USE OF A TELEVISION ANALYZING SYSTEM IN MICROCIRCULATORY RESEARCH

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To investigate quantitative structural and functional characteristics of the microcirculation *in vivo* a Leitz television analyzing system was used. In experiments on anesthetized rats the degree of development of the microcirculatory network and the geometric characteristics of the microvessels themselves were determined, changes in the blood volume were estimated, and the degree and spread of disturbances of vascular wall permeability were examined.

KEY WORDS: Microcirculation; automatic image analysis; permeability of the vascular wall.

Methods of quantitative morphometry [3], realized in concrete form as automatic image analysis [1, 7], are being increasingly used in recent years to study structural and functional correlations and the precise quantitative characteristics of spatial organization of biological objects in various functional and pathological states. However, this method is used principally to analyze fixed, static objects (histological preparations, electron micrographs, and so on). There is virtually no experience of the use of automatic image analysis for intravital investigations [2, 5]. The object of the present investigation was to study the possibility of using a television analyzing system (TAS) to study various parameters of the microcirculation during intravital microscopy. Apparatus from Leitz (West Germany) was used.

Since the TAS is intended for analysis of static images, its use for intravital investigations required the reconstruction of certain components and also a search for appropriate criteria for the estimation of the state of the microcirculatory system depending on the parameters measured by the instrument. The TAS was used to assess the degree of development of the microvascular network, to measure the diameter of the microvessels and the diameter of the blood flow in them, to estimate volume of blood flow, and also to determine the degree and distribution of disturbances of permeability of the vascular wall. Experiments were carried out on noninbred albino rats and the test object was the microvascular system of the mesentery of the small intestine (vessels with an internal caliber of 10-50 μ).

In the investigation the image to be analyzed is projected on a light-sensitive transmitting television tube and is scanned along the lines of the television grid. A measuring grid with a certain number of structural elements (scanning elements), hexagonal in shape, is superposed on the optical image electronically. In one television line 702 hexagonal structural elements can be counted, and in the image as a whole their number is 420,000, thus ensuring high resolving power during the measurements.

Determination of the Number of Particles Present in the Measuring Field. This determination is carried out by comparing signals from two consecutive lines of the television grid. The counting pulse arises whenever the line of the television grid crosses the dorsal side of the contour of each particle. If in the previous line there were no changes of signal at this point, this pulse is led into the contour. Otherwise the pulse is suppressed.

In the process of automatic image analysis one of the main criteria for recognition of objects is their optical density, and to distinguish some objects from others there must be a difference in the optical densities of these objects. This accounts for the main difficulty in the use of automatic analyzers for the microscopy of living objects, the images of which are known to be characterized by low contrast, i.e., by a limited range of optical densities. In addition, in unstained tissues, widely different formations and cells may have identical

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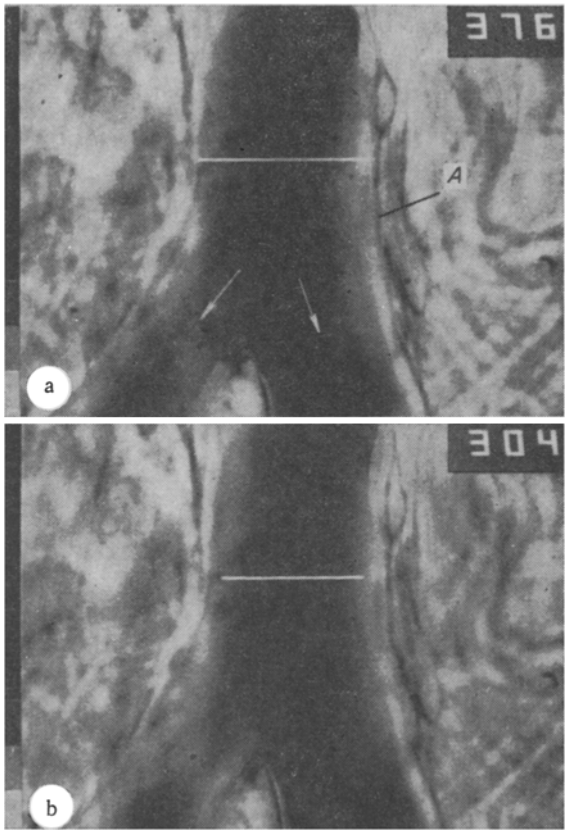


Fig. 1. Arteriole (A) of mesentery of rat small intestine. Television biomicroscopy. Photograph from screen of monitor, objective SW 50, ocular 10. Measurement of internal diameter (a) and diameter of blood flow (b) in arteriole. Arrows indicate direction of blood flow. Scale of levels of gray shown on left border of frame.

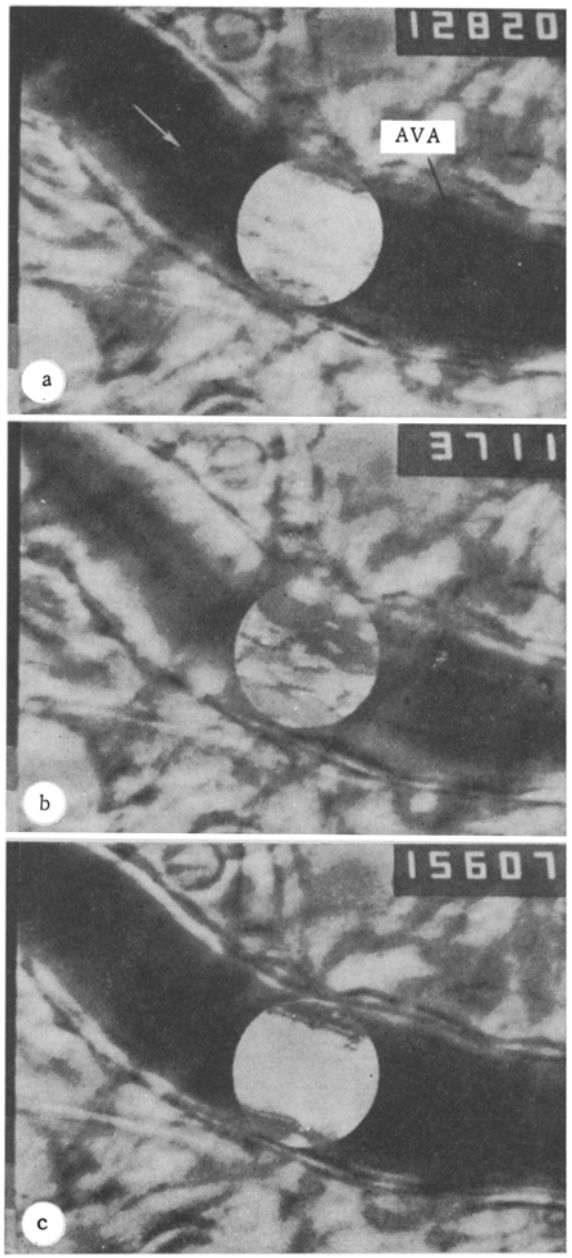
density, and this interferes considerably with their classification by means of the instrument.

To distinguish the image of an object which interests us for the purpose of measurement, various methods have to be used and, in particular, the measuring field must be restricted by means of masks of appropriate shape and size. The details of an image selected for measurement are distinguished on the screen of the monitor — they "light up."

Measurement of the Area of the Chosen Region. In this measurement, the number of structural elements of the grid corresponding to this region is determined. The results of measurement are displayed on the screen of the monitor in digital form in conventional units. The switch to named units of measurement is possible given appropriate calibration and conversion of the readings of the instrument allowing for the magnification of the microscope. Measurement of the total area of the image of microvessels in different parts of the mesentery (in assigned standard dimensions) enables the degree of development of the vascular system to be judged. The instrument can also be used to determine the perimeter of selected regions of the image. Measurement of the total perimeter of granules of mast cells was used by the authors as a means of characterizing their physiological activity.

Essential information on the state of the microvessels and cells is given by determination of their linear dimensions and, in particular, the internal and external diameter of the vessels and the diameter of the blood flow in them. Measurement of diameters was carried out in two ways: by means of rectilinear and circular masks. An advantage of the first method is that it can be used to determine desired measurements directly in the course of measurement and counting their values from the screen of the monitor (Fig. 1). However, by means of a rectilinear mask, the dimensions of objects studied (in particular, microvessels and cells) can be determined only in the direction of the lines of the television grid, so that strictly definite orientation of the object in the field of vision of the microscope is required.

Since opportunities for rotating the object are limited during biomicroscopy, and optical rotation of the image requires the use of a special device, an indirect method was used to determine linear dimensions by means of a circular mask. The value to be measured was found as the diameter of a circle inscribed in the desired dimension (for example, in the lumen of a vessel). The instrument enables the area of the circular mask to be determined, and



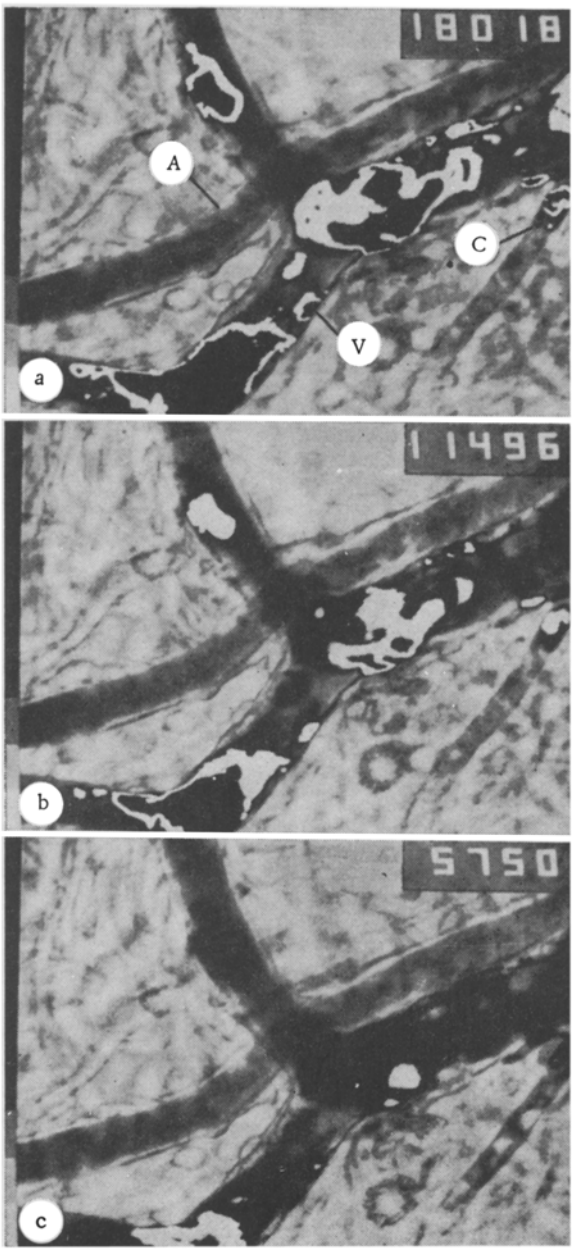


Fig. 2

Fig. 3

Fig. 2. Evaluation of volume blood flow in arterio-venular anastomosis (AVA). Conditions of photography as in Fig. 1. a) Initial level; b) 30 sec after injection of 0.02 mg adrenalin into rat's aorta. Slowing of blood flow and decrease in its volume velocity; c) 120 sec after injection of adrenalin. Increase in volume velocity of blood flow. Arrow indicates direction of blood flow.

Fig. 3. Determination of degree and distribution of disturbances of permeability of mesenteric microvessels. Television biomicroscopy, objective SW 50, ocular 4. Deposition of ink in places of increased permeability of wall of venule (V) and capillary (C); a, b, c) distribution of lesions of three degrees inorder of increasing intensity; A) arteriole.

the program compiled for the computer enables the diameter of the mask (and, consequently, the dimension corresponding to it on the object) to be calculated and recorded automatically. The greater complexity of this method is compensated by an important advantage: the possibility of determining dimensions irrespective of the orientation of the object relative to the lines of the television grid. The ratio between the diameters of the blood flow and vessels in different parts of the microcirculatory system was studied by this method.

Evaluation of the Volume of Blood in Microvessels. In the course of the experimental work, it was found that a relationship exists between visually observed changes in the blood volume of the arterioles and venules on the one hand, and changes in the optical density of the image of these vessels on the other hand (Fig. 2). Such correlation is found only when the velocities of the blood are sufficiently high. At flow velocities close to zero, with a jerky or pendulum-like current, this relationship does not hold good.

Changes in the optical density of images of microvessels, it is considered [6], may be connected with changes in the spatial orientation of the blood cells due to fluctuation in the velocity of the blood flow. The optical density of the image of the blood flow evidently also depends on the number of blood cells in the given region of the vessel and the density of their distribution. Analysis of these factors suggests that the optical density of the image of a microvessel can reflect the volume velocity of the blood flow in it, and changes in the blood flow relative to a certain initial level can be measured quantitatively. However, this is a matter for further research.

Disturbances of Permeability of Microvessels. These have been widely studied by a method based on the use of colloidal carbon (ink) or other substances as indicator. An essential defect of this method is the absence of precise quantitative criteria for visual assessment of the intensity and distribution of the indicator at places where permeability is disturbed. Accordingly the writers compared the results of quantitative assessment of disturbances of permeability of microvessels by a visual method and by means of the TAS [4]. Experiments were carried out with two groups of animals: 1) rats with aseptic peritonitis (3 h after intraperitoneal injection of 0.2% silver nitrate), 2) animals with aseptic peritonitis treated with antikinin preparation pyridinol carbamate (50 mg/kg by mouth).

To determine the intensity of ink labeling, the necessary level of optical density was chosen by means of a discriminator. Measurements were made at five levels, so that a sufficiently wide range of changes in intensity of the label could be obtained. The degree of severity of the disturbances of permeability was judged from the intensity of label in the region of the lesion, and the extent of the lesion was estimated from the area occupied by label of a particular level of intensity (Fig. 3). The experiments showed that the results of determination of permeability by the visual method and by means of the analyzing system agreed qualitatively, although a significant advantage of evaluation by the TAS is automation of the measuring process and recording of the data, ensuring greater accuracy and objectivity of the estimates.

It was thus shown experimentally that the use of a television method of image analysis during the study of the microcirculation ensures high accuracy of the quantitative data and their objective character, permits high reproducibility of the measurement, and considerable rapidity of action. However, the effective use of automatic image analyzers is possibly if the investigator has a clear idea of how the problem he is presenting to the automatic system should be solved and if he can correctly correlate the values obtained by means of the analyzer with the physical or biological significance of the process he is studying.

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