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RHEOLOGIC PROPERTIES OF RED CELL SUSPENSIONS FROM PATIENTS WITH ACUTE MYOCARDIAL INFARCTION WITHIN THE PHYSIOLOGICAL TEMPERATURE RANGE

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KEY WORDS: rheology; viscosity; phase transition; myocardial infarction.

Investigations of structural changes in erythrocyte membranes under the influence of temperature within the range 34-43°C have shown that a structural phase transition takes place in them [4, 11, 14, 15]. The viscosity of suspensions of red cells and their "ghosts" varies as a nonlinear (stepwise) function of temperature between 34 and 42°C and pH 5.5 to 8.5 under normal conditions in the region of phase transition. Inflections lie between temperatures of 37 and 40°C and pH between 7.3 and 7.5 [12]. Consequently, the nonlinear character of the change in membrane viscosity depending on temperature or pH means that phase transitions in membranes, just as in liquid crystal polymers [9], can be identified sufficiently definitely.

We used viscosity as the parameter with which to investigate red cell membranes from patients with acute myocardial infarction, during its course. Special attention was paid to the presence of inflections on the ηT curves, for they can be recorded by a technique developed by one of us (V.G.K.).

EXPERIMENTAL METHOD

Altogether 75 patients with acute myocardial infarction (40 during treatment and 35 in the acute period) and also 20 healthy subjects aged 20-80 years were investigated. Blood (0.3-0.5 ml) was taken from a vein or finger three or four times during treatment (1st, 9th, and 30th days). The red cells were washed free from plasma with phosphate buffer containing 0.103 M Na_2HPO_4 and 0.155 M NaH_2PO_4, pH 7.4 [10], without the addition of complex-forming compounds, by centrifuging three times at 2-3°C. The speed of centrifugation was 1500-2000 rev/15 min. A viscosimeter with capillary tube 0.54 mm in section, 100 mm long, and with the height of the column of liquid Δh = 20 mm above the capillary, was inserted into a centrifuge tube containing the suspension in a volume of V = 0.2-0.3 ml (Fig. 1). The shear velocity was 2.5 sec^{-1}. The tube was immersed in a type UT-15 ultrathermostat or a waterbath to the extent of three-quarters of its volume. The viscosity of the red cell suspension was measured between 34 and 42°C at intervals of 1°C from below upward. The incubation time of the suspen-

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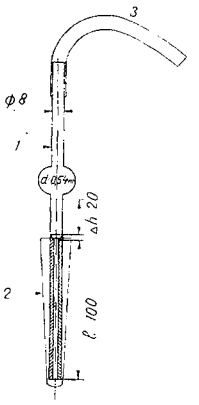

Fig. 1. Construction of viscosimeter. 1) Viscosimeter; 2) tube for red cell suspensions; 3) rubber tube (Δh indicates height of suspension above capillary, l the length of the capillary).

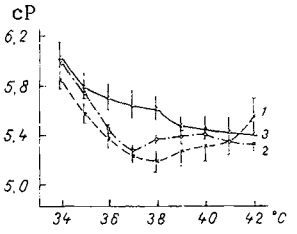

Fig. 2

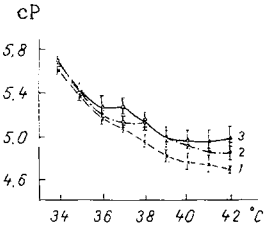

Fig. 3

Fig. 2. Averaged dependence of viscosity of red cell suspensions on temperature for patients with acute myocardial infarction (complicated form). Here and in Fig. 3: 1) 1st day of myocardial infarction, 2) 9th day, 3) 30th day of myocardial infarction.

Fig. 3. Averaged dependence of viscosity of red cell suspensions on temperature for patients with acute myocardial infarction (uncomplicated form).

sion at each temperature point was 8 min. The flow time of the suspension was measured with a stopwatch with an accuracy of 0.1 sec. The initial viscosity of the red cell suspensions at 34°C must be of the order of 6 cP (30 sec within the range Δh = 20 mm), H = 50-55%. Viscosity was calculated by the equation in [7]:

$$\eta = \eta_0 \frac{t_x \rho_x}{t_0 \rho_0},$$

where η_0 and η are the viscosity of the buffer and suspension, respectively (in cP, $\eta_0 \approx 1$ cP throughout the temperature range 34-42°C); t_0 and t_x the flow time of the buffer and suspension (in sec). The ratio between the densities gives a correction for viscosity in the second decimal place, so that the value of ρ and ρ_x can be disregarded, for it is sufficient to measure viscosity with an accuracy of one decimal place. The ultrathermostat maintains temperature with an accuracy of ±0.2°C. The rleative error of measurement of viscosity was 0.4-0.5%.

EXPERIMENTAL RESULTS

Dependence of viscosity on temperature $\eta(T)$ of the red cell suspensions from patients with acute myocardial infarction in a complicated form is shown in Fig. 2, on the 1st, 9th, and 30th days. Curve 1 has an inflection in the 39-40°C region, which is indistinct and ill defined, and is parabolic in shape as a result of an increase in viscosity in the region 40-42°C.

On curve 2 (9th day) the inflection is clearly defined and lies within the temperature range 38-40°C. Curve 2 differs from curve 1 by the shift of the point of inflection and also by its shape; the curve ceases to be V-shaped between 34 and 42°C.

TABLE 1. Statistically Analyzed Results

Statistical parameter	T, °C								
	34	35	36	37	38	39	40	41	42
Standard deviation, S	0,33	0,23	0,21	0,16	0,29	0,33	0,42	0,32	0,43(1)
	0,12	0,14	0,17	0,30	0,24	0,29	0,38	0,49	0,45(2)
	0,29	0,34	0,30	0,34	0,32	0,27	0,28	0,21	0,35(3)
Normalized deviation, t	1,77	2,0	2,21	1,86	1,61	2,39	2,20	1,74	1,68(1)
	2,1	2,06	2,01	1,49	2,05	1,41	1,51	1,83	1,93(2)
	1,83	1,77	2,01	2,01	1,87	1,48	1,87	2,68	1,98(3)
P	0,92	0,95	0,97	0,92	0,89	0,98	0,97	0,92	0,91(1)
	0,96	0,96	0,96	0,86	0,96	0,84	0,87	0,93	0,95(2)
	0,93	0,92	0,96	0,96	0,94	0,86	0,94	0,99	0,95(3)

TABLE 2. Statistically Analyzed Results

Statistical parameter	T, °C								
	34	35	36	37	38	39	40	41	42
Standard deviation, S	0,08	0,08	0,13	0,12	0,13	0,16	0,20	0,19	0,08(1)
	0,08	0,08	0,09	0,12	0,11	0,13	0,17	0,24	0,25(2)
	0,14	0,27	0,26	0,25	0,31	0,38	0,31	0,31	0,29(3)
Normalized deviation, t	1,85	2,25	1,59	1,97	2,16	2,14	2,1	2,13	1,90(1)
	2,02	1,86	1,40	1,90	2,69	2,24	1,87	1,54	1,67(2)
	1,91	1,60	2,08	1,80	2,07	2,24	2,08	1,81	1,84(3)
P	0,94	0,98	0,89	0,95	0,97	0,97	0,96	0,97	0,94(1)
	0,96	0,94	0,84	0,94	0,99	0,97	0,94	0,88	0,90(2)
	0,94	0,89	0,96	0,93	0,96	0,98	0,96	0,93	0,93(3)

Curve 3 has an inflection at 38°C and the V-shape has disappeared. The curves were plotted from data obtained as a result of investigation of seven patients.

The relationship $\eta(T)$ for patients with acute myocardial infarction in an uncomplicated form is shown in Fig. 3. In curve 1 the inflection in the 37°C region is very weak and viscosity decreases sharply with a rise of temperature. In curve 2 (9th day) an inflection appears in the 38°C region and is relatively clearly visible. On the 30th day of treatment the inflection is still present but has shifted to 37°C (curve 3). Characteristically in patients of both groups the viscosity of the red cell suspensions increased during the patients' recovery over the whole range of temperature (34-42°C).

Graphs of $\eta(T)$ of normal subjects aged 20-80 years (10 aged 20 years and 10 aged 70-80 years) are given in Figs. 4 and 5. All curves have inflections in the 37-40°C region. The inflection of the $\eta(T)$ curves in young individuals was more clearly defined and the points of inflection of the $\eta(T)$ curves for suspensions of whole erythrocytes and their "ghosts" coincide (Fig. 4).

Statistical analysis of the results showed that the empirical series of distribution obeyed the normal law. Statistically analyzed results obtained by a study of patients with acute myocardial infarction, in complicated and uncomplicated forms, respectively, are given in Tables 1 and 2.

Comparison of graphs showing dependence of viscosity of the red cell suspensions on temperature with data obtained previously by optical methods (Raman and IR spectroscopy, interferometry, EPR spectroscopy, and fluorescence [4, 11, 14, 15]), and also by methods of surface tension and calorimetry [12], indicates that inflections on the $\eta(T)$ curves reflect a phase transition in the erythrocyte membranes [12].

The phase transition is due to a stepwise change in anisotropy (orderliness) of the membranes [5]. On the $\eta(T)$ curves in the 37-40°C interval for patients with myocardial infarction in the acute stage no phase transition was present or, if it was it was very indistinct. Moving on to the subacute stage (after 7-8 days) the transition either appeared or became correspondingly more acute. The change in shape of the inflection on the $\eta(T)$ curve continued also on the 30th day of treatment.

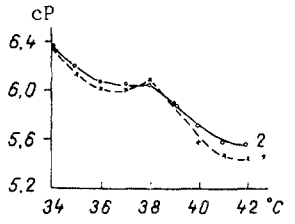

Fig. 4

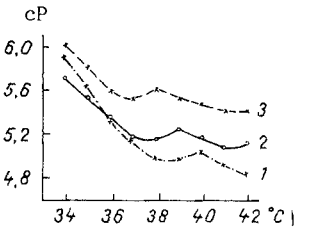

Fig. 5

Fig. 4. Dependence of viscosity of suspensions of red cells (1) and their "ghosts" (2) on temperature of normal subjects aged 20 years.

Fig. 5. Dependence of viscosity of red cell suspensions on temperature for normal subjects aged 70-80 years.

The increase in viscosity in the 40-42°C region in patients with complicated acute myocardial infarction (Fig. 2, 1) was due to hypercoagulation of the red cells, for a further rise in temperature to 46-48°C was accompanied by a sharp rise in viscosity, and this process was irreversible. Normal human erythrocytes are resistant under these conditions. No such sharp increase in viscosity is observed with them. The phenomenon of hypercoagulation is probably connected with a reduction in the ζ-potential [2].

It can be postulated on the basis of analysis of the data that the phase transition in the physiological temperature range (37-40°C) is a biological necessity for normal functioning of the membranes [4, 11, 13-15].

Disappearance, loss of definition, or shift of the phase transition in myocardial infarction point to considerable structural changes in the red cell membrane. In particular, the considerable increase in anisotropy of the membranes, especially with the participation of stabilizing agents, may lead to disappearance of the phase transition, for such a system is less capable of fluctuations [5]. An increase in anisotropy also leads to a reduction in deformability of the cells, characteristic of patients with infarction [6]. Another fact to be noted is that a change in anisotropy of the membranes may modify the thromboplastin activity of the red cells [1].

The absence or loss of definition of the phase transition may also be the result of elevation of the cholesterol level in the membranes. A shift of the transition temperature (T_k), which may be observed both in normal individuals and in patients with myocardial infarction in the course of treatment, is often connected with a change in nature of the polar groups, the length of the carbon chains, the ratio of saturated and unsaturated lipids and phospholipids, *cis-trans* isomerism, and so on [13]. Special investigations are necessary to give a more exact answer.

Changes in anisotropy and, as a result, changes in viscosity, deformation, aggregative resistance, and thromboplastin activity of red cell membranes and red cells are factors affecting the microcirculation of blood. Together with a change in the microcirculation, there is also a disturbance of function of the red cell membranes. In particular, their ATPase activity is altered [8]. A change in hemolytic and osmotic resistance is possible, and also a change in transport functions, as is observed at temperatures of 18-19°C [3].

The $\eta(T)$ curves of red cell suspensions from patients with myocardial infarction in the acute period thus have no inflection or only a very weak one in the 36-40°C region, and in our opinion this is evidence of the disappearance, indistinctness, or shift of the phase transition compared with normal.

In the subacute period an inflection appears on the $\eta(T)$ curves, evidence of the appearance of a phase transition in the 37-40°C region.

Changes in the viscosity of suspensions of red cells or their "ghosts" in the region of phase transition can be used, in the writers' view, for the prognosis of myocardial infarction and determination of the stage in the course of its development.

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EFFECT OF ULTRASOUND ON ADHESIVENESS OF *Escherichia coli*

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KEY WORDS: *Escherichia coli*; adhesion; ultrasound; pili.

Ultrasound has been widely used recently to destroy bacterial cells and to isolate intracellular components and various antigens [5]. However, despite many investigations into the action of ultrasound on microorganisms, the problem of how low-frequency ultrasound, not causing destruction of bacteria, as reflected in the activity of the bacterial cell, has not been adequately discussed in the recent literature. Since low-frequency ultrasound is being used more and more frequently [2, 3] in the treatment of some pathological processes accompanied by bacterial invasion (peritonitis, suppurative wounds), it is important to discover whether such treatment affects the virulence of bacteria.

The object of this investigation was to study one of the factors of virulence of *Escherichia coli* responsible for its adhesiveness during treatment with low-frequency ultrasound of low amplitude, such as is used at the present time to sterilize the peritoneal cavity of patients with suppurative peritonitis in the Department of Clinical Surgery, I. M. Sechenov First Moscow Medical Institute.

EXPERIMENTAL METHOD

The effect of ultrasound on adhesiveness of bacteria was studied with particular reference to *E. coli*, one of the most common pathogens causing suppuration in peritonitis [4].

Strain *E. coli* 815 was obtained from the L. A. Tarasevich State Research Institute for Standardization and Control of Medical Biological Preparations and specially selected for the presence of type I pili. A 24-h culture of bacteria was diluted to a concentration of

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