculatory phenomena is given by the possibility of combining descriptive methods with a quantitative analysis in single

Table 2. Data on flow velocity in *capillaries* as reported in the literature

Species	Specimen	Velocity mm/s	Anesthesia	Reference
Cat	Mesentery	0.5 – 1.0	75 mg/kg α-chloralose + pentobarbital if needed	[14]
Cat	M. sartorius	0.38 ± 0.02	75 mg/kg α-chloralose	[3, 15]
Cat	M. tenuissismus	0 – 1.5	Chloralose 50 mg/kg	[8]
Cat	M. tenuissismus	3.8 ± 1.0	Ketamine 20 mg/kg and chloralose 50 – 70 mg/kg	[9]
Frog	M. pectoralis	0.46 ± 0.37	Urethane 25 – 30 mg/10 g	[10]
Rabbit	Omentum	1.32	Thiopental 45 mg/kg	[24]
Rabbit	Omentum	0.72 – 0.92	Thiopental 45 mg/kg	[7]
Rat	Mesentery	0.68 – 0.96	Halothane	[5]
Rat	s.c.	0.13 – 0.22	Pentobarbital 20 mg/kg	[6]
Human	Nailfold	0.3 – 2.35	No anesthesia	[4]
Hamster	s.c.	0.35 ± 0.07	No anesthesia	[this study]

Table 3. Data on flow velocity *in venous segments of the microcirculation* as reported in the literature

Species	Specimen	Velocity mm/s	Segment	Anesthesia	Reference
Cat	Mesentery	1.22 – 2.2	Postcapillary	Pentobarbital 35 mg/kg	[24]
Cat	Mesentery	0.88 – 2.17	Venules	Pentobarbital 35 mg/kg	[24]
Rabbit	Omentum	0.80 ± 0.05 (S.D.)	Postcapillary	Thiopental 40 – 50 mg/kg	[20]
Rabbit	Omentum	1.10	Postcapillary	Thiopental 50 mg/kg	[24]
Rabbit	Omentum	0.78	Collecting venules	Thiopental 50 mg/kg	[24] [24]
Rabbit	Omentum	2.74	Venule	Thiopental 50 mg/kg	[24] [24]
Rabbit	Omentum	0.74 ± 0.07 (S.D.) 0.72 ± 0.04 (S.D.)	Venules	Thiopental 40 – 50 mg/kg	[20] [20]
Rat	s.c. tissue	0.19 – 0.32	Venules	Pentobarbital 20 mg/kg	[6] [6]
Hamsters	s.c. tissue	0.39 ± 0.07 0.51 ± 0.09	Postcapillaries Venules		[this study]

microscopic blood vessels. In fact, alterations in flow rate, distribution of flow down to the capillary level, and in total volume of flow to a vascular module can be readily quantitated by television techniques [9, 12, 13, 24]. The most important reason for direct microvascular studies refers to the fact that this segment of the circulation is able to function independently with an extreme sensitivity of terminal arterioles and precapillaries. As a consequence, numerous studies on vascular reactivity have established the capacity of different agents to induce a

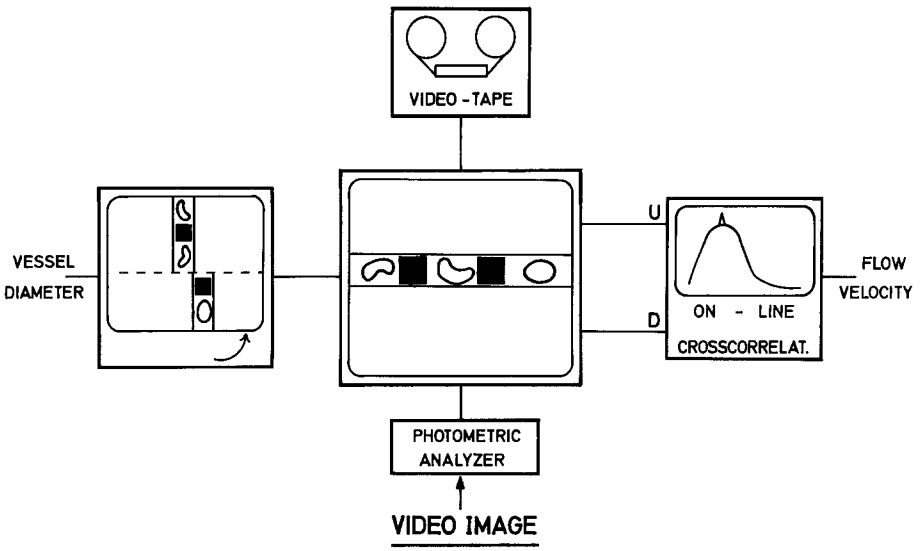

Fig. 3. Diagram of the electronic system for measuring blood cell velocity and vessel diameter in the microcirculation (for further details see [12, 13])

constrictor or dilator response in the microcirculation by measuring blood flow, vessel diameter, and microvascular pressure. To relate these findings to systemic pressures, most of the experiments have been carried out with the animal anesthetized [3, 8, 10, 14, 15, 18], hence adding additional (undesired) effects to the phenomenon under study.

In unanesthetized, small animals transparent chambers were used for observation of microcirculation and surrounding tissue. This technique provides the unique opportunity to study dynamic phenomena, such as neovascularization or vessel reactivity over a prolonged period in the same animal [11, 16].

It is self-evident that in these animals the exact mechanism of microvascular reactivity can be precisely established only when data gathered at the microcirculatory level are correlated to measurements of (at least) systemic pressures and heart rate. In the model presented, measurements of mean arterial pressure, central venous pressure, and heart rate as well as microhemodynamic measurements can be combined with the daily intravital, microscopic observation.

Data on mean arterial pressure and heart rate as well as microhemodynamic data for the hamster's s.c. tissue are listed in Table 1. Our results of blood cell velocity and vessel diameter in the microcirculation are consistent with data reported in the literature (Table 2 and 3). However, it should be noted that most of these analyses were done from "acute" experiments with the animal anesthetized and the preparation exposed only for a few hours. When compared to a "chronic" model, the blood cell velocity data in capillaries of the s.c. tissue of hamsters are elevated compared to skin flap preparations in rats [6] indicating a smaller cross-sectional area of capillaries for s.c. tissue of hamsters.

Immediately following surgical trauma induced by the chamber implantation the changes occurring in the microcirculation can be listed in approximate temporary sequence as follows:

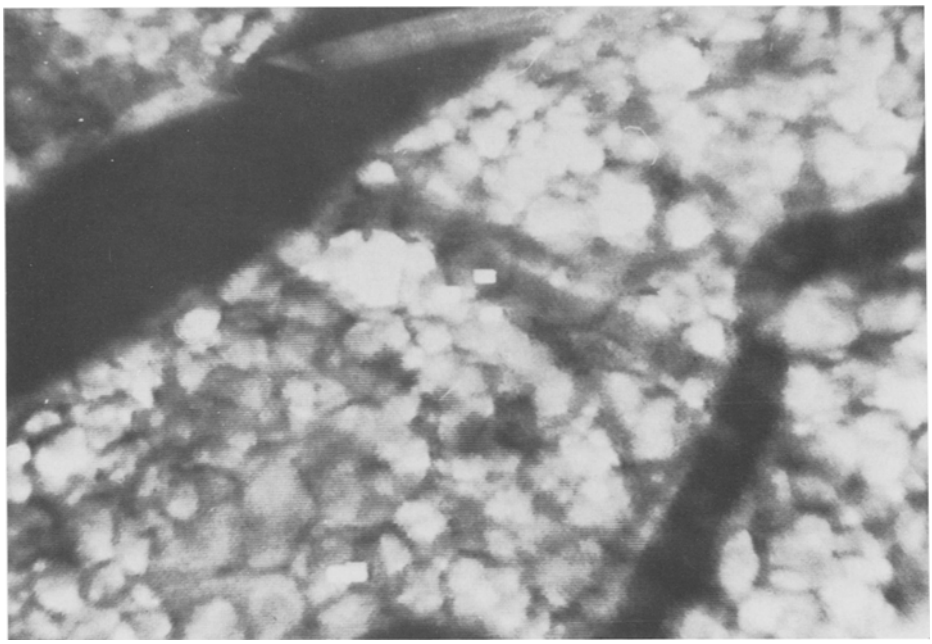

Fig. 4a. Televised scene of the microvasculature prior to the injection of FITC Dextran (magnification ca. 120×). Note the abscence of capillary structures

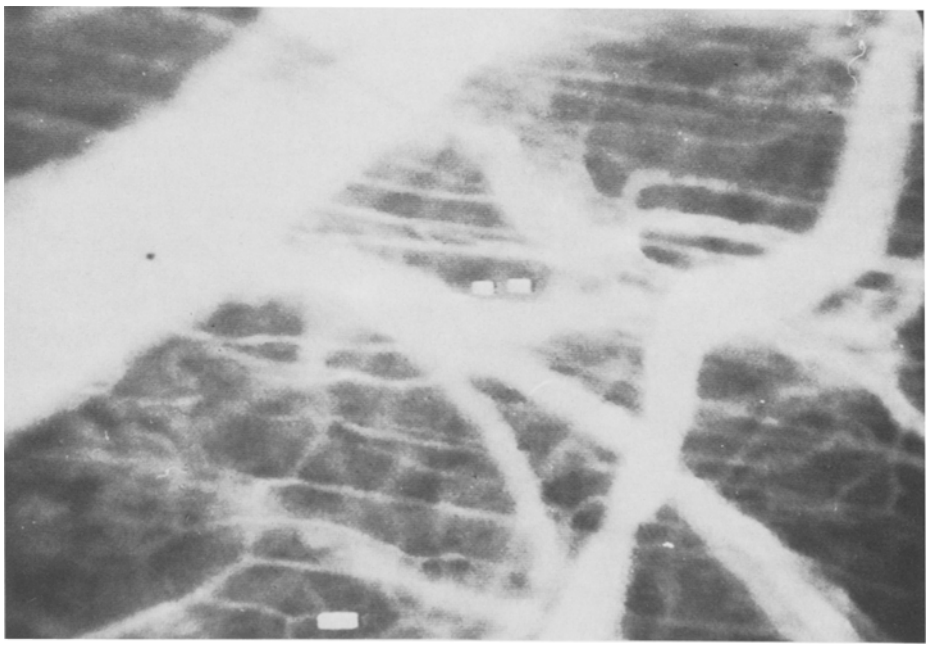

Fig. 4b. The same area about 5 min after the injection of 0.2 ml 5% FITC Dextran 150000. It should be noted that no leakage of fluorescent material was observed at the venous side of the microcirculation

1. Transient vasoconstriction of arterioles resulting in some instances in a partial and temporary ischemia.

2. Arteriolar dilation accompanied by increased flow through arterioles after 2–5 days.

3. Increased flow through capillaries and venules, as well as opening of „inactive" capillary beds.

4. Signs of increased permeability of the microvasculature as indicated by leakage of FITC-Dextran and erythrocytes in some preparations. The increased permeability first affects venules and might extend to capillaries.

5. Local slowing of blood flow within capillaries and venules, sometimes to the point of complete stagnation. In the healing wound, as given in our preparation, this was accompanied by other inflammatory components, such as white blood cell adherence.

These observations are in good agreement to findings reported in the literature [21]. However, our hemodynamic data and morphologic observation suggest that the inflammatory response due to surgical trauma is less pronounced in this hamster preparation because a general, progressive venular and venous dilation accompanied by a lowering of blood cell velocity was observed in none of the preparations studied.

Techniques used in the past have limited the microscopic observation to tissue anywhere between 30–150 µm and thus considered the microvascular analysis to be a more or less twodimensional problem [7, 8, 9, 20, 23]. However, most microvascular structures, e.g. in s.c. tissue layers or in the skeletal muscle, are three-dimensional with a rather poor contrast between blood vessels and surroundings (Fig. 4a). Microvascular beds under study could, however, be visualized using incident light for fluorescent microscopy after i.v. injection of 0.2 ml of 5% FITC Dextran (MW 150000). The combination of optical elements and very low amounts of FITC-Dextran improved the contrast of the televised image without changing macro- and microhemodynamic parameters to such an extent that blood plasma was delineated as bright structure against the substantially darker background of red blood cells and surrounding tissue [19].

Furthermore, it should be noted that the chamber design allows for repeated access to the tissue under observation and thus permits implantation of malignant tissue or micromanipulative maneuvers as indicated earlier [17].

In summary, an experimental model was designed for direct, quantitative studies at the microcirculatory level in small, unanesthetized animals.

It includes (1) a transparent skin flap access chamber and (2) two indwelling catheters in the jugular vein and carotid artery, respectively.

It allows for (1) measurements of systemic pressures and withdrawal of blood, (2) microscopic observation of living tissue, (3) quantification of hemodynamic parameters in the microvasculature at high optical magnification, and (4) analyses of capillary length and density in s.c. tissue.

Acknowledgements. The development of this technique and the measurements performed would not have been possible without the generous advice of Prof. Dr. M. Intaglietta and Prof. Dr. B. W. Zweifach, Department of AMES-Bioengineering, University of California San Diego, La Jolla, CA, USA.

The authors also wish to thank Mrs. R. Pfeiffer and Mrs. A. Kreisle for their valuable technical assistance and Mrs. I. Moll for typing the manuscript.

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Received May 16, 1980 / Accepted June 20, 1980