To inject perfusate into the central canal of the spinal cord a Teflon cannula was used (Fig. 1). The cannula was introduced into the central canal of the upper lumbar segments after total transection of the spinal cord at the level L2-L3 under visual control, as shown in Fig. 1, and was fixed inside the canal by means of the ligature 2. Free drainage of the perfusate from the central canal was ensured by dividing the terminal part of the spinal cord (filum terminale) without damaging the sacral segments or their roots. To collect perfusate the stump of the filum terminale 3 was drawn inside a special polyethylene funnel 4. Airtightness of the space between the inner surface of the funnel and the filum terminale was ensured by covering the spinal cord with a layer of mineral oil 5. Perfusate collecting in the funnel was withdrawn by means of a water-jet pump 6. The inlet cannula was connected to the vessel containing perfusate, which consisted of artificial cerebrospinal fluid with the composition given by Merlis [1], by means of a thin vinyl chloride tube 7. About 15 cm of this tube was immersed in the warm (36-38°C) mineral oil surrounding the spinal cord, which warmed the perfusate entering the spinal cord. Our 3 years of experience of the use of this method of perfusing the central canal of the lumbosacral segments of the spinal cord has shown that it ensures stable fixation of the tip of the inlet cannula in the central canal, with reliable airtightness of the perfusion system, so that a continuous flow of perfusion fluid along the central canal for many hours is possible; the sacral segments and their roots likewise are absolutely undamaged, so that the standard electrophysiological technique can be used to study the functions of the spinal centers controlling the muscles of the lower limb, so that selective stimulation of cutaneous or muscular afferent nerves of the hind limb is possible and potentials can be recorded from the ventral and dorsal roots of the lumbar and sacral segments.

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USE OF A MICROPRISM GRATING TO MEASURE ERYTHROCYTE VELOCITY IN MICROVESSELS

V. S. Shinkarenko and S. É. Morozov

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The blood flow rate in the microvessels (BFRM) is one of the most important parameters which determine the metabolic level in the tissues [2]. In pathological states it undergoes considerable changes, which correlate with the degree of damage, indicate the severity of the pathological process, and can be used as an early diagnostic sign of disease [3, 7, 8]. Determination of BFRM in living objects is connected with considerable technical difficulties. Methods based on the use of cross correlation, the Doppler effect, and frame analysis of motion pictures and videograms [1, 4-6, 9], while providing important information on the microcirculation nevertheless have disadvantages in the form of limitations of measurable velocity or range of diameters of the microvessels, complexity and cumbersomeness of the apparatus, its high cost, the impossibility of obtaining the results of measurements during recording (in the case of motion picture and video filming), etc. This has necessitated the designing of improved instruments for BFRM measurement. One such instrument is the PRIM (Ernst Leitz, West Germany), which is intended for measuring the velocity of erythrocytes and is based on spatial image filtration by means of a microprism grating [11]. An experi-

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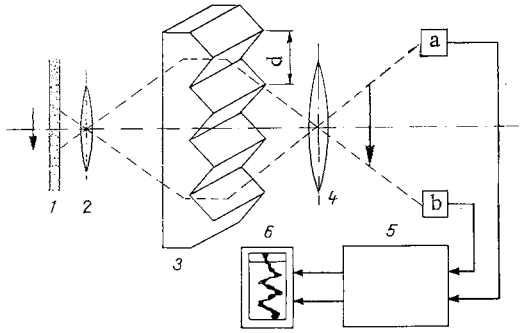

Fig. 1

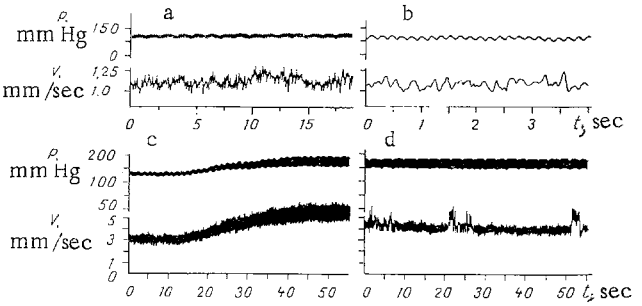

Fig. 2

Fig. 1. Diagram showing measuring part of PRIM instrument. 1) Microvessel, 2) optical system of microscope, 3) microprism grating, 4) intermediate lens, 5) amplifier-transformer, 6) recorder, *a* and b) photodiodes; d) constant of grating. Arrows indicate direction of movement of erythrocytes and of their image.

Fig. 2. Results of synchronous recording of systemic arterial pressure (P) and velocity (v) of movement of erythrocytes in microvessels. *a* and b) With no external influence (arteriole 15 μ in diameter). Expanded trace (b) reveals pulse waves of blood flow in microvessel; c) after intravenous injection of vasopressin arterial pressure rises and BFRM increases in arteriole 13 μ in diameter; d) fluctuations of blood flow velocity in same arteriole against the background of stable systemic pressure during passage of microemboli.

mental specimen of this instrument has been tested by the writers on model objects and also during intravital experiments on animals.

EXPERIMENTAL METHOD

The PRIM consists of a measuring unit, mounted on the barrel of the microscope, and a control panel with needle indicator (Fig. 1). The image of the microvessel 1 is projected through the optical system of the microscope 2 on to the microprism grating 3, consisting of a transparent plate, one side of which, facing the microscope, is flat whereas the other consists of microprisms mounted tightly one against another. The image of the microvessel is divided by the surfaces of the prisms facing in different directions, into strips; even strips are projected on one photodiode, odd on the other. The photodiodes are connected together so that if erythrocytes in the vessel and, consequently, their image on the grating are stationary, the resultant output signal will be zero. If, however, the blood cells in the vessel are moving, and their image correspondingly moves over the grating, this image, refracted by the different surfaces of the prisms, will fall successively on one and then on the other photodiode. As a result an electrical signal appears at the output with frequency f, which is directly proportional to the velocity of movement of the erythrocytes (v): $f = (v \cdot M)/d$, where M is the magnificiation of the microscope and d the distance between the apices of the microprisms.

We used gratings with constants $d_1 = 0.2$ and $d_2 = 0.4$ mm.

The signal from the measuring unit is amplified and filtered, and after transformation, is issued as a steady voltage proportional to the frequency of the original signal. The results of measurements of velocity were read on a needle indicator or recorded graphically. In addition, an acoustic output enables sound monitoring of the velocity measurements.

EXPERIMENTAL RESULTS

In model experiments a suspension of erythrocytes in physiological saline was passed through glass capillary tubes 120-550 μ in diameter, placed beneath the microscope objective. From the capillary tube the suspension passed into the measuring device, by means of which the volume of suspension flowing in a certain time was determined, and from it the actual mean linear velocity (v_a) of movement of the suspension in the test capillary tube was calculated. The value of v_a was compared with the velocity v_i, shown by the instrument. It

was found that v_i agrees well with v_a over a wide range of velocities: from 1 to 180 mm/sec (coefficient of correlation r = 0.98).

Experiments on intravital measurement of erythrocyte velocity in microvessels were carried out on the mesentery of Wistar rats weighing up to 250 g and anesthetized with pentobarbital (6 mg/100 g body weight). Biomicroscopy was performed by the technique adopted in the writers' laboratory [2].

The results of measurement of the velocity of movement of erythrocytes in a microvessel with parallel recording of the systemic arterial pressure are given in Fig. 2. Waves connected with cardiac contractions, and also waves of higher frequency superposed on them and due to local factors influencing the flow rate (redistribution of the blood flow within the territory supplied by the given vessel, closing of the lumen of the capillaries by leukocytes, etc.), can be seen on the velocity curve. During long-term recording longer waves connected with the animal's respiratory movements appear on the curve. In these experiments BFRM could be measured by the PRIM even in the smallest vessels: arterioles, capillaries, venules under 5-15 μ in diameter. Velocities in them were of the order of 1-5 mm/sec.

If the measuring field covers the whole lumen of the vessel, readings of the instrument correspond to a certain average or resultant velocity of movement of the erythrocytes in that particular vessel. If, however, the measuring field corresponds to only part of the lumen of the vessel, the velocity of movement of individual layers of cells is determined, and successive measurements of this parameter in the direction perpendicular to the axis of blood flow will yield a distribution profile of velocities by cross-section of the vessel. Various disturbances of the BFRM under pathological conditions are clearly recorded by the instrument. In some cases changes in the velocity curve have a specific character for a given type of disturbance. The record of velocity during intravenous injection of the synthetic vasopressin analog L-8 (from Zandoz, 1.5 pressor units) is shown in Fig. 2c. The character of the blood flow during passage of emboli along a test arteriole is indicated in Fig. 2d.

The PRIM instrument records very fine changes in BFRM in the smallest terminal blood vessels with great accuracy and enables the function of local regulatory mechanisms of the microcirculation to be studied.

Readings of the instrument are a linear function of the measured value over a wide range of velocities and diameter of microvessels. By its use very rapid fluctuations of velocity — up to 250 mm/sec — could be recorded. According to the results of previous investigations [10], as regards the field of application and accuracy of measurement the microprism grating method is comparable with cross-correlation, laser Doppler anemometry, and high-speed motion picture filming methods.

The method of measuring the velocity of movement of erythrocytes by means of a microprism grating thus enables one of the most important parameters of the microcirculation, namely the blood flow velocity in microvessels, to be estimated quantitatively with a high degree of accuracy.

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