Biological systems always contain several different proteoglycans [5, 12]. Probably, therefore, during aggregation and adhesion of cells the action of one of them, bound relatively specifically with the outer surface of the cell membrane, is reinforced by the nonspecific stereochemical (displacing) effect of other proteoglycans contained in the given system, as revealed by model systems and tissue cultures [2, 11]. The interactions noted above may be of essential importance in processes of morphogenesis, when sharp changes take place in the absolute content of the proteoglycans and also in the relations between the quantities of these biopolymers and glycoproteins [1, 5, 11, 12].

LITERATURE CITED

1. S. M. Bychkov, Vopr. Med. Khim., No. 6, 726 (1981).
2. S. M. Bychkov, Vopr. Med. Khim., No. 6, 2 (1983).
3. S. M. Bychkov, V. N. Bogatov, and S. A. Kuz'mina, Byull. Éksp. Biol. Med., No. 12, 680 (1981).
4. S. M. Bychkov, V. N. Bogatov, and S. A. Kuz'mina, Byull. Éksp. Biol. Med., No. 11, 52 (1982).
5. S. M. Bychkov and M. M. Zakharova, Vopr. Med. Khimii, No. 3, 227 (1979).
6. S. M. Bychkov and S. A. Kuz'mina, Byull. Éksp. Biol. Med., No. 3, 284 (1977).
7. S. M. Bychkov and S. A. Kuz'mina, Byull. Éksp. Biol. Med., No. 11, 562 (1977).
8. S. M. Bychkov and S. A. Kuz'mina, Byull. Éksp. Biol. Med., No. 6, 58 (1983).
9. J. F. F. Walker, Formaldehyde [Russian translation], Moscow (1957).
10. W. T. Butter, J. Immunol., 90, 663 (1963).
11. L. A. Culp, B. J. Rollins, J. Buntel, et al., J. Cell. Biol., 79, 788 (1978).
12. J. E. Morris, Exp. Cell. Res., 120, 141 (1979).

CHANGES IN PASSIVE ELECTRICAL PROPERTIES OF ERYTHROCYTES DURING HEMOPERFUSION

F. Pliquett, V. I. Sergienko,
Z. Wunderlich, and V. E. Kagan

UDC 615.38.015.2:615.246.2].015.4:
612.111.014.423

KEY WORDS: hemoperfusion; erythrocytes; electrical properties of membranes; fatty acids.

The widespread use of hemoperfusion in clinical practice has necessitated the development of rapid methods of monitoring the state of the blood cells actually during hemoperfusion changes take place in the chemical composition and physical characteristics of the plasma membranes of the blood cells [1, 3]. One such method that may be suggested is to measure the passive electrical properties of blood cells and blood plasma, which depend on the functional state of the cells and tissues and may be drastically changed during the development of various pathological states (aging, malignant transformation, low temperature injury, and so on) [7-9].

The aim of this investigation was to study the electrical characteristics of erythrocytes (total impedance, capacitance and conductance of the membrane, conductance of the intra- and extracellular medium) under hemoperfusion conditions on intact rabbits *in vivo* and on erythrocytes from normal human blood donors *in vitro*.

EXPERIMENTAL METHOD

Hemoperfusion was performed on intact rabbits with an anteriovenous circuit, using columns with a capacity of 50 cm^3, packed with synthetic SKN-2K activated charcoal (Medical

Institute of Biophysics, Karl Marx University, Leipzig, East Germany. N. I. Pirogov Second Moscow Medical Institute. Biological Faculty, M. V. Lomonosov Moscow University.

(Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 10, pp. 414-416, October, 1984. Original article submitted August 22, 1983.

0007-4888/84/9810-1334$08.50

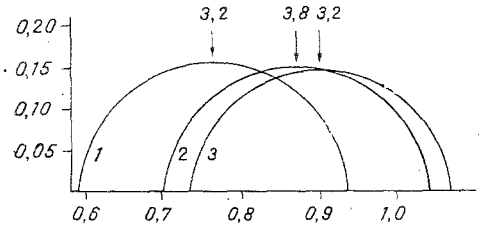

Fig. 1. Dependence of virtual part of conductance on actual (Cole-Cole diagram) during hemoperfusion in rabbits. Abscissa, virtual conductance (in Ω^{-1}); ordinate, actual conductance (in Ω^{-1}). 1) Control, 2) 30 min of hemoperfusion, 3) 60 min of hemoperfusion. Arrows indicate peaks of semicircles and corresponding values of characteristic frequencies (in mHz).

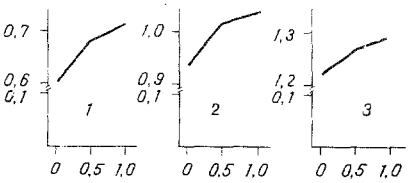

Fig. 2. Changes in macroscopic parameters of whole blood and plasma during hemoperfusion in rabbits. Abscissa, time (in h); ordinate conductance (in $\Omega^{-1} \cdot m^{-1}$). 1) Specific conductance $\varkappa$ of blood at low frequencies relative to f_0, 2) specific conductance $\varkappa$ of blood at high frequencies relative to f_0, 3) conductance $\varkappa$ of blood plasma.

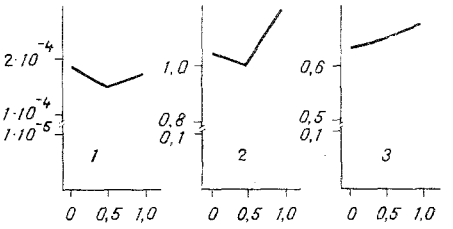

Fig. 3. Changes in electrical parameters of erythrocytes during hemoperfusion in rabbits. Abscissa, time (in h); ordinate, conductance (in $cm \cdot m^{-1}$), capacitance (in mF/cm^2). 1) Conductance $\varkappa$ of plasma membrane of erythrocytes, 2) capacitance C_s of plasma membrane of erythrocytes, 3) conductance $\varkappa$ of intracellular medium of erythrocytes.

Preparations Factory, Kiev). The rate of perfusion was 10 ml/min and the duration of hemoperfusion 60 min. The operation was performed under pentobarbital anesthesia (30 mg/kg, from Spofa, Czechoslovakia), and before hemoperfusion heparin (Weddell Pharmaceuticals Limited, England) was injected intravenously in a dose of 500 U/kg body weight.

Experiments *in vitro* were carried out on a suspension of erythrocytes obtained from human blood donors after resedimentation twice (3000g, 10 min) in 0.14 M NaCl, pH 7.4. The fatty acids — chromatographically pure oleic acid (from Serva, West Germany) and lauric acid (from Boehringer, West Germany) — were injected in alcoholic solution from a microsyringe, so that the final concentration of ethanol in the suspension did not exceed 2%. Hemoperfusion *in vitro* was carried out by passing the erythrocyte suspension (50 ml) through a column with SKN-2K activated charcoal.

The electrical parameters of the blood were measured in a specially constructed cylindrical constant temperaure cell (25°C), with gilded electrodes, possessing minimal parasitic capacitance (6×10^{-13} F) and inductance (9×10^{-9} H). Frequency dependences of complex conductance were measured on a BM-508 impedance meter (from Tesla, Czechoslovakia). The following parameters were determined from a "Cole-Cole" diagram (dependence of the virtual part of conductance on the actual part): the characteristic frequency (f_0), conductance at low frequencies relative to f_0 ($\varkappa_0$), and conductance at high frequencies relative to f_0 ($\varkappa_\infty$). The

TABLE 1. Passive Electrical Properties of Erythrocytes on Incubation with Free Fatty Acids and after Hemoperfusion

Experimental conditions	Specific conductance of cell membrane ($\varkappa_s$), $\Omega^{-1} \cdot m^{-1}$	Capacitance of cell membrane, C_s, mF/cm^2
Control	$1{,}1 \cdot 10^{-3} \pm 1 \cdot 10^{-3}$	$1{,}3 \cdot 10^{-2} \pm 0{,}1 \times \times 10^{-2}$
Oleic acid, 2.5 mg	$1{,}4 \cdot 10^{-3} \pm 0{,}1 \cdot 10^{-3}$	$1{,}4 \cdot 10^{-2} \pm 0{,}1 \cdot 10^{-2}$
2.5 mg Lauric acid,	$1{,}4 \cdot 10^{-3} \pm 0{,}2 \cdot 10^{-3}$	$1{,}6 \cdot 10^{-2} \pm 0{,}2 \cdot 10^{-2}$
2.5 mg Oleic acid (2.5 mg) + hemoperfusion	$8{,}1 \cdot 10^{-5} \pm 1{,}4 \cdot 10^{-5}$	$8{,}2 \cdot 10^{-3} \pm 1{,}1 \cdot 10^{-3}$

value of specific conductance of the intracellular medium ($\varkappa_i$), and of the specific conductance ($\varkappa_s$) and the capacitance (C_s) of the cell membrane were calculated from the experimental data by the following equations:

$$\varkappa_i = \frac{2\varkappa_a(1-P) - \varkappa_\infty(P+2)}{\frac{\varkappa_\infty}{\varkappa_a}(1-P) - 2P - 1}, \tag{1}$$

$$\varkappa_S = \frac{d}{r} \cdot \frac{1 - \frac{\varkappa_0}{\varkappa_a} \cdot \frac{1+\frac{1}{2}P}{1-P}}{\frac{\varkappa_0}{\varkappa_a} \cdot \frac{1+\frac{1}{2}P}{1-P}\left(\frac{1}{\varkappa_i} + \frac{1-P}{1+2P} \cdot \frac{1}{2\varkappa_a}\right) - \left(\frac{1}{\varkappa_i} + \frac{1+2P}{1-P} \cdot \frac{1}{2\varkappa_a}\right)}, \tag{2}$$

$$C_S = \frac{1/f_0}{r}\left(\frac{\varkappa_i \cdot \varkappa_a(2+P)}{\varkappa_a(2+P) + \varkappa_0(1-P)} + \frac{r\varkappa_s}{d}\right), \tag{3}$$

where d denotes the thickness of the membrane (10^{-8} m); r the effective radius of the erythrocytes (3.5×10^{-6} m); $\varkappa_a$ the conductance of the external medium; P the bulk concentration of the cells.

Values of $\varkappa_a$ were determined for supernatants obtained after sedimentation of the erythrocytes (3000g, 10 min) in each series of experiments.

EXPERIMENTAL RESULTS

A "Cole-Cole" diagram for whole blood during hemoperfusion in rabbits in shown in Fig. 1. Measurements were made during hemoperfusion for 60 min. It will be clear from Fig. 1 that hemoperfusion led to changes in the electrical parameters of the erythrocytes, manifested as a shift of the curve to the right. Average results of measurement of electrical parameters of erythrocytes and plasma in the course of hemoperfusion are given in Fig. 2. The data showed that the hemofusion procedure leads to the following changes in the macroscopic parameters of whole blood which were measured; an increase in specific conductance ($\varkappa_0$, $\varkappa_\infty$) and an increase in conductance of the blood plasma ($\varkappa_a$) relative to the characteristic frequency (f_0). On the basis of the experimental data conductance ($\varkappa_s$) and capacitance (C_s) of the plasma membrane of the erythrocytes and also the conductance of the intracellular medium of the erythrocytes ($\varkappa_i$) were calculated. It will be clear from Fig. 3 that hemoperfusion led to a decrease in conductance of their intracellular medium. These results are evidence of stabilization (an increase in specific resistance) of the erythrocyte membrane during hemoperfusion. The method used thus not only enables the effectiveness of the hemoperfusion procedure to be monitored, but its membrane effects to be studied also.

Since we know that the appearance of free fatty acids in the blood is a characteristic sign of the development of many pathological states (stress, hypoxia, ischemia, and so on) [5, 6], the action of fatty acids on the acid electrical properties of erythrocytes and the possibility of correcting changes which arose by means of hemoperfusion were studied in a separate series of experiments. As will be clear from Table 1, oleic and lauric acids, built into the plasma membrane of the erythrocytes, induce an increase in capacitance and conductance

of the membrane, but have virtually no effect on intracellular conductance. Perfusion of erythrocytes "loaded" with fatty acids through a column containing the sorbent leads to opposite changes in their electrical characteristics: a sharp decrease in conductance and capacity of the membrane, much below the control levels obtained for intact erythrocytes. It can be postulated that not only the added fatty acids are removed from the plasma membranes of erythrocytes during hemoperfusion, but also membrane destabilizers present in them initially (hydrolysis products of phospholipids, peroxidation products of polyenic fatty acids, etc.) [2, 4].

It can thus be concluded from these results that the method of recording passive electrical properties of blood is a highly sensitive and informative means of monitoring the effectiveness of hemoperfusion. Further investigations will demonstrate to what extent the method can be used to select optimal conditions of hemoperfusion for different pathological states.

LITERATURE CITED

1. V. S. Efimov, V. I. Sergienko, M. Ya. Rozkin, et al., in: Current Problems in Hemoperfusion [in Russian], Moscow (1981), pp. 72-76.
2. V. E. Kagan, Yu. V. Arkhipenko, V. A. Pisarev, et al., in: Biophysics of Membranes [in Russian], Moscow (1981), pp. 88-95.
3. Yu. M. Lopukin and M. N. Molodenkov, Hemoperfusion [in Russian], Moscow (1978).
4. A. M. Katz and F. C. Messineo, Circ. Res., 48, 1 (1981).
5. A. M. Katz, H. Dunnett, D. Repke, et al., FEBS Lett., 67, 207 (1976).
6. F. Z. Meerson, V. E. Kagan, Yu. P. Kozlov, et al., Basic Res. Cardiol., 77, 465 (1982).
7. F. Pliquett, M. Haferkorn, and A. Weiss, Z. Alternforsch., 33, 523 (1978).
8. F. Pliquett, Dtsch. Gesund.-Wes., 35, 853 (1980).
9. F. Wiese, F. Pliquett, H. Wegher, et al., Z. Alternforsch., 32, 127 (1977).

REACTION OF NONENZYMIC FIBRINOLYSIS TO INTRAVENOUS INJECTION OF SMALL DOSES OF SALMONELLA ENDOTOXIN INTO RABBITS

D. T. Esartiya and V. E. Pastrova

UDC 612.118-221.3-06:615.919-579.824.14

KEY WORDS: salmonella endotoxin; nonenzymic fibrinolytic activity of blood; heparin.

Acute intestinal infections are accompanied by endotoxemia, acute renal failure (ARF), and changes in hemostasis and the water-electrolyte balance.

The response of the blood clotting system to intravenous injection of endotoxins into animals has been studied in fair detail. One of the main symptoms reflecting the character of the effect of endotoxin on hemostasis is a state of hypercoagulation which arises when the endotoxin circulates in the blood stream, and if the toxin is injected continuously, intravenously over a period of several hours characteristic features of disseminated intravascular clotting (DIVC) develop [6, 10].

The main trigger mechanism leading to temporary thrombin formation in the circulating blood in response to intravenous injection of endotoxin may originate from different sources. For instance, according to some workers blood cells and, in particular, leukocytes of many species of animals, on contact with endotoxin, become the source of procoagulant activity. Platelets stimulated by endotoxin are one source of both procoagulant and proaggregating activity [8]. Experiments *in vitro* have demonstrated the cytotoxic action of granulocytes treated with endotoxin on a culture of endothelial cells [9]. Injury to the endothelium by endotoxin, mediated through granulocytes, is another factor in the stimulation of hypercoagulation and thrombin formation when endotoxin enters the blood stream.

Laboratory of Physiology and Biochemistry of Blood Clotting, Biological Faculty, M. V. Lomonosov Moscow University.

(Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 10, pp. 416-418, October, 1984. Original article submitted April 28, 1983.

0007-4888/84/9810-1337$08.50