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EFFECT OF LOW TEMPERATURE ON MEMBRANE PERMEABILITY OF RED BLOOD CELLS RECONSTITUTED IN MEDIA OF VARIED IONIC COMPOSITION

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The role of physicochemical changes in the medium in the mechanisms of low temperature injury to cells, the most important manifestation of which is a disturbance of their permeability [9], has now been studied sufficiently completely [4]. Meanwhile the importance of the structural and functional state of the plasma membranes still remains largely unexplained although existing information [1, 8] suggests that it is this factor which largely determines the optimal program for freezing the cells and the choice of cryoprotective agents. For these reasons it was decided to study the effect of low temperatures on permeability of red blood cells whose membranes were modified by means of various agents. In the investigation described below the action of mineral cations (Na^+, K^+, Ca^{++}, Mg^{++}) was studied; as a result of adsorption on the membrane these cations significantly alter its permeability for water and ions and also its mechanical properties [2, 7].

EXPERIMENTAL METHOD

A convenient model with which to study this problem is reconstituted red blood cells, for the ionic composition on both sides of their plasma membrane can be varied within wide limits [6]. Reconstituted red blood cells were obtained from human donors' blood kept for 2-5 days, as described in [3], with some modifications to the composition of the lytic and reconstituting media, which are indicated in the appropriate captions to the figures. The red blood cells were quickly frozen in reconstituting medium by immersing polyethylene ampuls, each containing 0.5 ml of the sample, in liquid nitrogen. Thawing was carried out on a waterbath at 37°C. The rate of freezing was 200-400°C/min.

Membrane permeability was assessed by the ability of the reconstituted red blood cells to retain hemoglobin, K^+ and Na^+ ions, and ^{14}C-sucrose (Czechoslovakia, specific activity 340 mCi/mmole). The hemoglobin concentration was determined after centrifugation in the residue and supernatant by a spectrophotometric method [5]. The K^+ and Na^+ concentration in the residue of the reconstituted red blood cells was determined by flame photometry [5].

^{14}C-sucrose was added to the lytic solution in a dose of 1 μCi/ml. After reconstitution, sucrose not incorporated into the red cells was washed out by centrifugation in reconstituting solution under the same conditions as the hemoglobin. Radioactivity of the labeled sucrose was determined in the acid-soluble fraction of the residue and supernatant of the reconstituted red cells after freezing and thawing. The samples were counted on an SL-40 scintillation counter. ZhS-7 fluid (1 liter dioxane, 5 g PPO, 100 g naphthalene) was used as the scintillator. The specific volume of the reconstituted red blood cells was determined on a microhematocrit centrifuge (Adams Readacrit).

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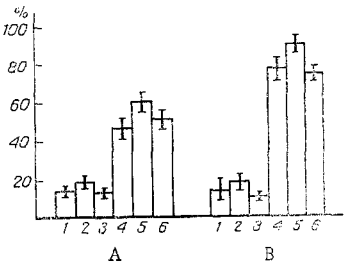

Fig. 1. Hemoglobin concentration and specific cell volume of red blood cells reconstituted in different ionic media. Lytic medium contained: 1, 4) 10 mM Tris-HCl, pH 7.4; 2, 5) 10 mM Tris-HCl (pH 7.4) and 4 mM $MgCl_2$; 3, 6) 10 mM Tris-HCl (pH 7.4), 4 mM $MgCl_2$, and 50 μM $CaCl_2$. In addition to 10 mM Tris-HCl buffer (pH 7.4), reconstituting medium contained: 1) 150 mM KCl; 2) 150 mM KCl and 4 mM $MgCl_2$; 3) 150 mM KCl, 4 mM $MgCl_2$, and 50 μM $CaCl_2$; 4) 150 mM NaCl; 5) 150 mM NaCl and 4 mM $MgCl_2$; 6) 150 mM NaCl, 4 mM $MgCl_2$, and 50 μM $CaCl_2$. A) Hemoglobin concentration, B) specific cell volume. Total hemoglobin concentration in residue and supernatant of sample taken as 100%.

TABLE 1. Hemoglobin Concentration and Specific Cell Volume of Red Cells Reconstituted in Reconstituting Medium with Different Na^+ and K^+ Concentrations, after Rapid Freezing and Thawing (M ± m)

Concentration of NaCl and KCl during reconstitution and freezing–thawing, mM		Hemoglobin concentration in residue, %	Specific cell volume, %
NaCl	KCl		
150	—	63,6±2,9	92,5±6,4
125	25	68,2±2,3	94,4±9,7
100	50	66,6±4,2	76,5±3,0
75	75	58,3±3,0	78,8±5,3
50	100	34,4±3,7	34,5±1,6
25	125	21,6±3,8	22,0±4,8
—	150	14,6±3,0	11,1±0,6

EXPERIMENTAL RESULTS

The investigations showed (Fig. 1a) that red blood cells reconstituted and frozen in the presence of KCl, after thawing lose 85.6% of the hemoglobin contained inside them. The presence of Mg^{++} ions, in a physiological concentration, in the reconstituting medium instead of K^+ ions caused a very small increase in the hemoglobin concentration in the residue after freezing and thawing, whereas addition of Ca^{++} ions reduced its concentration. Replacement of the K^+ ion by Na^+ in the reconstituting medium had a significant effect on the preservation of the initial impermeability of the membranes of the reconstituted red blood cells for hemoglobin. In this case, after thawing 46.9% of the hemoglobin (relative to the control) remained in the samples. Addition of Mg^{++} ions to Na^+-reconstituting medium significantly increased the hemoglobin concentration in the residue, whereas Ca^{++} ions had the opposite action in this case also (Fig. 1A).

K^+ and Ca^{++} ions thus endow reconstituted red blood cells with cryolabile properties, whereas Na^+ and Mg^{++} make them cryoresistant. Comparison of the results (Fig. 1A, B) shows that the disturbance of permeability for hemoglobin correlates on the whole with the reduction in specific cell volume. This suggests that an important role in the mechanism of injury is played by permeability of the red cell membrane for water at the freezing–thawing stages.

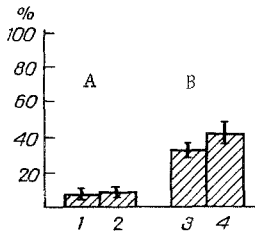

Fig. 2

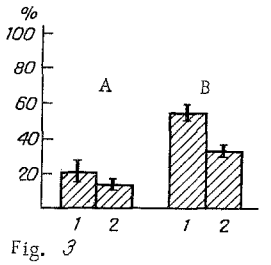

Fig. 3

Fig. 2. Concentrations of ^{14}C-sucrose, K^+, and Na^+ in reconstituted red blood cells after rapid freezing and thawing. Lytic medium contained: 10 mM Tris-HCl (pH 7.4) and 4 mM $MgCl_2$. Reconstituting medium contained: A) 150 mM KCl, 4 mM $MgCl_2$, 10 mM Tris-HCl (pH 7.4); B) 150 mM NaCl, 4 mM $MgCl_2$, and 10 mM Tris-HCl (pH 7.4). Concentrations of: 1) K^+, 2) ^{14}C-sucrose, 3) Na^+, 4) ^{14}C-sucrose. Total concentration of labeled sucrose in residue and supernatant of sample and concentration of cations in control taken as 100%. Control samples of K^+ and Na^+- reconstituted red blood cells contained 112.5 ± 6.9 millimoles K^+ and 112.2 ± 10.9 millimoles Na^+ per liter intracellular water. Total radioactivity of residue and supernatant of sample of K^+- and Na^+-reconstituted red cells was 59,034 and 53,104 cpm respectively.

Fig. 3. Content of hemoglobin and ^{14}C-sucrose in reconstituted red blood cells subjected to rapid freezing and thawing, after replacement of external medium. Lytic medium contained 10 mM Tris-HCl (pH 7.4) and 4 mM $MgCl_2$. A) Reconstituting medium contained 10 mM Tris-HCl (pH 7.4), 4 mM $MgCl_2$, and 150 mM KCl. External medium during freezing and thawing contained 10 mM Tris-HCl (pH 7.4), 4 mM $MgCl_2$, and 150 mM NaCl. B) Reconstituting medium contained 10 mM Tris-HCl (pH 7.4), 4 mM $MgCl_2$, and 150 mM NaCl. External medium during freezing and thawing contained 10 mM Tris-HCl (pH 7.4), 4 mM $MgCl_2$, and 150 mM KCl. Concentration of: 1) hemoglobin, 2) ^{14}C-sucrose. Total concentration of hemoglobin and ^{14}C-sucrose in residue and supernatant taken as 100%.

It will be clear from Fig. 2A that after freezing and thawing the K^+-reconstituted red cells almost completely lost the ^{14}C-sucrose and K^+ contained in them, whereas Na^+-reconstituted red cells retained 31.8% and 41.2% respectively of their initial content of ^{14}C-sucrose and Na^+. The fact will be noted that losses of labeled sucrose and ions (Fig. 2) were much greater than the loss of hemoglobin (Fig. 1). This is evidence that red cell membranes after freezing and thawing possess defects of different sizes.

Investigation of the action of rapid freezing and thawing on the escape of hemoglobin from red blood cells reconstituted in isotonic solutions containing NaCl and KCl in different proportions (Table 1) showed that high cryoresistance is observed even when the reconstituting solution contains both Na^+ and K^+ ions added in the ratio of 1:1. The results are evidence against strict specificity of the action of Na^+ and K^+ ions on the properties of the plasma membrane. Characteristically in this case also disturbance of permeability for hemoglobin correlates with a reduction in the specific cell volume.

In the next experiments the escape of hemoglobin and ^{14}C-sucrose from Na^+-reconstituted red blood cells was studied during freezing and thawing in Na^+-reconstituting medium, endowing the membrane, with which it was in contact on both sides, as was pointed out above, with cryolabile properties. K^+-reconstituted red cells, on the other hand, were subjected to freezing and thawing in Na^+-reconstituting medium. It was found that loss of hemoglobin and ^{14}C-sucrose was on a much smaller scale if the internal medium contained Na^+ (Fig. 3).

The manifestation of cryoresistance by Na^+-reconstituted red cells is thus connected to a greater degree with changes in the inner surface of the plasma membrane.

It can be tentatively suggested that disturbance of the permeability of red cell membranes after rapid freezing and thawing is largely dependent on the initial state of the plasma membrane.

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TYROSINE HYDROXYLASE ACTIVITY IN THE BRAIN OF RATS WITH DIFFERENT LEVELS OF INITIAL ALCOHOL MOTIVATION

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KEY WORDS: alcohol; alcohol motivation; tyrosine hydroxylase; rat hypothalamus.

Investigations have shown that animals of the same population differ in their attitude to alcohol [2, 3]. The activating effect of alcohol on the structure of positive reinforcement at the hypothalamic level has been shown to be related to a high level of alcohol intake in a certain number of animals [1]. In other animals, by contrast, absence of any activating effect of alcohol on the structure of positive reinforcement correlated with a low level of alcohol consumption. The development of a liking for alcohol in rats can thus be taken to depend on its effect on the structure of positive reinforcement.

Considering that the chief neurochemical substrate of the structures of positive reinforcement is the catecholaminergic system of the brain [11-13], in the investigation described below tyrosine hydroxylase (TH) activity in the hypothalamus was studied in rats with different levels of initial alcohol motivation.

EXPERIMENTAL METHOD

To determine the initial level of alcohol motivation in rats without prolonged contact with this substance, a method based on the difference in the rate of alcohol metabolism in rats with high and low initial levels of alcohol motivation was used. Animals with a mean duration of sleep of 80 min (predisposed to taking alcohol) and 180 min (rejecting alcohol) were selected for the experiments after intraperitoneal injection of a 25% solution of ethanol in a dose of 4.5 g/kg body weight.

Noninbred male albino rats weighing 200-250 g were used.

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