INTRAVITAL RECORDING OF THE DIAMETER OF SMALL BLOOD VESSELS BY SCANNING PHOTOMETRY

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By means of the suggested method of scanning photometry and intravital recording it was shown that application of 0.1 ml of 0.1% histamine solution to the small blood vessels of the rat's mesentery causes an increase of 30% in the diameter of the arterioles and an increase of 25% in the diameter of the venules after 30 sec. Application of 0.1 ml of 0.1% adrenalin solution after the action of histamine reduced the diameter of the blood vessels to its initial value.

The great importance of microcirculatory disturbances in the pathogenesis of many diseases is no longer in doubt. In a study of the terminal vascular bed new and important morphological and functional results have been obtained by the use of such precision techniques as luminescence, electron, and television microscopy, and by the use of unique instruments such as microangiometers, velocitometers, microviscosimeters, and so on, enabling the diameter, viscosity, pressure, velocity of the blood flow, and so on to be determined in small blood vessels (arterioles, venules, and capillaries).

An important criterion of the functional state of these small blood vessels is their diameter, which varies under the influence of nervous impulses and also of vaso-active substances of natural and synthetic origin, and is thus an essential factor regulating the local blood flow. It is obvious that the objective study of microvascular constriction and dilatation resulting from the action of the above factors is impossible without some means of recording quantitatively the diameter of the vessels. For this purpose, several solutions have been suggested abroad. Complex and expensive instruments are available, incorporating television microscopy, allowing resolution of the image [4]; optical scanning photometry [5, 6] and also simpler methods with a graduated ocular micrometer and photomicrography [3] have been used.

A method using photoelectric recording of the intensity of illumination of a given area occupied by a small blood vessel has been described in the Soviet literature [2]. In this case it is not the true diameter of the vessel which is recorded, but a relative parameter, increasing with constriction and decreasing with dilatation. Two methods have been developed for recording the diameter of small blood vessels in the Laboratory of General Pathophysiology and Experimental Therapy of our Institute. The first method uses a television microscope to make accurate measurements on these microscopic objects, while the second is based on the principle of scanning photometry [1]. Since television microscopes are not yet available at all laboratories engaged in the study of the microcirculation, we suggest a comparatively simple and readily available method for intravital recording of the diameter of small blood vessels based on the use of scanning photometry.

The apparatus incorporates a recording cytofluorimeter, consisting of a luminescence microscope, a photoelectronic multiplier (FÉU) with a high-voltage stabilized rectifier, and an amplifier with automatic writer [1]. The luminescence microscope can be replaced by any other microscope with a sufficiently bright source of light, to enable images to be examined under high magnifications (2000-3000 times).

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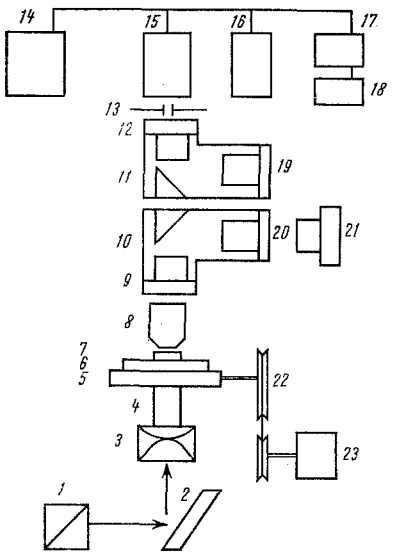

Fig. 1

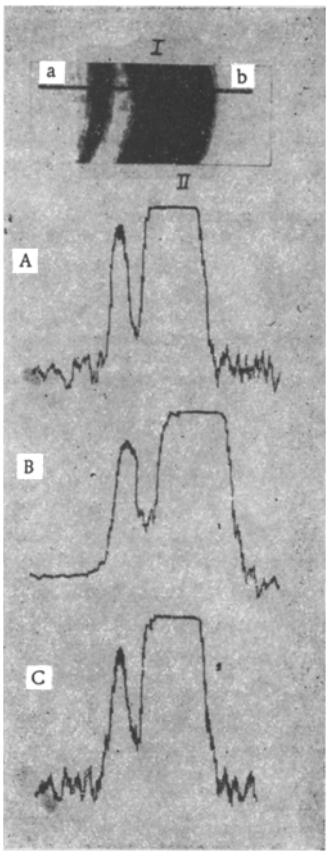

Fig. 2

Fig. 1. Diagram showing the principles of the apparatus for recording the diameter of small blood vessels. Explanation in text.

Fig. 2. Photographic record (I) and trace of diameter of small blood vessels (II). The scanning line is denoted by the segment a-b. A) Original trace; B) 30 sec after local application of 0.1 ml of 0.1% histamine solution; C) 30 sec after corresponding application of 0.1 ml of 0.1% adrenalin solution.

To record the diameter of the small blood vessels, the cytophotometer was slightly modified (Fig. 1): a heated stage (6) with light guide (4) was fixed to the object stage (5) of the microscope, and the Warren's motor was replaced by a type SD-54 reversing motor. The shaft of the motor (23), making 38 revolutions per minute, was connected through a rubber band drive with a pulley (22) operating the mechanism moving the heated stage backward and forward in the horizontal direction. The velocity of movement of the object is 100 μ/sec. To obtain high degrees of magnification and to photograph the image, a tube and sighting device (10) from the MFN-7 photomicrographic attachment was used; the tube and sighting device (11) from the camera attachment to the luminescence microscope was fixed to it. In this way a magnification of 250-2250 times could be obtained on the film of the "Zenit" reflex camera (21) and a magnification of 500-4500 times on the photocathode of the FÉU if objectives with magnification of between 10 and 90 were used. The FÉU (15) is powered from a high-voltage stabilized rectifier VSV-2 (14). The photocurrent generated in the FÉU is proportional to the optical density (intensity of illumination) and can be measured by means of the type M-95 microammeter (16) or recorded through an amplifier (17) on the heat-sensitive paper of the automatic writer (18).

The order of work with the apparatus is as follows. The anesthetized rat (urethane, 2 g/kg, or Nembutal, 40 mg/kg body weight, intramuscularly) is placed on the heated stage at 38°C. Laparotomy is performed and a loop of intestine, with its mesentery and blood vessels, is withdrawn and placed on the light conductor. A blood vessel (arteriole, venule, capillary) is found under the microscope, an objective (8) with a magnification of between 10 and 90 and oculars (9 and 12) are chosen to obtain the necessary magnification.

The FÉU is then set to a particular level of sensitivity, a certain voltage is applied to it, the automatic writer is switched on, the paper winding mechanism is started at a speed of between 2 and 200 mm/sec, and the motor which moves [under visual control through the ocular (19)] the highly enlarged image of the vessel relative to the stationary diaphragm (13), located in front of the photocathode of the FÉU, is set in motion. In this way mechanical scanning is combined with photometry (measurement of the change in optical density of the vessel and surrounding tissues) and simultaneous recording on paper.

The record thus obtained of the diameter of the small blood vessels, together with their photograph, is shown in Fig. 2. The scanning line is denoted by the line of section a-b. Analysis of the original record of the vessels (Fig. 2A) indicates that the diameter of the arteriole is 45 μ and the thickness of its wall 5 μ. The diameter of the venule is 135 μ and the thickness of its wall 10 μ. After application of 0.1 ml of 0.1% histamine solution (Fig. 2B), the diameter of the arteriole increased to 58 μ (by 30%) in 30 sec, while the diameter of the venule increased to 170 μ (by 25%). Thickening of the wall of the arteriole to 9 μ and of the wall of the venule to 18 μ was observed. Application of 0.1 ml of 0.1% adrenalin solution (Fig. 2C) against this background was accompanied by a decrease in the diameter of both vessels back to the initial level, and the thickness of the wall of the arteriole was almost doubled.

LITERATURE CITED

1. P. N. Aleksandrov and A. M. Chernukh, Pat. Fiziol., 82 (1967).
2. E. P. Smolichev and V. M. Volodin, Pat. Fiziol., No. 3, 72 (1968).
3. B. M. Altura, Am. J. Physiol., 212, 1447 (1967).
4. S. Baeez, J. Appl. Physiol., 21, 299 (1966).
5. P. S. Johnson and W. H. Greatbaten, Meth. Med. Res., 11, 220 (1966).
6. P. S. Johnson, J. Appl. Physiol., 23, 593 (1967).