AN AUTOMATIC IMAGE ANALYSIS STUDY OF THE MICROCIRCULATION IN INFLAMMATION

V. S. Shinkarenko

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Despite extensive study of inflammation [1, 7, 9], information on its connection with the state of the microcirculation is still inadequate. There are few data also on the development of inflammation at the cellular level obtained by direct observation of this process under intravital conditions. Research in this direction is mainly confined to qualitative estimates, because the quantitative description of changes in the microcirculation under these conditions is beset with considerable technical difficulties. Some of these problems can be overcome by the use of a new method of investigation, namely automatic image analysis [3, 4, 10].

In this investigation an attempt was made by intravital microscopy and the use of an experimental model to discover changes in the microcirculation arising during inflammation, and also to obtain quantitative characteristics of those changes by the method of automatic image analysis.

EXPERIMENTAL METHOD

A model of experimental peritonitis was obtained in male Wistar rats weighing 150-250 g by intraperitoneal injection of carrageenan (from Sigma, USA) in a dose of 5 mg in 5 ml of physiological saline. Control animals received an intraperitoneal injection of 5 ml physiological saline. The animals were anesthetized with pentobarbital (50 mg/kg, intramuscularly) and the state of the peritoneal microcirculation of the small intestine was studied between 15 min and 7 days after injection of the test substances. The internal (D_i) and external (D_e) diameters of the microvessels were measured 24 h after injection of the substances and their coefficient of dilatation $K = (D_e - D_i)/D_i$, the blood filling index, the intensity and spread of disturbances of permeability of the walls of the microvessels, and also the number of mast cells (MC) per unit area of surface of the mesentery were determined. The blood filling index was determined by the equation:

$$BF = \frac{OD_f - OD_b}{OD_b \cdot D_i} \cdot 100\,(\%\ \mu),$$

where OD_f is the optical density of the blood flow in the given vessel in a direction perpendicular to the axis of blood flow, OD_b the optical density of the background, i.e., of tissues surrounding the vessel. The intensity of disturbances of permeability of the vascular wall was estimated from the optical density of accumulations of colloidal carbon (label) in the affected areas of the vessels, whereas the extent of spread of these disturbances was estimated from the area occupied by accumulations with a given intensity. Colloidal carbon (Encre de Chine, Pelican, West Germany) was injected before the beginning of the measurements by the intra-aortic route through a cannula in the left carotid artery (0.25 ml/100 g body weight). Fuller details of the technique of measurmenet, using the Leitz TAS Texture Analysis System (Ernst Leitz, West Germany) were described previously [4].

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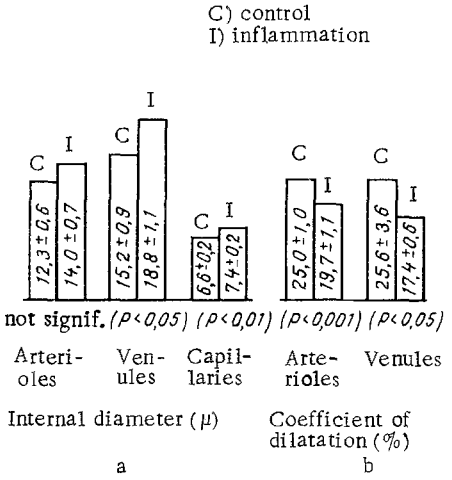

Fig. 1

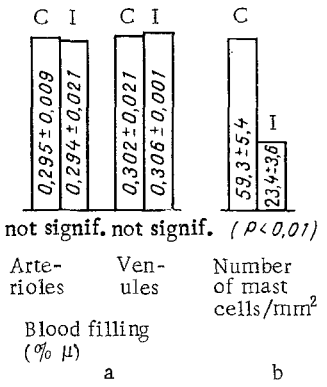

Fig. 2

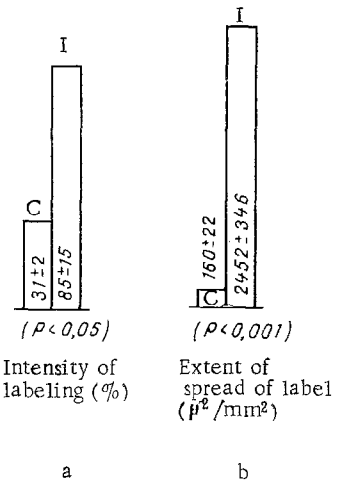

Fig.3

Fig. 1. Diagrams of changes in internal diameter (a) and coefficient of dilatation (b) of different parts of mesenteric microcirculation during inflammation.

Fig. 2. Diagrams of changes in blood filling index (a) and number of mast cells (b) during inflammation.

Fig. 3. Diagrams of changes in intensity (a) and extent of spread of disturbances of vascular permeability (b) during inflammation.

EXPERIMENTAL RESULTS

Damage caused by injection of carrageenan into the peritoneal cavity of the rats led to the development of a marked inflammatory response in the microcirculation of the mesentery. Pavementing of the leukocytes and the initial stages of MC degranulation were observed in the microvessels as early as 15 min after injection of carrageenan. After 1 h the response of the tissue and vessels intensifed, vascular permeability increased, as shown by deposition of colloidal carbon, leukocytes escaped from the vessels and degranulation of MC was more marked. After 24 h a distinct response to injury could be seen, with the development of a picture of clearly defined inflammation. On the following days the intensity of the inflammation diminished and by the 5th-7th day the state of the microvessels returned to normal.

The period of greatest changes, namely 24 h after injury, was chosen for undertaking the measurements. For this purpose the vessels were divided into three classes: 1) arterioles; 2) venules; 3) capillaries. Data on arteriolo-venular anastomoses were included in one of the first two classes, depending on which part of the anastomosis — arteriolar or venular — was in the field of vision.

Comparison of the measurements of the internal diameter of the mesenteric microvessels in the control animals and in the course of inflammation revealed a statistically significant increase in the mean diameter of the venules from 15.2 ± 0.9 to 18.8 ± 1.1 μ ($P < 0.05$) and capillaries from 6.6 ± 0.2 to 7.4 ± 0.2 μ ($P < 0.01$; Fig. 1a). This is evidence of dilatation of these portions of the microcirculation in animals with inflammation, although this could not be judged visually. The difference in the mean diameters of the arterioles in the control and experimental animals was not significant. Does this mean that dilatation was not present in the arteriolar portion? Some help with the answer to this question may be given by the use of a coefficient of dilatation (Fig.1b): A statistically significant increase in this coefficient indicates that dilatation of arterioles was present in animals of the experimental group.

The mean values of the blood filling indices in the experimental and control animals did not differ significantly (Fig. 2a). This index is largely dependent on the blood flow velocity in the microvessels: An increase in velocity is accompanied by an increase in the optical density of the vessel, and this is expressed as an increase in the blood filling index. This relationship may be due to changes in the scattering of light passing through the vessel to the sensitive element (television camera) with a change in the blood flow velocity.

The existence of this relationship has also been mentioned in the literature [5, 6, 8]. Absence of change in the blood filling index in the tissue during inflammation may thus be evidence that the linear velocity of blood flow is unchanged in the blood vessels studied. In that case, assuming equal linear velocity of the blood, the dilatation of the microvessels mentioned above must lead to an increase in the volume blood flow, i.e., although the linear velocity was unchanged the volume blood flow in the microvessels was increased during inflammation.

To describe the process of MC degranulation quantitatively the degranulation index (DI) was introduced; this is the ratio between the number of MC per unit area of tissue under normal conditions and their number in inflammation. In the present experiments the inflammatory reaction was accompanied by a decrease in the number of MC (Fig. 2b) on account of their degranulation. The intensity of degranulation corresponded to DI = 2.53, i.e., more than half the MC became fragmented, with liberation of the physiologically active substances (PAS) contained in them: histamine, serotonin, and heparin. The vasodilator response mentioned above arises and is maintained through the presence of PAS or, more exactly, by the result of their stochastic interaction. In addition, through the action of PAS and, in particular, histamine on the vessel wall its permeability may be modified.

The measurements showed that during inflammation the permeability of the microvessel walls increased by 2.74 times (Fig. 3a); these disturbances, moreover, affected a large part of the microcirculatory system (Fig. 3b). In different experiments the intensity of MC degranulation was found to correlate directly with the intensity of disturbances of permeability. This relationship is also clearly reflected in the overall results: When DI was 2.53 permeability was increased by 2.74 times. This relationship is evidently due to the fact that the intensity of disturbances of permeability is proportional to the quantity of PAS acting on the vessel wall, and this, in turn, depends on the number of degranulated MC.

Several types of disturbances of the microcirculation arising during inflammation were thus reproduced in the model experiments and quantitative characteristics of these disturbances affecting several parameters are given. The model and its quantitative description can provide a basis for the study of the pathophysiological mechanisms of the inflammatory reaction at the microcirculatory level and also for research into the experimental treatment of this form of pathology.

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