TRAJECTORIES OF RED BLOOD CELL FLOW IN MICROVESSELS STUDIED BY AUTOMATIC IMAGE ANALYSIS

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Changes in structure of the blood flow lie at the basis of many physiological and pathological processes taking place in the vascular system. They are especially important in the microcirculatory system, in which changes in the rheologic properties of the blood may become abnormal even in the presence of trivial disturbances of homeostasis. Since blood is a suspension of formed elements in plasma, which is a Newtonian fluid, abnormal non-Newtonian properties of blood flowing along microvessels are due mainly to the presence of red cells in the blood, their behavior, and their interaction with one another, with other blood cells, and with the blood vessel wall.

The structure of the blood flow is most commonly recorded by microfilming or videofilming and subsequent analysis of the information obtained. This method has been used in model experiments on glass tubes [5, 6]. The development of a biological model to study the microcirculation [3] has widened the scope of such research, for it enables several parameters of the blood low in living microvessels to be controlled: blood flow velocity, concentration of red cells, intravascular pressure, and so on.

The aim of the present investigation was to develop an automatic method of frame-by-frame analysis of the movement of red cells in microvessels on the basis of the "Leitz-TAS" computerized image analysis system (Ernst Leitz, West Germany) [4]. To solve this problem an algorithm was worked out and a "TRACE" program written in BASIC language.

EXPERIMENTAL METHOD

Experiments were carried out on the mesentery of 12 frogs, immobilized with pentobarbital and diplacin (0.02 mg/g body weight of each). The object was arranged on the special stage of an MBI-13 microscope with OSF 26P objective (Leningrad OpticoMechanical Combine) and ocular. Filming, at a speed of 32 frames per second, was carried out with a "Konvas automatic" camera. Enlargement of the image on the film was 83. Red cell flow in the mesenteric microvessels under 45 μm was studied when the hematocrit reading was 2-3% and the mean axial blood flow velocity was from 0.2 to 0.7 mm/sec.

The algorithm for frame-by-frame interpretation of the motion pictures was based on the principle of finding the coordinates of the centers of the red cells in each consecutive frame. The outline of the vessel on the first frame recorded was traced with a light pen on the display screen, stored in the memory of the instrument, and used during analysis of subsequent frames to determine the initial coordinates. The diameter of the vessel over the whole length of the region studied was determined and the coordinates of its axis calculated automatically. The centers of the red cells chosen for study (usually from 5 to 15 cells) were then noted on interactive mode. Measurement of the coordinates of the test red cells, and determination of their number and of the local hematocrit, allowing for the total volume of the red cells and the volume of the given segment of the vessel, were then carried out automatically.

A diagram of a red cell in three successive segments of a vessel (frames of a microfilm) is given in Fig. 1. The system of coordinates was chosen so that the abscissa coincided with the axis of the vessel and the ordinate with its diameter at the extreme left point of the frame. After determination of the coordinates X and Y (in μm) of the centers of the erythrocytes in

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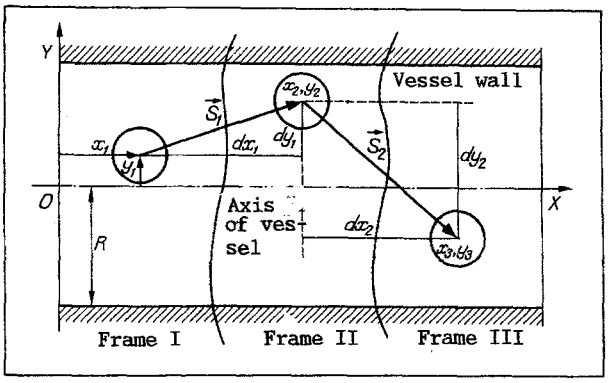

Fig. 1. Diagram showing trajectory of a red blood cell in three successive frames of the microfilm. Explanation in text.

TABLE 1. Results of Analysis of Microfilms of Trajectories of Red Cells in a Microvessel

Cell No.	X	Y	dX	dY	dY/dX	Y/R	V, μ/sec
				Frame II			
1	12,7	6,7	3,5	1,4	0,400	0,417	121,1
2	16,9	7,3	4,9	1,4	0,286	0,445	163,8
3	10,5	—3,6	2,8	0,7	0,250	—0,227	92,8
4	18,3	—9,1	4,2	0,7	0,167	—0,569	136,9
5	21,1	—0,6	3,5	0,7	0,200	—0,038	114,7
				Frame X			
1	49,2	6,7	2,1	1,4	0,667	0,417	81,1
2	54,1	5,4	2,8	0,7	0,250	0,341	92,8
3	54,8	—3,6	2,2	0,7	0,250	—0,227	92,8
4	57,0	—9,1	3,5	0,7	0,200	—0,569	114,7
5	68,9	0,0	3,5	0,0	0,000	0,000	112,5

Legend. *V*) Velocity of movement of red cell during time *t*; hematocrit (in %), frame II, 1.44; frame *X*, 1.36.

each frame the projections of the displacement vector (*S*) on both axes of coordinates, i.e., *dX* and *dY* (in μm). The ratio *dY/dx*, reflecting displacement of the red cells in a direction perpendicular to the flow relative to their displacement along the flow; and the ratio of the distance of the red cell center to the axis of the vessel (*Y*) to the radius of the vessel (*R*), i.e., *Y/R*, which is a parameter indicating the position of the red cell on the diameter of the vessel, were calculated automatically.

The velocity of movement of the red cells in the test segment of the vessel was determined (in μm/sec) from these data, after which the results were averaged for the number of frames analyzed, and, after statistical analysis, they were printed out in the form of mean values with standard deviations (Table 1). Table 1 gives the results of analysis of a microfilm of the trajectories of five red cells moving along a vessel 37 μm in diameter. The results of measurements on frames II and X of the film are given. From these data it is possible to obtain the distribution of velocities along the diameter of the vessel (i.e., the velocity profile) and also to judge changes in the pattern and structure of flow and the rheologic properties of the blood in one concrete vessel.

The algorithm of this program is based on the principles of frame-by-frame analysis, which are used during manual analysis of motion picture frames by projecting them on a screen and then measuring the coordinates of the centers of the red cells with the aid of a measuring rule [1, 2]. In this way the results of measurements made manually and automatically can be directly compared and the efficiency of the method of automatic analysis evaluated.

Special comparative studies showed that, using the manual method of analysis, the time required to prepare each frame and to obtain data, for example, for 5 red cells in each of 10 frames, is 2.0-2.5 h. The subsequent quantitative processing of the data requires another 4-5 h. Using analysis of the microfilms by the method of automatic image analysis, the time required to study the same number of red cells and frames, including measurements and quantitative analysis of the data, is not more than

25-30 min, i.e., the saving of time amounts to 10-15 times. The method also is much less laborious, but at the same time it increases the accuracy and objectivity of the investigations.

Thus although the manual method of analysis of microfilms can yield several important characteristics of blood flow structure under various physiological and pathological conditions, it has important disadvantages, namely the great laboriousness of analysis (interpretation) of the frames and the possibility of subjective assessments of the results of the measurements. Moreover, with manual analysis the available information on flow structure is not fully utilized. The program of automatic frame-by-frame interpretation of microfilms which we have developed not only makes it possible to analyze them quickly and qualitatively, but also yields a greater number of parameters reflecting the behavior and interaction of red blood cells moving in microvessels.

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MICROSPECTROFLUOROMETRIC ANALYSIS OF THE ACTION OF MECLOFENOXATE ON LIPOFUSCIN GRANULES OF HYBRIDOMA (RETROVIRUS-TRANSFORMED) CELLS

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It is now almost 30 years since attention was first drawn to the stimulating action of a newly synthesized compound ANP-235, a derivative of the natural plant growth factors (auxins) on the vertebrate CNS [11]. This substance, an ester of dimethylaminoethyl-*p*-chlorophenoxyacetic acid, was later called meclofenoxate (MF), with synonyms centrophenoxine, acephen, lucidril, cerutil, etc.

MF lowers the intracellular potassium concentration [14], increases the rate of synthesis of total and mRNA [15], increases the density of adrenergic receptors [12], influences the adenylate cyclase system [9], acts on phospholipid metabolism [6, 8], and modifies the lipid composition of the cell membrane so that it becomes more fluid [13].

This perturbation in the lipid composition of the cell membrane is naturally reflected also in the state of intracellular structures. There is evidence that MF reduces the number of lipofuscin granules (aging pigment) in cells both during natural

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