SCANNING ELECTRON MICROSCOPY OF INJECTION REPLICAS IN THE STUDY OF THE VASCULAR SYSTEM OF MOUSE AND RAT LIVER

N. I. Ryzhova, N. A. Baryshnikova,
T. M. Povalii, and S. A. Gusev

UDC 611.36:611.13/.14]-019-086.3

KEY WORDS: angioarchitectonics of the animal liver, scanning electron microscopy of injection replicas, injection medium.

A new experimental approach to the study of the vascular bed of organs is the method of scanning electron microscopy of injection replicas (SEMIR), by means of which it is possible, unlike by light and transmission-electron microscopy, to study the three-dimensional organization of blood vessels and their microrelief [2, 6, 8]. Due to the use of SEMIR much new and original information on the 3-dimensional arrangement of the network of microvessels of the heart, lungs, kidneys, spleen, liver and many other organs has been obtained [1-5, 9]. At the same time, it will be noted that many of the investigations were carried out by completely filling the vascular bed of the organ with injection medium (IM). However, when all the vessels are filled completely with IM; and this is especially true of organs such as the liver, it is virtually impossible to study the size, character of ramification, and the relief of the various components of the vascular bed.

It is thus clear that elucidation of the structure of the vascular system of the liver is possible only by the use of different methods and degrees of filling of the microcirculatory bed (MCB) with IM.

EXPERIMENTAL METHODS

Experiments were carried out on 18 noninbred male rats and 12 female (C57Bl × CBA)F_1 hybrid mice. Batson's 17 resin (Polyscience, USA) was used as the IM. The blood vessels were filled with the resin either through the cannulcated abdominal aorta or through the portal vein after preliminary flushing out of all the blood from the vascular bed by means of Earle's solution (pH 7.4) at 37°C for 5-10 min. The quantity of IM injected corresponded to 2.5; 5, or 10 ml per animal. The liquid in both vessels was drained through the inferior vena cava. After the end of injection the aorta, portal vein, and inferior vena cava were ligated to prevent the resin from escaping from the vessels. The resin was polymerized under a layer of liquid at room temperature for 2-3 h. Tissue was removed from the replicas in a 25% solution of KOH for several hours. Portions of fresh alkali were alternated with distilled water until all the tissues had been removed. The process of corrosion was carried out at 56°C. The replicas were then dried in air, exposed to OsO_4 vapor for 24 h, glued to the microscope stages, and sprayed with gold in the ELKO-IB-3 apparatus (Hitachi, Japan). The specimens were analyzed on the Philips 501 scanning electron microscope.

EXPERIMENTAL RESULTS

The study of vascular replicas of the animal's liver after injection of the resin through the abdominal aorta showed that the resin first enters arterial microvessels and sinusoids and also the peribiliary vascular plexus. According to the results, the hepatic artery in rats at its origin had a diameter of 240-360 μ (Table 1). The diameter of the lobar arteries after branching of the hepatic artery was 180-210 μ. Vessels of the next orders of magnitude were given off approximately at an angle of 45°, with a gradual reduction of their diameter: from branches of the second to branches of the seventh order 150-170, 100-120, 80-95, 60-70,

Laboratory of Carcinogenic Substances, Research Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. Laboratory of Cytology, Research Institute of Physicochemical Medicine, Ministry of Health of the RSFSR, Moscow.
(Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Trapeznikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 6, pp. 746-750, June, 1988. Original article submitted May 6, 1986.

0007-4888/88/0006-0888$12.50

TABLE 1. Diameter of Replicas of Portal System of Vessels of Rat and Mouse Liver (diameter of vessels, in μ)

Species of animals	Portal vein	Branches of portal vein		Branches of subsequent orders								Venules	Sinusoids
		right	left	2nd	3rd	4th	5th	6th	7th	8th	9th		
Rats	1200—1450	950—1100	1000—1300	800—950	600—700	450—550	300—350	150—200	90—120	50—70	30—40	15—20	5—12
Mice	950—1000	550—650	600—750	425—450	300—350	200—250	150—180	100—120	60—70	30—40	18—25	12—15	3—10

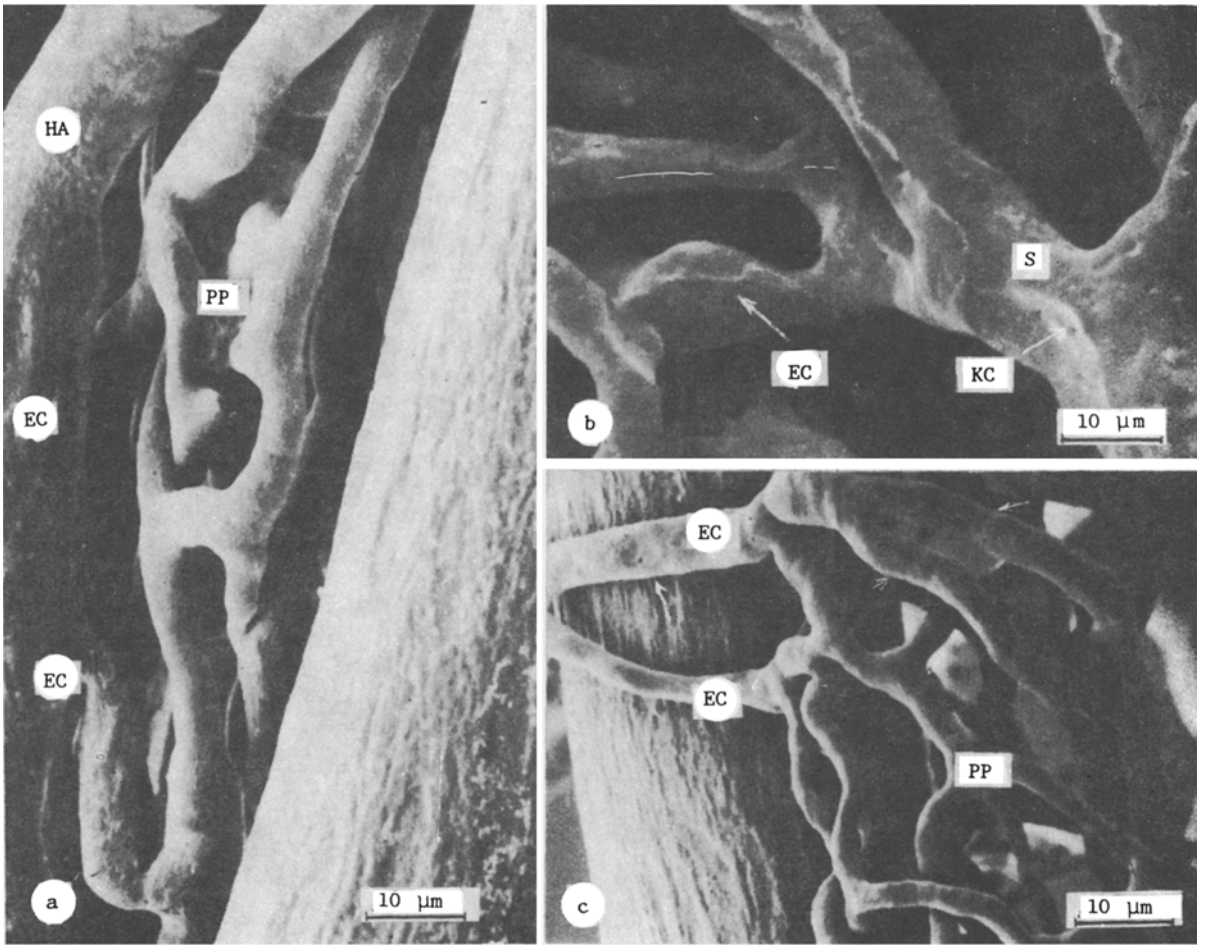

Fig. 1. Three-dimensional organization of arterial division of vascular bed of mouse and rat liver. a) Rat liver: branch of hepatic artery (HA), peribiliary vascular plexus (PP), impressions of nuclei of endothelial cells (EC); b) mouse liver: sinusoids (S), impressions of nuclei of endothelial cells (EC), impressions of nuclei of Kupffer cells (KC); c) rat liver: peribiliary vascular plexus (PP), peribiliary venule (PV), impressions of nuclei of endothelial cells (EC), transverse folds on surface of capillaries (arrows).

40-50, and 30-35 μ respectively. Arterial branches of the 8th order (20-25 μ) were interlobular arteries. Branches on the 9th order, arising from them (septal arteries) had a diameter of 16-20 μ, and they broke up in the lobule into arterioles (7-15 μ), which then changed into sinusoids. The diameter of individual sinusoids in the lobule was 5-12 μ.

The study of the relief of the arterial vessels revealed nucleus-containing zones of endothelial cells, oval or spindle-shaped and oriented, as a rule, along the axis of the vessels or at a small angle to it, on their surface (Fig. 1a). The small arteries had long folds on their surface, and the arterioles branching from them had circular folds with characteristic constriction bands. On the surface of the sinusoids both small oval depressions, which were impressions of nucleus-containing zones of endotheliocytes, and also irregularly

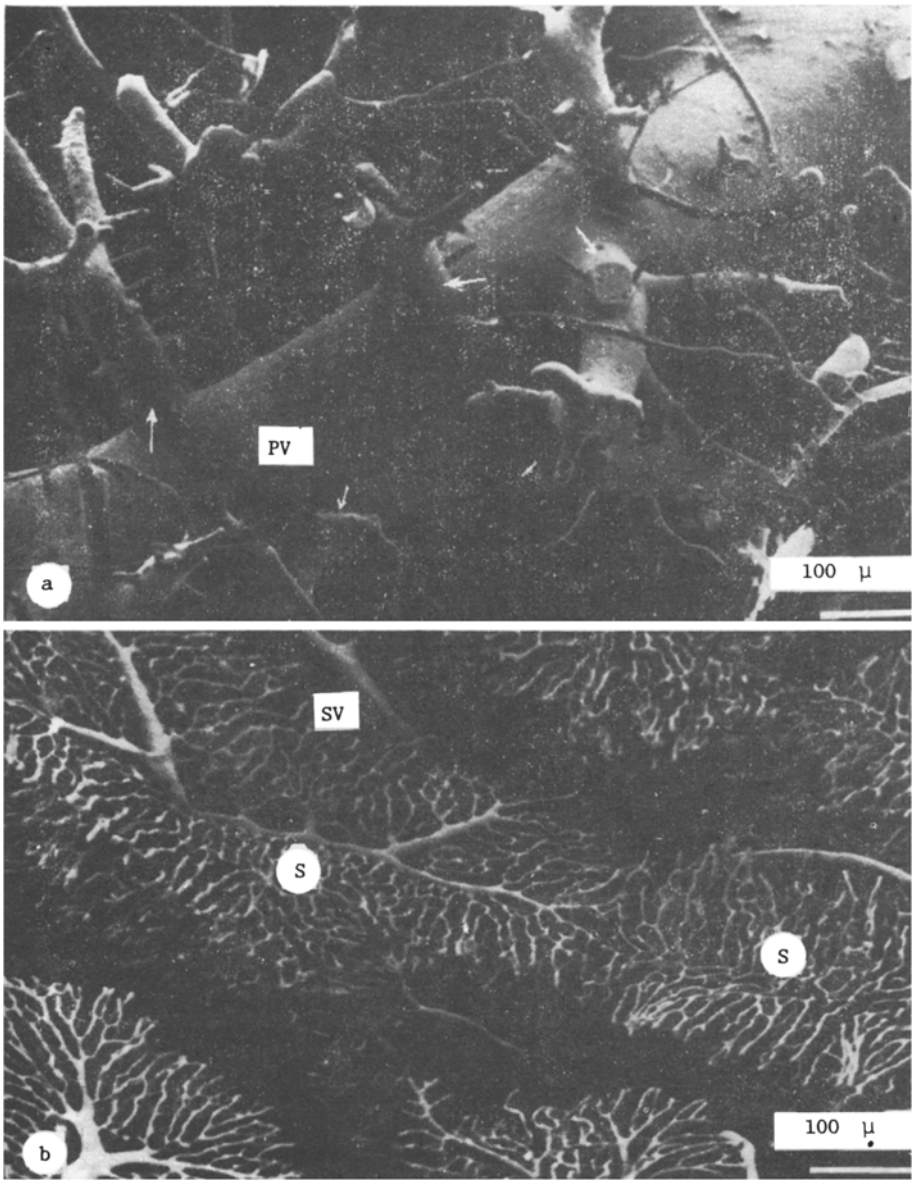

Fig. 2. Three-dimensional organization of portal system of mouse liver. a) Branch of portal vein (PV) and smaller branches arising from it (arrows), b) septal vein (SV), sinuoids (S).

shaped large, deep depressions, evidently impressions of Kupffer cells, were clearly visible (Fig. 1b). Ribbon-like capillaries of the peribiliary plexus 5-10 μ in diameter, surrounding the bile ducts, formed distinctive polygonal cells (Fig. 1c). The diameter of the peribiliary venules, connecting the peribiliary plexus with the sinusoids, was 15-20 μ. Transverse folds and circular nucleus-containing zones were found on the surface of the capillaries studied.

Investigation of injection replicas of the hepatic vessels of animals obtained by injection of IM through the portal vein showed that the portal vein of rats has a diameter of 1,200-1,450 μ, and it branches dichotomously into right (950-1100 μ) and left (1000-1300 μ) trunks. Later the lobar veins (800-950 μ) also divide dichotomously into branches of succesive order, including the 8th and 9th, 30-40 μ in diameter (Table 1).

Besides dichotomous branching and a gradual reduction in the diameter of the vessels from branches of each order (from the 2nd to the 9th), numerous small branches of varied diameter, including venules (15-20 μ), were given off in all directions, later to joint sinusoids directly (Fig. 2a). Interlobular veins of the 8th order were seen in vascular constructions in direct contact with the capsule of the liver. They branched at an angle of 45°

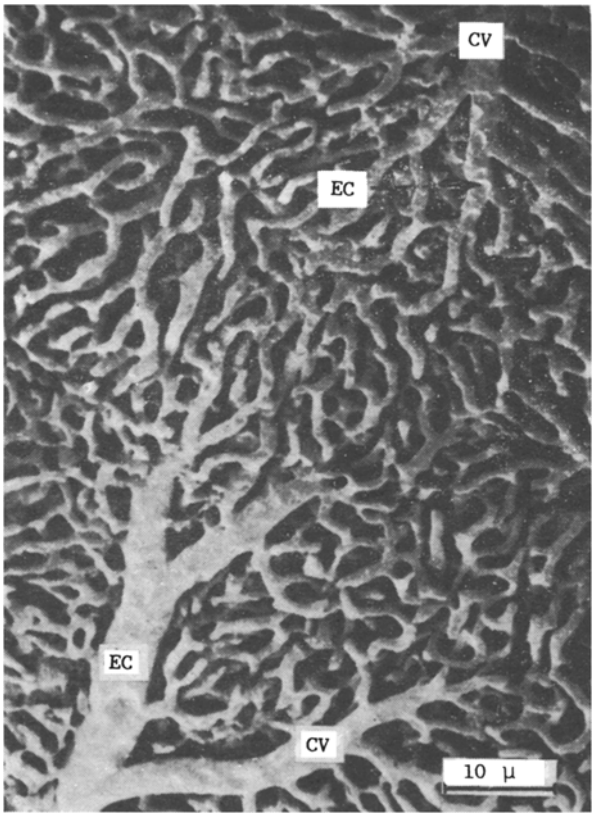

Fig. 3. Three-dimensional organization of origin of system draining blood from the liver. Mouse liver: sublobular vein (SV), central vein (CV), impressions of nucleus-containing zones of endothelial cells (EC), sinusoids (S).

into septal veins, running parallel to the lateral surface of the lobules, from which venules, later turning into sinusoids, arose (Fig. 2b).

The data given above, incidentally, were obtained by the use of incompletely injected vascular constructions.

The study of injection replicas of peripheral parts of the liver with complete filling of the vascular bed with IM enables the character of branching and the microrelief of the initial segments of the outflow system of blood from the liver (central and sublobular veins) to be studied. The results of the measurements showed that the diameter of the central veins is 15-20 μ. They are surrounded by an anastomotic network, formed by numerous branching sinusoids (Fig. 3). The diameter of the sublobular veins reached 25-30 μ. The microrelief of the efferent vessels was characterized by numerous irregularly oriented nuclear impressions of endothelial cells.

To conclude, the SEMIR method has certain unique facilities which can be adequately utilized to study the angioarchitectonics and microrelief of the vascular system of the liver of experimental animals. The angioarchitectonics of the rat and rabbit liver has been studied by the above method [3, 7, 8]. Data on the 3-dimensional organization of the vascular bed of the mouse liver are described for the first time in the present publication. However, in previous investigations no detailed differential account was given of the 3-dimensional features of the arterial and venous systems of the liver.

It must accordingly be emphasized that the SEMIR method, with the use of different degrees of injection of the vessels with IM and different injection techniques yielded additional information on the 3-dimensional organization of the arterial and venous portions of the vascular bed of the liver, and this will evidently have to be taken into account in the future when data of the liver is involved are analyzed.

LITERATURE CITED

1. Ya. L. Karaganov, S. A. Gusev, V. A. Mironov, and N. F. Pugachevskaya, Arkh. Anat., No. 6, 111 (1979).
2. Ya. L. Karaganov, V. A. Mironov, and S. A. Gusev, Arkh. Anat., No. 6, 90 (1980).
3. Ya. L. Karaganov, V. A. Mironov, A. A. Mironov, and S. A. Gusev, Arkh. Anat., No. 8, 5 (1981).
4. A. A. Mironov, S. I. Kataev, and Yu. I. Aleksin, Abstracts of Proceedings of the 11th All-Union Conference on Electron Microscopy [in Russian], Vol. 2, Tallin (1979), p. 13.
5. T. M. Povalii, S. A. Gusev, and V. A. Mironov, Krovoobrashchenie, No. 3, 3 (1980).
6. T. Fujita, J. Tokunawa, and H. Inoue, Arch. Histol. Jpn., 30, 321 (1969).
7. T. Fujita and T. Murakami, Arch. Histol. Jpn., 35, 255 (1973).
8. P. Motta and K. Porter, Cell Tissue Res., 148, 111 (1974).

EFFECT OF HELIUM-NEON LASER IRRADIATION OF THE GASTRIC MUCOSA ON ITS EPITHELIAL CELLS

I. M. Baibekov, É. Sh. Musaev, and D. T. Alimov

UDC 616.33-002.44-085.849.19-036.8-07:616.33-018.73-003.9

KEY WORDS: laser irradiation, stomach, epithelial cells.

Laser treatment of gastroduodenal ulcers has now been successfully introduced into clinical practice [3-5]. Helium-neon lasers (HNL) with an emission wavelength of 632.8 nm are most widely used for this purpose [4, 5]. However, the morphological basis of biological stimulation of ulcer healing has been inadequately studied [5]. There have been virtually no investigations of the action of low-intensity laser-radiation on the intact gastric mucosa (BM) in the case of endogastric irradiation.

This paper gives the results of a study of the action of HNL radiation on the microrelief, morphology, and proliferation of epitheliocytes in the gastric fundus during irradiation of GM.

EXPERIMENTAL METHODS

Experiments were carried out on 50 male Wistar albino rats weighing not less than 140 g. The technique of irradiation of GM by means of a light guide, coupled with an LG-75 laser, and introduced perorally into the stomach with the aid of a special tube [6], developed previously, was used. Only a zone of the gastric fundus selected beforehand was irradiated in this method. The power of the radiation at the exit of the light guide was 8 mW, the diameter of the zone of irradiation was 3 mm, the duration of irradiation 1, 3, and 5 min, and the doses of irradiation given were 6.78, 20.34, and 33.9 J/cm^2 respectively. For the autoradiographic investigation ^{3}H-thymidine was injected intraperitoneally into the starving animals at 10 a.m. in a dose of 18.5 kBq/g body weight, and irradiation began 10 min later. The animals were killed by instant decapitation 1 h after the injection of ^{3}H-thymidine, and the stomach was removed and fixed by injection of 3-5 ml of 10% formalin into its cavity. Paraffin sections of circular fragments of the stomach, including the zone of irradiation (ZI) and the adjacent area (AA) were stained with hematoxylin and eosin, and by the PAS method, and sections for autoradiography were covered with type M emulsion. The index of labeled nuclei (ILN) of epitheliocytes of each type was determined by counting 1,000 cells in longitudinal sections through the fundal glands, and expressed as percentage. Statistical analysis was carried out by the Fischer–Student method. Fragments of ZI and AA for scanning and transmission electron microscopy were taken from the stomach immediately after irradiation and prepared for study by the usual method. Specimens were examined in H-600 and S-405 A

Department of Pathological Anatomy, Branch of the All-Union Scientific Center for Surgery, Academy of Medical Sciences of the USSR, Tashkent. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 6, pp. 750-752, June, 1988. Original article submitted June 10, 1987.

 0007-4888/88/0006-0892$12.50