

METHODS

A SIMPLE METHOD OF OBJECTIVE EVALUATION OF THE STATE OF THE BLOOD FLOW IN SMALL BLOOD VESSELS

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UDC 612.135-087

A simple method is described for comparing several photomicrographs, prepared by intravital microscopy, one after the other at short intervals in order to evaluate the state of the blood flow in the microvessels.

KEY WORDS: microcirculation; blood flow; photographic recording.

An extremely important parameter in the study of the microcirculation is the state of the blood flow in the capillaries, precapillaries, arterioles, venules, and shunts. In most cases the investigator can only make an approximate estimate of its value on the basis of biomicroscopic observation, and the documentation is often a simple written statement or a series of photographs. Measurements of the velocity and objective recording of the state of the blood flow in these vessels have not yet obtained general application because of technical difficulties.

Special microfilming [3] and, in particular, television biomicroscopy with videorecording [4] are perhaps the best methods of obtaining and preserving full and objective information on the state of the microcirculation obtained experimentally. However, the need for specialized and expensive equipment and materials have so far restricted the extensive use of these methods. Different methods of measuring or calculating the velocity of movement of blood in capillaries [1, 2, 5, 6, 8, 9] are only relatively accurate, or they cannot be used for continuous observation and recording of changes in the blood flow in microvessels.

The simplest and most widely used method of recording the state of the microcirculation is photomicrography with a very short exposure. However, the photographic print obtained by this method gives information only on the degree of dispersion of the blood cells and their shape, the character of aggregation of the red cells or its absence, and the ratio between cells and plasma in the vessels. No information on movement of the blood in the vessels is given by a separate photograph.

For a number of years we have used a simple method of obtaining serial photographs one after the other at definite intervals to provide additional objective information on the blood flow in very small vessels. Observations are made and photographs taken of the microcirculation in the rat mesentery or in the mucous membrane of the retrobuccal pouch of the hamster on an apparatus for intravital microscopy, mounted on the base of an MBI-6 microscope, using a camera ocular giving a magnification of 10 times and objectives with magnifications of 10, 20, 40, and 60 times (the last three with oil or water immersion). The mesentery of the small intestine or appendix, removed under urethane anesthesia, is quickly placed in thermostable medium consisting of the biologically neutral silicone fluid PMS-500, forming a thin watertight film on the surface of the mesentery which prevents drying. This liquid acts at the same time as the immersion medium. High-sensitivity aerial photographic film is used for photography, and the source of light is a type ISSh-500 photoflash giving flashes of the order of several microseconds so that individual blood cells can be recorded on the film and distinguished whatever the velocity of blood flow. An ordinary photoflash bulb can also be used, but in that case fewer photographs can be taken and the interval between suc-

Laboratory of Pathophysiology of Extremal States, Institute of Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 79, No. 5, pp. 120-123, May, 1975. Original article submitted September 20, 1974.

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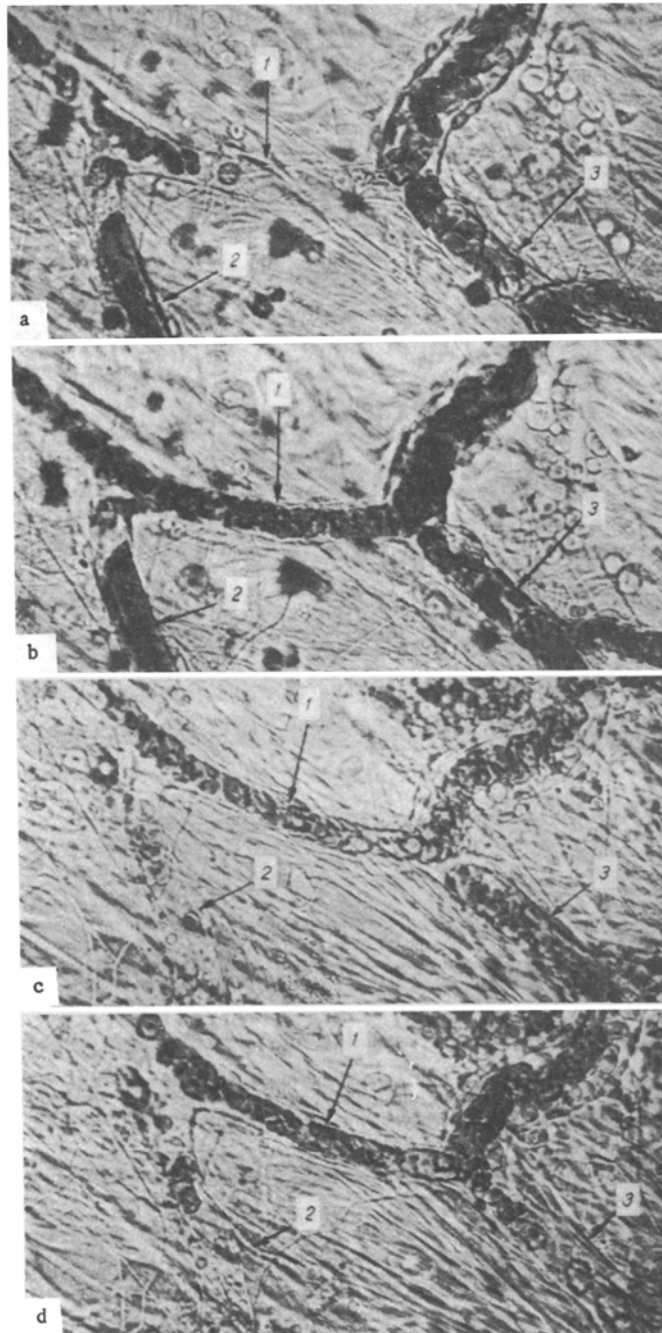


Fig. 1. Venous collector loop (1) and mouths of two venules (2, 3) in rat mesentery in acute phase of burns: a, b) serial photographs before treatment; c, d) 2 h after treatment (subcutaneous infusion of gemodez).

cessive photographs is longer. By means of the ISSh-50 lamp, frequent photographs can be taken, i.e., every 5 sec — the time required to wind the film after taking the previous photograph. These intervals can be increased.

Comparison of the position of individual cells or clumps of cells in a series of photographs makes it possible to determine the forward movement of the blood in a particular vessel or its cessation, and the linearity of the movement and its slowing; if the interval between the frames is very short and can be precisely measured and the photographs are standardized, the velocity of movement of the blood cells can also be calculated.

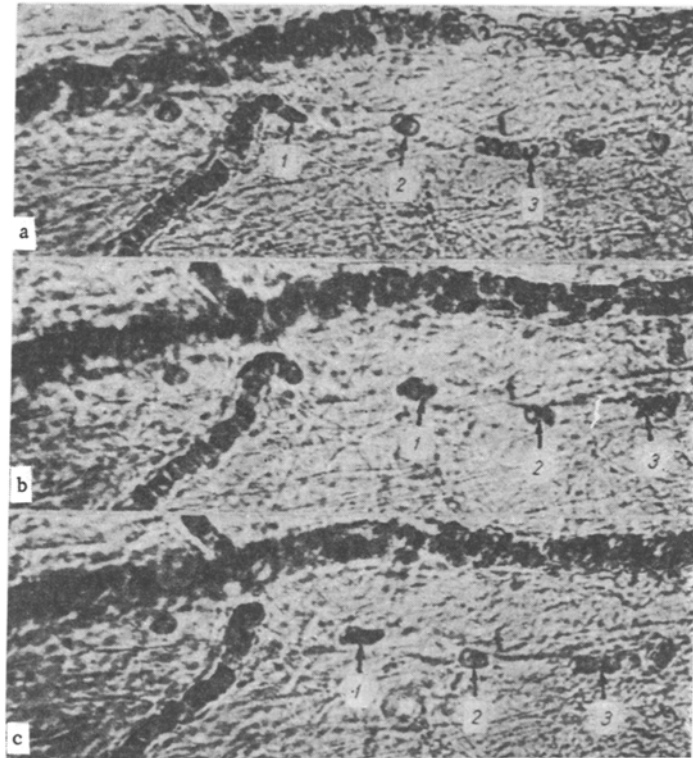


Fig. 2. Serial photographs, a, b, c) of part of microcirculation in rat mesentery in acute phase of burns: 1, 2, 3) clumps of red cells moving very slowly in shunt vessel.

Several serial photographs of two areas of the rat mesentery 24 h after an extensive, deep burn of the skin of the trunk are given as examples. The photographs a and b in Fig. 1 were taken at an interval of 20 sec. Substantial changes taking place during this interval, i.e., in the position of the blood cells and their aggregations in the venous collector loop 1, can be seen which reflect the forward movement in the loop, although signs of a disturbance of the normal structure of the blood flow will be apparent. No changes in the position of the blood cells took place during the 20 sec in the mouths of the venules 2 and 3. Large clumps of cells block these vessels and prevent the flow of blood among them. Photographs c and d of the same area were also taken at an interval of 20 sec, but 2 h after infusion of the product gemodez, a Soviet preparation of low-molecular-weight polyvinyl-pyrrolidone, into this rat. In this case changes are evident also in the venules 2 and 3, indicating that under the influence of treatment, the large aggregates had broken up into smaller clumps or into separate red cells, and that the forward movement of blood had resumed in the venules.

Three successive photographs of the same area in the mesentery of a rat in the acute phase of burns, taken at 10-sec intervals, are shown in Fig. 2. During this time aggregates 1, 2, and 3 moved a negligible distance along the vessel. Consequently, forward movement of blood is present in this vessel, but it is very slow. By means of such photographs the approximate velocity of movement of aggregates in a given vessel can be calculated.

The method can also be used to demonstrate an oscillating, to-and-fro movement of blood if present, and so on.

The method described above must not be regarded as a precision method. However, it is a comparatively simple and convenient method of obtaining objective information on the movement of blood in the microcirculatory system and can be a valuable addition to the written records of experiments and also used for the preparation of illustrative material.

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