QUANTITATIVE EVALUATION OF DISTURBANCES OF VASCULAR PERMEABILITY BY MEANS OF A TELEVISION ANALYZING SYSTEM

Academician A. M. Chernukh,* M. P. Gorizontova, and V. S. Shinkarenko

UDC 616.16-008.6-07

A method of quantitative evaluation of disturbances of permeability of microvessels for particles of colloidal carbon with the aid of a television analyzing system is described. Advantages of the method include automation and high accuracy of the measurements.

KEY WORDS: *automatic image analysis; microvessels; permeability.*

One method of studying vascular permeability is that of "tagged vessels," in which particles of colloidal carbon are used as the indicator. These particles are deposited at sites of disturbed permeability after intravenous injection of ink (0.2-0.25 ml/100 g body weight). Majno et al. [5] showed that the ultrastructural mechanisms of deposition of colloidal carbon are based on the escape of its particles from the blood stream through transendothelial spaces or channels and their retention at the basement membrane. The ink method of studying vascular permeability, despite its disadvantages [2, 4], is widely used. One of its disadvantages is the absence of quantitative assessment of the disturbances of vascular permeability. Clark [3] suggests assessing the extent of the disturbances of the permeability by measuring the length of the affected vessels. However, this method is extremely laborious and not sufficiently accurate.

The writers suggested previously a method of quantitative assessment of disturbances of vascular permeability in mesenteric preparations which takes into account the number of affected vessels and the intensity of the label and recognizes four degrees of disturbance of permeability [1]. In the investigation described below, this method is compared with that of quantitative assessment of disturbances of vascular permeability with the aid of a television analyzing system (TAS).

The process of analysis of the preparation was carried out as follows. In a microscope the image of the preparation examined was projected on the cathode of a transmitting television tube, transformed into an electronic signal, and reproduced on the screen of the monitor. Special electronic circuits made it possible to analyze this signal and, in particular, to determine the optical density of different regions of the preparation and the surface area of those regions. To determine the intensity of the ink label, the necessary level of optical density was selected by means of a regulator relative to a scale located at the edge of the television picture. The scale had ten gradations ranging from the lightest to the darkest region of the picture. When setting the chosen level of optical density, regions of the picture corresponding to this level lit up on the screen of the monitor (Fig. 1), so that it was possible to judge which particular elements of the object were being measured at that moment.

Measurements were made at five levels of optical density, so that a sufficiently wide range of changes of intensity of the label could be included. The results of the measurements were led to a Hewlett-Packard 9810A computer and recorded by an automatic writer. The extent to which the microcirculation was affected could be judged from the area occupied by the ink tag of a certain intensity. To estimate the extent, a coefficient of extent of the lesion (K) was introduced:

*Academy of Medical Sciences of the USSR.

Laboratory of General Pathology and Experimental Therapy, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 84, No. 10, pp. 507-509, October, 1977. Original article submitted May 12, 1977.

 0007-4888/77/8410-1526$07.50

TABLE 1. Effect of Pyridinol Carbamate on Permeability of Mesenteric Microvessels in Aseptic Peritonitis

Experimental conditions	Number of mesenteric windows with labeled vessels, % of all examined			Number of rats with different degrees of labeling, % of total number of animals in experiment				
	0 labeled	1-10 labeled	over 10 labeled	0	I	II	III	IV
0.2% Silver nitrate, intraperitoneally	64	26	10	20	80	80	80	40
0.2% Silver nitrate intraperitoneally + pyridinol carbamate by mouth	80	13	7	30	70	70	60	20

TABLE 2. Distribution of Labeling by Intensity in Mesenteric Microvessels

Gradations of optical density on a 10-point scale	Relative percentage of gradations of density in animals	
	with aseptic peritonitis	with aseptic peritonitis receiving pyridinol carbamate
1*	14	4
2	12	11
3	16	14
4	21	24
5	37	47

*Corresponds to maximal intensity of labeling.

$$K = \frac{S_p}{S_m},$$

where S_p is the total area of deposition of colloidal carbon in the vessel walls, S_m the total surface area of the mesenteric preparations examined.

The use of a TAS, especially in the first stages of the work, was beset by certain difficulties due to the search for appropriate criteria for assessment and of algorithms and the optimal programming of the computer. However, with increasing experience, the amount of time wasted gradually decreased.

In this investigation disturbances of permeability were determined in two groups of animals: 1) rats with aseptic peritonitis (3 h after intraperitoneal injection of 0.2% silver nitrate); 2) animals with aseptic peritonitis receiving the antikinin preparation pyridinol carbamate (50 mg/kg by mouth).

Visual analysis showed that in the rats receiving pyridinol carbamate the disturbances of permeability were less severe both in extent and in intensity (Table 1). Analysis of the preparations by the TAS showed that in animals receiving pyridinol carbamate the maximal density of labeling (degree 1) was less marked than in the control, whereas conversely, the minimal density (degree 5) of labeling was more marked (Table 2), evidence of the lower intensity of damage to the vessels.

The extent of the lesion in the animals receiving pyridinol carbamate was less than in rats not receiving the product. For instance, the coefficient of extent of the lesion in these animals was $7.3 \cdot 10^{-5}$, compared with $14.6 \cdot 10^{-5}$ in the control group, i.e., it was twice as high.

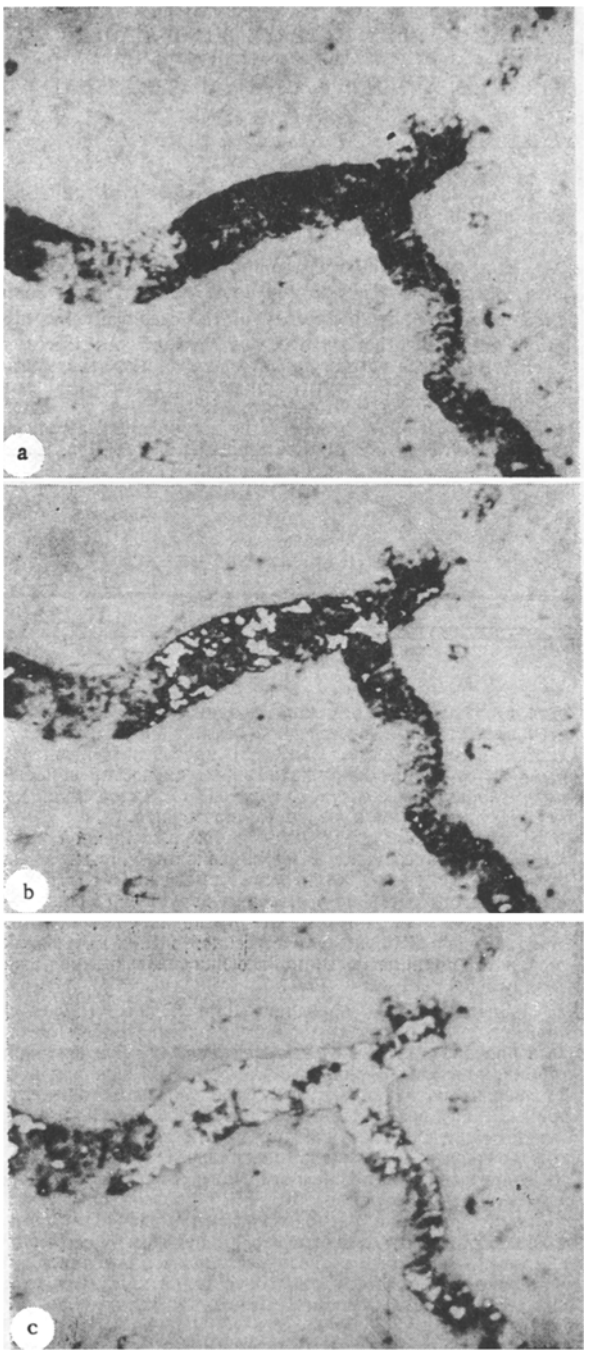

Fig. 1. Venule of mesentery of rat with aseptic peritonitis. Deposition of colloidal carbon particles in regions of disturbed permeability. a) Segment of venule chosen for analysis; b) "lighting up" of region with greatest disturbances of permeability; c) "lighting up" of region with lesser (by 1 degree) disturbances of permeability. Fixed preparation, 100×. Photographed from screen of monitor.

The results of the determination of disturbances of permeability obtained by the two methods thus agreed. However, an advantage of the method of quantitative assessment using the TAS is automation of the process of measurement and recording, resulting in high accuracy and objectivity of the estimates of the processes studied.

LITERATURE CITED

1. M. P. Gorizontova, O. V. Alekseev, and A. M. Chernukh, Byull. Éksp. Biol. Med., No. 3, 22 (1975).
2. P. J. Branemark, R. Ekholm and J. Lindhe, Med. Exp., 18, 139 (1968).
3. S. Clark, J. Pathol., 99, 93 (1969).
4. R. S. Cotran, E. R. Surer, and G. Majno, Vasc. Dis., 4, 107 (1967).
5. G. Majno, G. E. Palade, and G. I. Schoefl, J. Biophys. Biochem. Cytol., 11, 607 (1961).