INTRAVITAL METHOD OF STUDYING THE MICROCIRCULATION OF THE SCIATIC NERVE

I. L. Razumova and Academician A. M. Chernukh*

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A bloodless method of isolating the sciatic nerve in rats, placing it on a light guide, and adapting the MBI-6 microscope for the intravital study of the microcirculation of blood in the epineurium of the sciatic nerve is described. Variations in the microvascular network of the epineurium in rats are demonstrated.

KEY WORDS: *biomicroscopy; microcirculation; nerve.*

The microcirculation of the peripheral nerve has not been adequately studied. Such investigations as have been performed have dealt mainly with the study of the blood supply to the peripheral nerves. The authors concerned have used mainly methods of injecting the vessels with contrast materials and subsequent clearing of total preparations [1-4] or microradiography, microroentgenography, and stereoscopic photography [5]. No descriptions of intravital studies of the microcirculation of the sciatic nerve could be found in the accessible literature. Consequently, a method of intravital study of the microcirculation of the rat sciatic nerve was developed in the laboratory.

A square heating stage measuring 260 × 115 mm was fixed securely to the sliding stage of an MBI-6 microscope (Fig. 1). The stage (1) was connected to the electric power supply through an autotransformer to provide a stable temperature within the range from 30 to 50°C. A holder (2) for the case of the illuminating lamp with light guide was secured to the back left-hand corner of the stage. The arm of the holder (3) was fixed to a plate with a screw device (4) enabling the light guide to be moved in the horizontal plane for a distance of 20 mm. A coupling (5) with a horizontal arm fitted with a firm antivibration spring (6) is secured to the vertical arm of the holder. The coupling (5) enables the case of the lamp with the light guide to be moved through 100 mm in the vertical direction. The sleeve (8) of the metal case (7) of the K-21-150 illuminating lamp enables the angle of inclination of the light guide to be altered. The horizontal arm of the lamp case (9) is freely connected with the shortened case of the OI-18 illuminating lamp (10) with a double-lens collector. A heatproof filter protecting the light guide against overheating is glued to the front lens of the collector. Instead of light filters, a metal holder (11) with a polymethyl acrylate† light guide (12) fitted into it is fitted over the case (10) of the illuminating lamp. The case of the K-21-150 lamp is continuously cooled by a powerful jet of air from the fan (13) during operation of the lamp. To prevent the jet of air from striking the region of the heating stage, light paper screens (14) are fitted to both ends of the lamp case (9).

To prevent clouding of the lenses of the objective, instead of one objective, a specially made heating device (15) is screwed into the socket of the revolving head of the MBI-6 microscope; the heater is connected to the electric power supply through the transformer of the OI-19 illuminator and heats the objective to 30-36°C.

Preparation of the microscope for work with the light guide takes not more than 30 min. The heating instruments must be switched on 20-30 min before the work begins.

The actual process of biomicroscopy of the nerve is carried out as follows: The rats are anesthetized with urethane, an incision is made in the skin of the right hind limb along

*Academy of Medical Sciences of the USSR.

†The light guide was made in the Institute's workshops.

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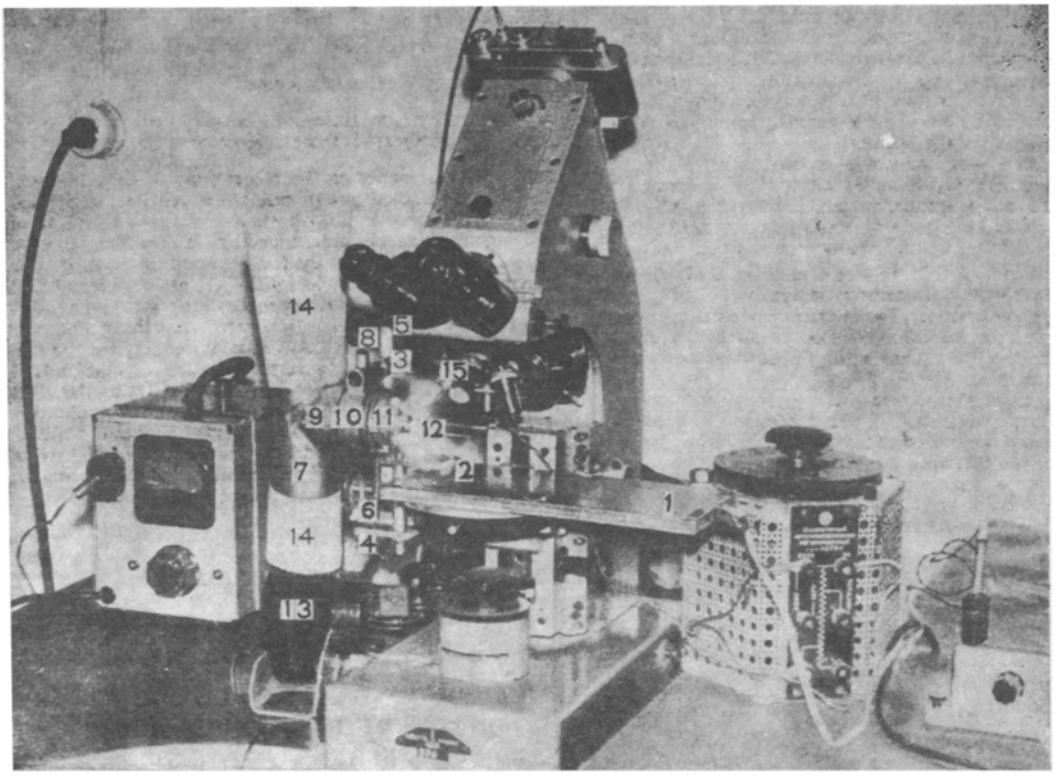

Fig. 1. Apparatus for intravital study of microcirculation of rat sciatic nerve: 1) heating stage; 2) holder of case of illuminating lamp; 3) arm holder; 4) plate with screw device moving lamp case with light guide horizontally; 5) coupling with horizontal arm; 6) antivibration spring; 7) case of illuminating lamp; 8) coupling of metal case of lamp; 9) horizontal arm of lamp case; 10) shortened case of OI-18 illuminating lamp with double-lens collector; 11) holder of light guide; 12) light guide; 13) fan; 14) paper screen; 15) device for heating objective.

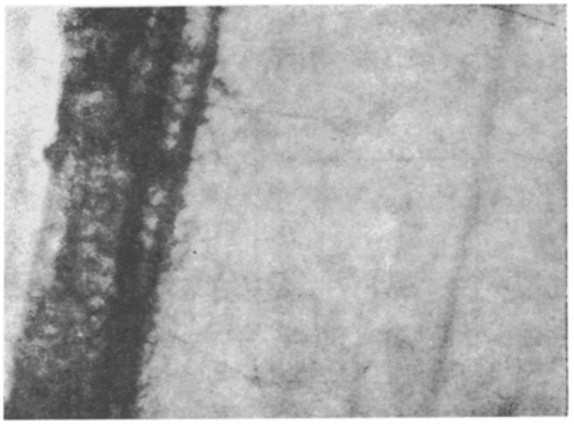

Fig. 2

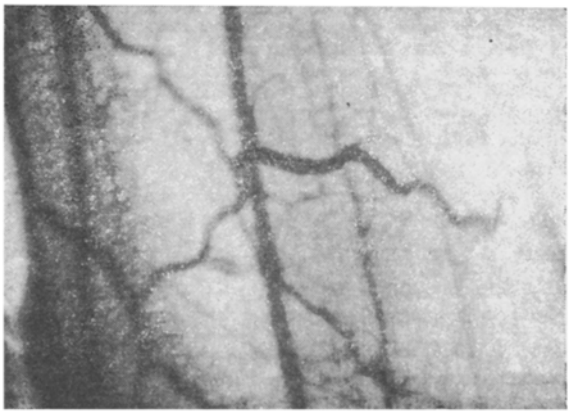

Fig. 3

Fig. 2. Rat No. 1. Normal. Almost unbranched vessels in epineurium. Here and in Fig. 2: biomicroscopy; objective 9, ocular 10.

Fig. 3. Rat No. 5. Normal. Densely branching vessels in epineurium of sciatic nerve.

the lateral surface of the leg and thigh as far as the middle of the lumbar region, and the incision is then continued transversally across the trunk as far as the vertebral column. The triangular skin flap thus obtained is reflected by careful dissection so as not to injure the vessels. The subcutaneous connective tissue is incised along the fascia at the boundary between the biceps femoris muscle, the gluteus maximus muscle, and the fascia lata of the thigh from the vertebral columns as far as the knee joint. The popliteal fascia around the joint is carefully incised without touching the vascular bundle. The biceps femoris muscle is detached with a blunt hook and reflected and the sciatic nerve is freed from the connective-tissue membranes without injuring the epineurium. If the dissection is done properly, the nerve is isolated bloodlessly. The rat thus prepared is carefully placed on the heating stage of the microscope. Having first placed the edge of the light guide in the center of the field of vision of the 3.5 × objective, the sciatic nerve is placed on the light guide by carefully removing the rat by its tail, and the nerve is straightened with a narrow, curved spatula. When it is certain that the nerve is lying correctly and conveniently on the light guide, the exposed muscles are covered with towels soaked in warm (36°C) physiological saline. A very narrow strip of wet gauze is placed on the light guide. Drops of physiological saline, applied constantly to this strip, gradually fall off and moisten the nerve continuously. With the slightest injury to the nerve, which is almost unavoidable when it is applied to the light guide and the vessels are compressed, the blood flow is slowed and stasis is formed temporarily. At the same time, blood can be seen to fill the collaterals, which were inactive before injury, but which now become visible and continue to function even after restoration of the normal blood flow in the vessels injured during manipulations with the nerve. In this connection, after the nerve has been successfully applied to the light guide, it must be covered with a narrow strip of moist gauze and moistened with several drops of warm physiological saline without pressing on the gauze. Usually the strip of gauze is applied closely and evenly to the nerve. When periodically irrigating the moist chamber thus formed around the nerve the animal must be kept at rest for 1.5-2 h until the blood flow is completely restored and the collaterals filled with blood as a result of the trauma have disappeared.

Two vascular bundles run to the sciatic nerve of the rat: one in the upper part of the thigh and another just above the knee joint. Two or three venules with blood flowing in different directions run along the dorsal surface of the epineurium; their diameter varies from 32 to 73 μ. Short branches of arterioles are rarely seen here. In the epineurium of the ventral aspect of the nerve smaller vessels are found (up to 32 μ in diameter). Most frequently there is one venule and one arteriole.

Histological study of transverse sections of the nerve showed that larger vessels (venules up to 102 μ in diameter and arterioles up to 51 μ) run along the lateral and medial aspects of the epineurium, where they are almost inaccessible for intravital observation. The vascular plexus of the epineurium is very variable. It may consist of almost unbranched microvessels (Fig. 2) or it may be a thickly branched network (Fig. 3).

By means of the method which the writers have developed it is possible to observe the circulation of the blood in the vessels of the dorsal surface of the epineurium for the period of 8 h.

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