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ANALYSIS OF THE ENERGY OF ELECTROSTATIC INTERACTION BETWEEN BLOOD CELLS IN EXPERIMENTAL MYOCARDIAL ISCHEMIA

M. P. Popov and V. V. Pichugin

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The diffusion and ζ potentials of red cells in blood flowing directly from a zone of myocardial ischemia along a branch of the great cardiac vein in the acute period of experimental infarction were studied by a microelectrode method and by microelectrophoresis. By this means the energy of electrostatic repulsion (EER) between the blood cells to be calculated and factors exerting a significant effect on this parameter in acute experimental myocardial infarction caused by ligation of the anterior interventricular branch of the left coronary artery in 20 dogs could be identified. The energetic state of the double electric layer was shown to be a leading factor in the change in EER and manifestation of the aggregation properties of the blood cells. A statistically significant decrease in the energetic potentials of the red cells was found in blood taken directly from the zone of myocardial ischemia.

KEY WORDS: binding energy; blood; ischemia.

Disturbances in the blood clotting system, together with changes in the state of the vascular wall and the hemodynamics, are factors of great importance in the development of ischemic heart disease [6, 7]. However, the problem of the intimate mechanisms of the changes in blood clotting during pathological shifts in the microcirculation and perfusion of the myocardium still remains a matter for discussion [8, 14].

It is accordingly interesting to study the dynamics of the energy of electrostatic repulsion (EER) between the blood cells, the biophysical basis for aggregation and adhesion processes in the initial stage of thrombosis.

EXPERIMENTAL METHOD

To calculate EER — an index which essentially characterizes the conditions of dissociation of the blood cells — the well-known Deryagin—Landau equation [2, 3, 5] is used

$$W = \frac{8(kT)^2 \varepsilon \cdot a}{e^2 \cdot Z^2} \cdot \left[\frac{\exp\left(\frac{Ze}{2kT} \cdot \psi_0\right) - 1}{\exp\left(\frac{Ze}{2kT} \cdot \psi_0\right) + 1} \right]^2 \exp(-\chi H_0), \quad (1)$$

where k is Boltzmann's constant; T the absolute temperature; ε the dielectric constant of the medium; a the radius of the cell; e the elementary charge; H_0 the distance between the cells; χ the Debye—Hückel function, corresponding to the reciprocal of the double electric layer of the cell; ψ_0 the surface potential determined from the density of electric charges of the red blood cells by the equation [3]

$$\sigma_0 = \left[\frac{\varepsilon N_i kT}{2\pi}\right]^{1/2} \cdot \left[\exp\left(\frac{Ze}{2kT} \cdot \psi_0\right) - \exp\left(\frac{Ze}{2kT} \psi_0\right)\right], \quad (2)$$

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TABLE 1. Changes in Some Energetic Parameters of Red Blood Cells, Platelets, and Whole Blood After Coronary Occlusion (M ± m)

Place of taking blood	Red blood cells		Platelets		Whole blood
	ζ- potential	surface potential	ζ-potential	surface potential	diffusion potential
Cardiac vein	0,023±0,0011	0,0049±0,0007	0,023±0,0024	0,0047±0,00002	0,93±0,003
	0,015±0,0027	0,0035±0,0009	0,016±0,0021	0,0041±0,00003	0,70±0,006
Femoral vein	0,022±0,0018	0,0047±0,0006	0,0029±0,003	0,005±0,0004	0,11±0,004
	0,22±0,0020	0,0048±0,0004	0,026±0,0021	0,054±0,0005	0,96±0,008

Legend. Here and in Table 2: numerator shows initial state, denominator the state 30 min after ligation of coronary artery.

TABLE 2. Changes in EER of Red Blood Cells After Coronary Occlusion (M ± m)

Place of taking blood	EER (in cal) disregarding H_0			EER (in cal) allowing for H_0		at $y(\psi_0)$	
	*y (ψ_0)	y (ξ)	y (φ_1)	H_0=4 mm	H_0=10 mm	H_0=20 mm	H_0=30 mm
Cardiac vein	$[24\pm1]\ 10^{-4}$	$[53\pm9]\ 10^{-3}$	[0,5±0,02)	$[3,0\pm0,01]\ 10^{-11}$	$[3,0\pm0,01]\ 10^{-12}$	$[0,9\pm0,02]\ 10^{-15}$	$[0,58\pm0,04)\ 10^{-19}$
	$[12\pm0,3]\ 10^{-3}$	$[23\pm9)\ 10^{-3}$	[0,34±0,03]	$[1,2\pm0,03]\ 10^{-11}$	$[0,8\pm0,02]\ 10^{-12}$	$[0,67\pm0,006)\ 10^{-15}$	$[0,34\pm0,04]\ 10^{-19}$
Femoral vein	$2,1\pm0,2\ 10^{-3}$	$5\pm0,1\ 10^{-2}$	0,6±0,04	$[2,9\pm0,01]\ 10^{-11}$	$[2,9\pm0,02]\ 10^{-12}$	$[1,02\pm0,03\ 10^{-15}$	$[0,5\pm0,08]\ 10^{-19}$
	$2,2\pm0,4\ 10^{-3}$	$4,6\pm0,8\ 10^{-2}$	0,63±0,05	$[2,8\pm0,02]\ 10^{-11}$	$[2,8\pm0,04]\ 10^{-12}$	$0,8\pm0,02\ 10^{-15}$	$[0,54\pm0,09]\ 10^{-19}$

Legend. $y=\left[\dfrac{\exp\frac{Ze}{2ki}\psi_0-1}{\exp\frac{Ze}{2ki}\psi_0+1}\right]^2$

where σ is the surface density of electric charges of the cell; N_i the ion concentration; ψ_0 the surface potential.

It follows from equations (1) and (2) that for the calculation it is first necessary to determine the electrophoretic mobility of the cells, on the basis of which the value of the Z potential and surface density of the electric charges of the red cells are calculated. The diffusion potential, from which the thickness of the double electric layer can be estimated qualitatively, was used to determine the Debye–Hückel function; the concentration of cells per unit volume of blood and the dielectric constant also were measured.

Electrophoretic mobility was determined in a chamber of original design [9]. The diffusion potential, characterizing the potential step in the double electric layer as a function of its thickness, was recorded by means of a dc amplifier and microparticle concentration meter (IKM-2) [12]. The dielectric constant was determined in the usual way [13].

Experiments were carried out on 20 dogs in which the anterior descending branch of the left coronary artery was ligated at the level of its middle third. Preliminary blood samples were taken from one branch of the great cardiac vein and the femoral vein in order to determine the original parameters of EER. Further blood samples were taken 30 min after ligation of the coronary artery. The volume of the blood samples was 3 ml.

All calculations in connection with equations (1) and (2) were carried out on the Mir-1 computer.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the ζ potential of the red cells and platelets of the coronary venous blood was statistically significantly reduced after ligation of the coronary artery ($P < 0.01$), whereas in blood from the femoral vein it remained at its former value, reflecting the local character of changes in the Z potential of the blood cells flowing from the zone of ischemia. The diffusion potential of whole blood taken from the zone of ischemia 30 min after ligation of the coronary artery also was reduced ($P < 0.05$) but, on the other hand, the diffusion potential of blood from the femoral vein was increased (Table 1).

The results show that after ligation of the coronary artery there was a very considerable decrease in EER in blood samples taken from the zone of ischemia and the distance between the cells was changed, whereas in blood samples taken from the femoral vein no such changes were observed (Table 2).

Allowing for H_0, EER was calculated by equation (1). EER of red blood cells in samples taken from a branch of the great cardiac vein before ligation of the coronary artery, allowing for the potential step in the double electric layer, characterizing its thickness [15], was 0.5 ± 0.02 cal, but allowing for the surface potential it was only 0.0024 ± 0.0001 cal. This indicates a definite role of the double electric layer of the blood cells in the manifestation of their aggregation and adhesion properties.

In our view, the results of these investigations shed some light on the intimate mechanisms of the disturbance of hemostasis when the blood flow in the myocardium is reduced.

Blood, being a heterogeneous system, must be regarded as a thixotropic, pseudoplastic, non-Newtonian fluid, in which the cells and platelets, in order to perform their specific function, must be dissociated and must lie at a certain distance from each other. Disturbance of this dissociation leads to intravascular aggregation and adhesion of the cells (the initial phase of thrombosis) [4]. The main factors determining dissociation of the blood cells are the electrostatic forces of repulsion or, in Chizhevskii's words [12], the "electrostatic spacer" between the cells. Determination of the size of this "spacer" is of great importance for the evaluation of the aggregating and adhesive properties of the blood cells, which are enhanced in many pathological states, including myocardial infarction [1, 8, 9, 14, 15]. Enhancement of the aggregating and adhesive properties of the blood cells is based on a decrease in the value of EER, so that sludging of the red cells is facilitated, and this may lead to the development of the state known as "red shock" [6].

The results of these experiments thus provide a closer understanding of the biophysical nature of the disturbances of hemostasis in ischemic heart diseases.

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ROLE OF LIPID PEROXIDATION AND α-TOCOPHEROL IN CONDUCTIVITY OF ARTIFICIAL MEMBRANES MADE FROM LIVER PHOSPHOLIPIDS OF RATS WITH BURNS

M. I. Agadzhanov, S. A. Badzhinyan, and V. G. Mkhitaryan

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The state of membrane permeability for potassium and calcium ions was studied in bilayer phospholipid membranes (BPM) from the liver of rats with burns. A sharp increase in the conductivity of BPM was found under these circumstances. This process was accompanied by an increase in lipid peroxidation. Administration of α-tocopherol (1 mg/kg body weight) restored these indices to normal. Model experiments with methyl oleate and cumyl hydroperoxide confirm the peroxide mechanism of injury to membrane formations.

KEY WORDS: burns; conductivity of membranes; lipid peroxides; α-tocopherol.

Although burns are serious conditions, there is as yet no general agreement regarding the pathogenesis of the disturbances they cause [4]. Considerable attention has been paid to the neurogenic theory [5, 7]; in recent years the theory of an autoimmune mechanism of the pathological changes in burns has been researched intensively [10].

Burns cause serious disturbances in the whole of the body, which are based on a disturbance of homeostasis. Displacement of sodium, water, and protein from the blood stream, hypovolemia, an increase in the blood concentrations of metabolic and breakdown products, hypoxemia, tissue hypoxia, and other disturbances are observed. All these disturbances may be largely attributed to pathology of membranes, for which there is much indirect evidence [8, 9].

It was shown previously that in various stress states there is intensification of free-radical processes [3], accompanied by elevation of the level of lipid peroxidation. Burn trauma is known to be a powerful stressor, leading to a considerable increase in the lipid peroxide level [6]. This increase, in turn, increases the permeability of cell membranes [2].

The object of this investigation was to study permeability of membranes in burns at the molecular level in model experiments.

Department of Biochemistry, Erevan Medical Institute. Department of Biophysics, Central Research Laboratory, Erevan Postgraduate Medical Institute, Ministry of Health of the USSR.
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