SURFACE ELECTRICAL PROPERTIES OF BLOOD CELLS AND HEMOCOAGULATION

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Iso-osmotic solutions of lithium, potassium, and rubidium chlorides facilitate hemocoagulation whereas sodium chloride does not affect blood clotting. No direct relationship was found between changes in blood coagulation and the magnitude of the surface electric charge of the red cells and platelets. Changes in hemocoagulation are evidently connected with a whole range of surface phenomena taking place at the interphase boundary between blood cell membrane and plasma and also with the specific effect of the ions studied on the functional properties of the red cells and platelets.

Key words: hemocoagulation; surface charge; blood cells.

In the existing view blood is a colloidal system, the aggregative and sedimentation resistance of which is largely determined by electrostatic repulsive forces arising between the negatively charged blood cells, protein micelles, and the vascular endothelium [2, 5-7, 10, 11]. It is postulated that a decrease in the surface electric charge on the blood cells induces their aggregation and stimulates hemocoagulation, whereas an increase in the surface charge on the blood cells tends to maintain the dispersed state of the blood [3, 4, 12, 13].

The effect of a change in the surface electric charge on the platelets and red cells on hemocoagulation was studied.

EXPERIMENTAL METHOD

Changes in the surface electric characteristics of the blood cells were induced by means of iso-osmotic solutions of chlorides of the alkali metals and determined by microelectrophoresis [8]. The effect of 0.16 M solutions of Li, Na, K, and Rb chlorides on the coagulation of whole blood, of platelet-rich plasma, and of platelet-free plasma was studied. The thromboelastogram (TEG) and electrocoagulogram were recorded. For the former, 0.1 ml of a solution of the electrolyte was added in the cell of an ISK-1 thromboelastograph to 0.4 ml of whole blood or plasma stabilized with 3.8% sodium citrate. To record the electrocoagulogram, 0.05 ml of a solution of the electrolyte was mixed with 0.25 ml whole blood or plasma. In parallel tests the electrophoretic mobility (EPM) of the platelets and red cells was measured with the Olton cytopherometer. All measurements were carried out in Michaelis buffer, pH 7.35, with a specific resistance of 68.64 Ω/cm, at 25°C, and with a current of 5 mA. The EPM was calculated by the formula

$$B = \frac{l}{t \cdot E}\ \text{cm}^2 \cdot \text{V}^{-1} \cdot \text{sec}^{-1},$$

where l is the path traveled by the blood cell (in cm), t the time taken to pass along the path (in sec), and E the voltage of the electric field (in V).

Solutions of the alkali metals were added to 1 ml platelet-rich blood plasma or to a suspension of red cells in physiological saline, incubated for 10 min at room temperature, diluted with buffer, and poured into the measuring cell of the cytopherometer. At each measurement the EPM of 20 separate cells was re-

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corded. Altogether more than 2000 blood cells were investigated.

Aggregation was recorded in 25 experiments after the addition of 0.2 ml of the electrolyte solution to 0.7 ml plasma; after incubation at room temperature for 10 min the aggregation properties of the platelets were determined [9].

EXPERIMENTAL RESULTS

Addition of lithium chloride to the whole blood of intact rabbits facilitated the formation of thromboplastin and thrombin, reduced the density and elasticity of the blood clot, and caused virtually no change in the intensity of formation of the blood clot or its retraction time (Fig. 1).

Potassium and rubidium chlorides accelerated all three phases of blood clotting, facilitated the formation of thromboplastin, thrombin, and fibrin, accelerated formation of the blood clot, increased its density and elasticity, and shortened the retraction time. The action of potassium ions was the stronger of the two. An increase in the blood concentration of Na^+ ions had no significant effect on blood clotting.

The addition of iso-osmotic solutions of chlorides of the alkali metals to platelet-rich blood plasma accelerated coagulation whereas Na^+ ions, just as in the experiments with whole blood, did not change the time or dynamics of the clotting of platelet-rich plasma (Fig. 2). An increase in the concentration of all the ions studied in platelet-free plasma did not affect its coagulation. Li^+ and K^+ ions induced an increase in red cell mobility in an electric field, but platelet mobility was not significantly altered. Rb ions slightly increased the EPM of the platelets but did not change the mobility of the red cells, whereas Na^+ ions reduced the EPM of the platelets (Table 1).

The study of the aggregation properties showed that Li^+ and Rb^+ ions inhibited aggregation of the platelets whereas Na^+ and K^+ ions did not significantly alter either the time or the degree of aggregation (Fig. 3).

Iso-osmotic solutions of Li, K, and Rb chlorides thus accelerated the clotting of whole blood and of platelet-rich plasma. An increase in the concentration of these electrolytes in platelet-free plasma did not significantly affect its coagulation. This shows that the acceleration of coagulation was connected with changes in the functional state of the blood cells.

The effect of ions of the alkali metals on red cells and platelets might be supposed to be connected with differences in the degree of hydration of these ions and, consequently, with their effect on the ζ-potential of the blood cells. However, no direct relationship could be found between the EPM of the blood cells and blood clotting processes.

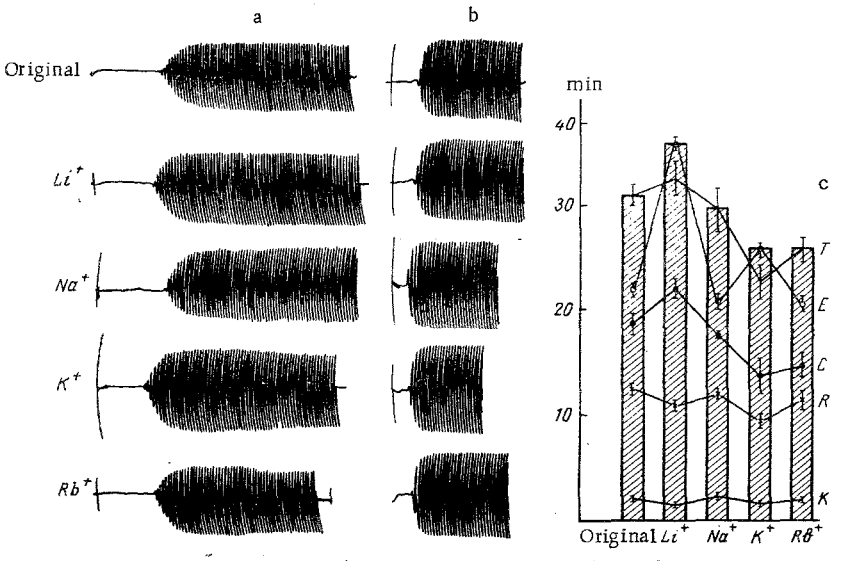

Fig. 1. Effect of iso-osmotic solutions of chlorides of alkali metals on coagulation of whole blood and platelet-rich plasma: a) TEG of whole blood; b) TEG of platelet-rich plasma; c) changes in parameters of TEG of whole blood during action of chlorides of alkali metals (M ± m).

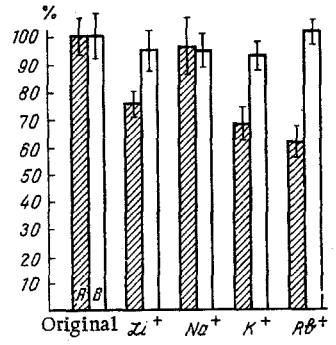

Fig. 2

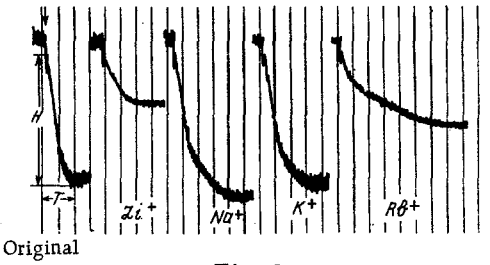

Fig. 3.

Fig. 2. Effect of iso-osmotic solutions of chlorides of alkali metals on coagulation of platelet-rich (A) and platelet-free plasma (B) (M ± m). Ordinate, total time of coagulation of platelet-rich and platelet-free plasma (in %).

Fig. 3. Effect of iso-osmotic solutions of chlorides of alkali metals on aggregation properties of platelets: H) degree of platelet aggregation; T) aggregation time of platelets (rate of retraction 12 mm/min). Arrow marks addition of ADP to plasma.

TABLE 1. Effect of Ions of Alkali Metals on EPM of Platelets and Red Cells

Electrolyte	Platelets		Red cells	
	t	B	t	B
Original	7,9± 0,09	$1,27 \cdot 10^{-4}$	12,43± 0,22	$0,80 \cdot 10^{-4}$
Li+	7,4± 0,32 $P<0,1$	$1,34 \cdot 10^{-4}$	11,27± 0,27 $P<0,01$	$0,80 \cdot 10^{-4}$
Na+	8,36± 0,15 $P<0,02$	$1,20 \cdot 10^{-4}$		
K+	7,67± 0,11 $P<0,1$	$1,14 \cdot 10^{-4}$	11,18± 0,23 $P<0,01$	$0,89 \cdot 10^{-4}$
Pb+	7,68± 0,08 $P<0,05$	$1,14 \cdot 10^{-4}$	12,76± 0,32 $P<0,1$	$0,82 \cdot 10^{-4}$

Legend. Significance of change P calculated by comparison with original values; t) time taken by platelet or erythrocyte to cover distance of 0.0032 cm; B) electrophoretic mobility of red cells (in $cm^2 \cdot V^{-1} \cdot sec^{-1}$). The original value for red cells was taken to be the EPM of cells suspended in physiological saline.

Li^+ and K^+ ions increased the EPM of the red cells and accelerated coagulation, whereas the EPM of the platelets was not significantly altered. Rb^+ ions increased the EPM of the platelets a little but did not change the EPM of the red cells; the clotting time of blood and platelet-rich plasma was considerably shortened.

Since the electrical conductivity, the dielectric constant, and the viscosity of the surrounding medium were unchanged in these experiments, determination of the mobility of the blood cells in an electric field gave some idea of the electrokinetic potential and the surface charge on the blood cells. Consequently, the surface electric charge of the red cells and platelets does not play an essential role in blood clotting.

The study of the aggregation properties of the platelets showed that Li^+ and Rb^+ ions inhibit aggregation of the platelets whereas Na^+ and K^+ ions have no significant effect on this process. No relationship was found between the aggregation function of the platelets and the magnitude of their EPM, on the one hand, and hemocoagulation processes on the other hand.

Electrostatic forces thus play only a secondary role in the maintenance of the colloidal state of the blood. It is more likely that the aggregative and sedimentation resistance of the blood and processes of hemocoagulation are determined by a whole range of surface phenomena taking place on the interphase boundaries of the blood: the adsorption properties of the contacting surfaces, their hydrophilicity, the con-

centration of free energy in the interphase layers, and the magnitude of the electric charge of the boundary surfaces [1].

Another possibility is that ions of the alkali metals interact with the lipid–protein complexes of the blood cell membranes; this interaction induces changes in the permeability of the cell membranes and facilitates the liberation of thrombogenic substances from their cytoplasm, accelerating hemocoagulation processes.

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