AN INSTRUMENT FOR THE GRAPHIC RECORDING OF CHANGES IN DIAMETER OF MICROVESSELS BASED ON OPTICAL DISPLACEMENT OF THE IMAGE

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The design of an instrument for intravital measurement of changes in the diameter of microvessels based on the principle of optical displacement of the image is described. A semiautomatic method is suggested for the graphic recording of changes in the diameter of microvessels with the aid of a reversing micromotor and intervalograph.

Comparison of various instrumental methods of intravital measurements of the diameter of microvessels at intervals over a period of time [1, 8, 9, 11, 13-15] shows that the method based on the principle of optical displacement ("splitting") of the image is the most accurate and has the highest resolving power [2, 10, 12].

A diagram of an instrument developed by the writers on the basis of this principle is shown in Fig. 1A. A beam of rays from the objective, passing through the semitransparent surface of a double prism (P_1), is reflected into the ocular by an oscillating mirror (M_1) and a similar prism (P_2). Another beam is directed into the ocular by the prism P_1, a stationary mirror (M_2), and the prism (P_2). When the plane of the mirror (M_1) is exactly parallel to the reflecting surface of the mirror (M_2) and prisms (P_1) and (P_2), the path of the beams coincides and a single image of the object is seen in the ocular (additionally magnified by 1.6 times because of lengthening of the optical path by the crown glass prisms). As the mirror (M_1) moves, besides the image I (Fig. 1B) an identical image II of the same object is formed. Depending on the direction of rotation of the mirror (M_1), the new image will be to the right or left of the image I. It has been shown [10, 12] that the diameter of the object measured is directly proportional to the angle through which the mirror (M_1) must be turned in order to shift the image from position I to position II.

The mirror (M_1) is rotated by means of a lever (1) fitted with ball bearing (2). This ball is kept in contact with the cam (4), shaped like an Archimedes' spiral, by means of the spring (3). In this way the angle of rotation of the mirror (M_1), fixed in cylindrical bearings, is a linear function of the angle of rotation of the worm gear (5). An indicator knob with dial graduated in divisions is fixed to the shaft (6) of the worm. The knob is rotated until one edge of the image I coincides with the edge of image II (Fig. 1B), and the number of divisions is counted. By multiplying the result by a constant (for a given objective and ocular), the diameter of the vessel in microns is obtained. This constant is determined by calibrating the system with reference to the division of a stage micrometer.

The instrument is built as a separate unit (Fig. 2) for use with the MBI-6 microscope [6]. It fits into the socket of the binocular adapter of the microscope or into the socket of its camera. The method of fixing the unit allows it to rotate through 360° in these sockets, so that the diameter of microvessels located along any azimuth can be measured.

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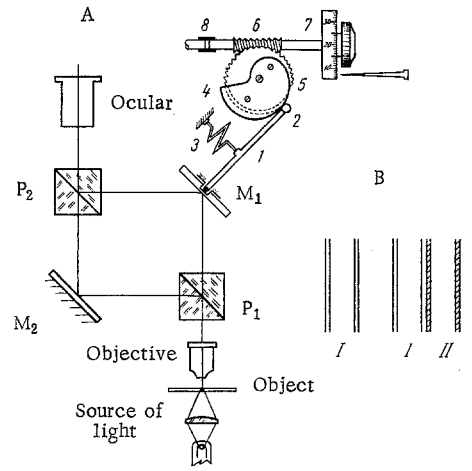

Fig. 1. Diagram of instrument (A) and principle of measurement of diameter of microvessel (B). Explanation in text.

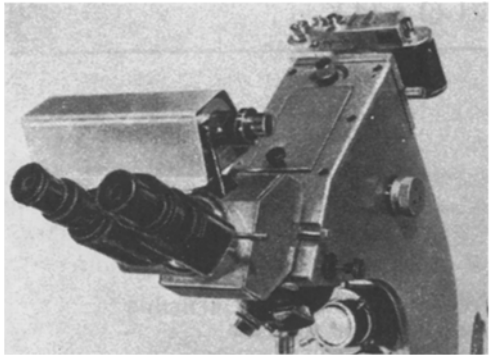

Fig. 2. Apparatus mounted in the socket of the binocular adapter of the MBI-6 microscope.

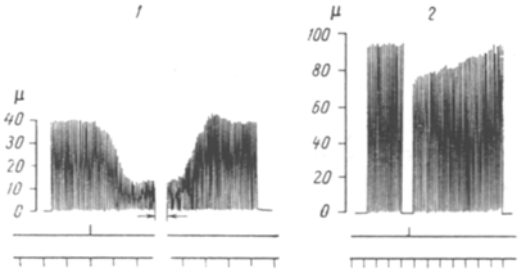

Fig. 3. Dynamics of constriction of arterioles of the mesentery (1) and submaxillary muscle (2) of a frog induced by noradrenalin. From top to bottom: record of diameter of arteriole; marker of time of noradrenalin application (10 μg/ml in 1, 0.01 μg/ml in 2); arrows mark stopping recording for 7 min (resumed 15 sec after stimulation); time markers 30 and 60 sec. Objective 9; total magnification 252×.

Displacement of the image is controlled through the binocular adapter which is fixed in the appropriate socket of the optical unit or (if secured in the socket for the camera) by reference to the screen of the videocontrol system (VCS) of an industrial PTU-26 television set [4]. In the latter case, the vidicon with its focusing and deflecting systems is fitted into the above-mentioned socket of the optical unit. The latter are taken out of their cumbersome factory cover and mounted in a light screening tube with a ring for attachment in the socket of the optical unit. The method of bringing out the image of the object to the VCS as suggested by Baez [8, 9] makes it possible to use the electronic contrast control in the video channel system [4] and thus to underline particular structural details of the object on the VCS screen.

It must be pointed out, however, that there is a considerable loss of light flux in the optical unit. (Neither the mirrors nor the prisms were coated.) For the investigation of vessels in objects of optically comparable density, the sensitivity of the LI-415 vidicon used in the PTU-26 apparatus may prove inadequate.

Changes in the diameter of the microvessels were recorded graphically in two ways. In the first way [8, 9] the shaft of the worm (6) (see Fig. 1A) is rigidly connected by a coupling (8) to a potentiometer which is included in the circuit of the dc source in series with the amplifier. A high-precision, multiple-turn PPML-1 linear potentiometer [5], a P-361 amplifier, and an N-372 ink-writing millivoltameter were used. The last of these records the angle of rotation of the potentiometer in a rectangular system of coordinates (total deflection 100 mm) and a record of the diameter of the vessel (Fig. 3: 1).

The second, semiautomatic, method speeds up the measurement process a little and reduces operator fatigue. The worm (6) is connected by the coupling (8) (see Fig. 1A) with the shaft of a miniature reversing step motor (SM) the speed of rotation of which can be varied within wide limits [3]. With a constant velocity of rotation of the SM the angle of rotation of the mirror (M_1) is directly proportional to time. Having secured complete superposition of the images of the vessel by means of the indicator knob (7), the SM and the electromagnetic coupling of the intervalograph are simultaneously activated by a tumbler switch [7]. The pen of the intervalograph records the ordinate on the kymograph at constant velocity. At the moment when the two opposite edges of the "split" image of the blood vessel come into contact (see Fig. 1B) the operator throws over the tumbler switch, thereby reversing the direction of rotation of the SM and disconnecting the electromagnetic coupling of the intervalograph. After 0.05 sec the time relay restores the connection and the intervalograph pen, which has returned to its initial position, begins to record the next ordinate. The height of the ordinates is directly proportional to the

diameter of the microvessel (see Fig. 3: 2). The records were calibrated against the divisions of a stage micrometer. With both methods of recording the height of the ordinates is a linear function of the number of divisions.

Calculation of the standard error of the records of 10–15 ordinates using the graduations of the stage micrometer (10, 30, 50, or 100 μ; objective 9 times, total magnification 252 ×) showed that the degree of error of the method is determined by the class of accuracy of the recording instruments used.

At the times of complete coincidence and separation of images of the object the light flux reaches its extreme values. This phenomenon enables the method to be completely automated, and its rapidity can be substantially increased by projecting the image on a photosensitive detector and if the micromotor is reversed at extremes of the light flux.

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