CONTINUOUS MONITORING OF HUMAN ERYTHROCYTE DESTRUCTION IN CIRCULATING BLOOD BY MULTIPLE LIGHT SCATTERING

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Various investigators have studied osmotic, temperature, acid, mechanical, and other types of hemolysis [2, 4, 5]. Many factors leading to erythrocyte destruction have been found, but in practice the effects of increased mechanical loading of erythrocytes and, perhaps also, of their osmotic swelling, accompanying surgical operations with the use of an artificial circulation apparatus (ACA), are particularly important. In some cases, during a heart operation lasting several hours hemolysis in the ACA may amount to 350 mg% [1], yet an increase in the degree of hemolysis to only 70 mg% may have a marked toxic effect [4].

In the investigation described below an optical diagnostic method was used, based on analysis of multiple light scattering in circulating blood, with the aim of monitoring the course of hemolysis. An experimental model of hemolysis was created by subjecting the erythrocytes to mechanical (shear) stresses or osmotic overloading.

EXPERIMENTAL METHOD

Citrated blood from healthy blood donors, stored for between 1 and 10 days, was used. For quantitative analysis results of experiments conducted on blood from the same donor for 24 h were compared. Hemolysate required for calibration was obtained by rapid freezing of whole blood in liquid nitrogen, followed by rewarming to 36°C. All measurements of light scattering were made on blood circulating under the influence of a peristaltic pump working in a closed circuit under conditions similar to those of blood flowing in human arteries: temperature 36 ± 0.25°C, diameter of silicone tubes 4-5 mm, average flow rate 4 cm/sec. The recirculation circuit contained an oxygenator, for saturating the blood with oxygen, a mixer, and also a capillary shunt with a narrow part 5 mm long and 0.3 mm in diameter. The capillary tube was used to simulate hemolysis developing in the ACA during high blood flow rates: on occlusion of the main path, the flow could be entirely directed through the shunt. In all the experiments the initial blood volume was 10 cm^3 and the total length of the circuit 30 cm. For continuous monitoring of processes taking place in the moving blood, a glass cylindrical cuvette 4 cm long was incorporated in the recirculation circuit. Light rays from a 20-W incandescent lamp were shaped by a spherical lens and diaphragms into a virtually parallel beam of white light with an angle of convergence of 7°. The beam of light was directed on the cuvette perpendicularly to its axis so that the focus, when no blood was present, was located at its center. After the circuit had been filled with blood, light was scattered on the erythrocytes, and some of it adsorbed [4]. The scattered radiation was recorded by external silicon photodetectors, equipped with light filters. Light reflected backward was recorded in the vicinity of the isobestic wavelength of 0.80 μ [3]. Two other photodetectors, by means of which radiation was recorded on both sides of the isobestic wavelength, were located at the side, symmetrically to the axis of the beam: they were intended to monitor the oxyhemoglobin concentration [2]. The fourth photodetector was used to monitor the intensity of the incident radiation. A glass light guide was used for spatial diversion of light scattered backward. All experiments wre carried out on completely oxygenated blood with an oxyhemoglobin concentration of 98-99%.

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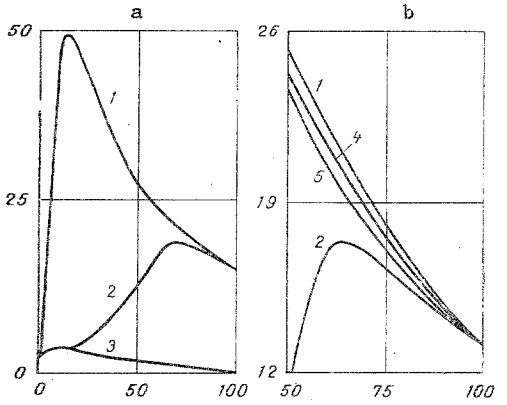

Fig. 1. Change in scattering of light depending on degree of dilution (in %); ordinate, intensity of reflected radiation (in relative units). $C = \frac{V_B}{V_B + V_d} \cdot 100\%$, where V_B denotes the volume of blood, V_d the volume of diluent. 1,2,4,5) Dilution of blood with NaCl solution in concentrations of 0.90, 0.75, and 0.8%, respectively; 3) dilution of hemolysate with NaCl solution in a concentration of 0.9%.

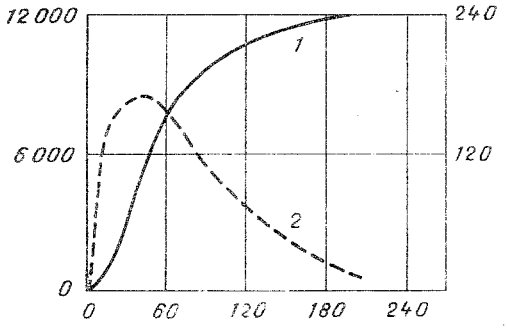

Fig. 2. Time course of hemolysis (1) and rate of hemolysis (2) during shear destruction of cells (shear velocity 10^3 sec^{-1}, time of one passage through region of exposure 7×10^{-4} sec). Abscissa, time (in min); ordinate: on right – degree of hemolysis γ (in mg%); on left – rate of hemolysis (in mg%/min).

EXPERIMENTAL RESULTS

Dependence of the intensity of backward scatter of light on the dilution of whole blood and hemolysate by different solutions is shown in Fig. 1. On addition of distilled water to blood to a concentration (C) of about 70%, curve 2 in Fig. 1a behaves just as after addition of an isotonic solution (curve 1), i.e., its behavior is mainly determined by a decrease in erythrocyte concentration. After C = 70% curve 2 begins to deviate appreciably from curve 1 toward diminution of scatter. This indicates that hemolysis has begun in the system. When all erythrocytes are hemolysed, curve 2, at C = 13%, joins curve 3, corresponding to dilution of the hemolysate with isotonic solution. The region of concentrations from 100 to 70% is prehemolytic, and scatter between curves 1 and 2 is a measure of hemolysis during osmotic shock.

On addition of hypotonic solutions to the blood the behavior of the light scattering curves (not shown here) depended on the size of the portions in which the solution was added. For example, if distilled water was added to the circulating blood in small droplets each

measuring 0.02 cm^3 the dilution curves changed their slope abruptly at C = 67% (effective NaCl concentration 0.60%), whereas if it was added in portions of 0.5 cm^3, the change of direction took place at C = 75% (NaCl 0.68%). In the latter case hemolysis was accelerated due to local osmotic overloads and could not be strictly assigned. Similarly, on the addition of solutions at a temperature which differed appreciably from that of the blood, local temperature gradients arose (temperature shock), leading to changes in the state of the erythrocyte membrane. Conditions under which mixtures with an NaCl concentration $\geq$0.6% were added to the blood in small doses, under isotemperature conditions, made it possible to study the prehemolytic stage (Fig. 1b). Deviation of curves 4 and 5 from curve 1 was probably due to diminution of light scattering as a result of osmotic swelling of the erythrocytes, with a corresponding change in their aggregation.

The time course of the degree of hemolysis γ (in mg% of free hemoglobin), taking place during passage of the whole blood flow through the capillary tube, is illustrated in Fig. 2. To obtain absolute values of γ, calibration was first carried out. The oxygenated blood was separated into two portions and one of them was hemolysed. Next, the two parts were mixed again in different proportions, the light scattering signal was recorded, and a calibration curve plotted. As will be clear from Fig. 2, the rate of hemolysis at the initial moment of shear stress was low. From time t = 1 min it began to increase and reached a maximum at t = 35 min.

Under the influence of shear stresses, local disturbances of curvature of the membrane and transient surges of intracellular pressure evidently arise. Similar factors, but quasi-stationary in character, must also act in the case of osmotic swelling of erythrocytes. The temporary appearance of fluctuation windows in the membrane, stimulated by internal pressure, and through which small portions of potassium ions, hemoglobin, and certain important enzymes are discharged into the external medium, can therefore be postulated as the mechanism preceding hemolysis [6-8]. In this case, the fact that shear-induced hemolysis is delayed by a time corresponding to several passages of the erythrocytes through the region of mechanical disturbances, can be explained by the existence of a certain value of loss of key molecular elements of the membranes structure that is critical for integrity of the membrane.

Thus continuous monitoring of hemolysis is possible in circulating blood. The parameter γ, introduced above, characterizes the degree of hemolysis in the artificial circulation circuit under the condition that hemoglobin does not leave the blood stream and additional erythrocytes from cell depots do not enter the blood stream. Furthermore, for correct application of the method under operation conditions, the possible appearance of disturbing factors affecting scattering of light, such as dilution and osmotic swelling of the cells, must be taken into account.

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