these experiments is evidently due to its greater vulnerability to trypsin compared with protein from the intact muscle, and it is a particularly important discovery because of the increased proteinase activity in SR of the affected muscle.

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# BIOCHEMICAL PARAMETERS OF ERYTHROCYTES PROTECTED FOR CRYOPRESERVATION BY 1,2-PROPANEDIOL AND GLYCEROL


M. M. Loevskii, A. M. Vorotilin, A. K. Gulevskii, and A. M. Belous



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KEY WORDS: erythrocytes; preservation; glycerol; 1,2-propanediol.


During long-term low-temperature conservation of blood cells [1, 2, 11, 13, 14] protective media containing glycerol are used most often. However, the need to remove the glycerol from the cells before transfusion limits the use of this method on a wide scale, and this has led to the search for less laborious and more efficient ways of preservation. The method of low-temperature preservation of erythrocytes, protected by 1,2-propanediol (1,2-PD), developed at the Institute for Problems in Cryobiology and Cryomedicine, Academy of Sciences of the Ukrainian SSR [3], is interesting in this respect. It allows considerable simplification of the procedure of removal of the cryoprotector from the cells before use after preservation.

This paper gives comparative results of a study of the time course of the concentrations of ATP, 2,3-diphosphoglycerate (2,3-DPG), and also $Na^+$ and $K^+$ ions in erythrocytes after keeping for 5 days at 4°C in suspending media 8b and 8c of the Central Research Institute of Hematology and Blood Transfusion [6] after freezing under protection of 1,2-PD and glycerol. These biochemical parameters determine the structural integrity of the erythrocytes and the degree to which they perform their oxygen-transport function in the recipient's blood stream [7, 15].

## EXPERIMENTAL METHOD

Blood from donors preserved at 4°C for not more than 24 h after collection was used. Low-temperature preservation of blood under protection by 1,2-PD and glycerol, including freezing to −196°C, warming on a water bath, and removal of the cryoprotectors, was carried out in accordance with the procedures described [3, 6]. The blood, after removal of the preservatives, was resuspended in media 8b and 8c. After storing for 0, 12, 36, and 120 h at 2-4°C the ATP [8] and 2,3-DPG levels in the cells were determined. The intracellular concentration of $Na^+$ and $K^+$ cations was determined at the same periods of storage by flame photometry. To remove extracellular $Na^+$ and $K^+$ the blood was washed three times at 4°C with a solution containing 30 mM Tris-HCl, pH 7.4, and 90 mM $MgCl_2$ [9].


Institute for Problems in Cryobiology and Cryomedicine, Academy of Sciences of the Ukrainian SSR, Khar'kov.


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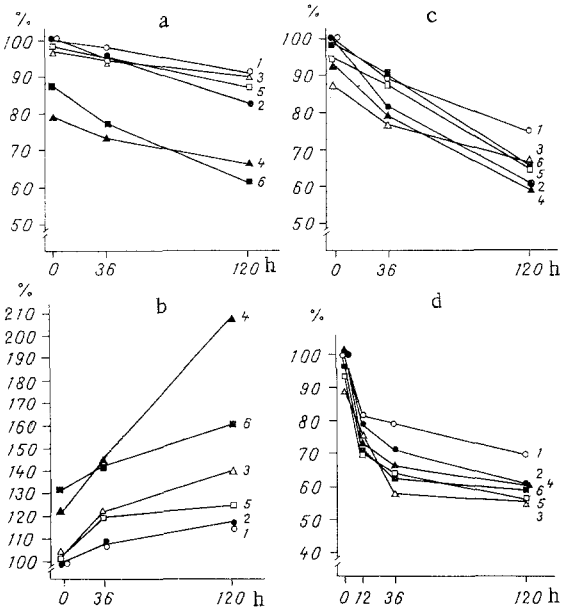


Fig. 1. Time course of intracellular $K^+$ (a), $Na^+$ (b), ATP (c), and 2,3-DPG (d) concentrations after freezing of erythrocytes with 1,2-PD and glycerol and keeping for 5 days in media 8c and 8b (Research Institute of Hematology and Blood Transfusion) at 4°C. Control level at 0 points, corresponding to 103.4 ± 13.4 meq/liter blood (a) and 10.67 ± 0.78 meq/liter blood (b), and also 5.41 ± 0.38 μmoles/g Hb (c) and 12.2 ± 1.3 μmoles/g Hb (d), taken as 100. 1, 2) Control in media 8c and 8b respectively; 3, 4) after freezing with glycerol in media 8c and 8b respectively; 5, 6) after freezing with 1,2-PD in media 8c and 8b respectively.

## EXPERIMENTAL RESULTS

It is generally accepted that loss of $K^+$ by cells and entry of $Na^+$ into them after low-temperature preservation are one of the reliable criteria of the presence of latent injuries to the plasma membrane [2, 14]. It will be clear from Fig. 1a that immediately after thawing and removal of the cryoprotectors, the outflow of $K^+$ from erythrocytes suspended in medium 8c was significantly less than from those suspended in medium 8b. This rule also applied during later observations, i.e., after keeping at 4°C for 36 and 120 h. When medium 8b was used, immediately after thawing and removal of the cryoprotector and intracellular $K^+$ concentration was significantly higher in the case of cells frozen with protection by 1,2-PD ($P < 0.05$) than in those frozen with glycerol. After keeping for 36 h, this difference was no longer significant ($P > 0.05$), and after keeping for 120 h, a rather better result was obtained by analysis of blood samples cryopreserved under glycerol protection, although the difference likewise was not significant.

A similar picture also was observed when the inflow of $Na^+$ into the cells was studied. It will be clear from Fig. 1b that the permeability of erythrocytes frozen with glycerol for $Na^+$ was far higher, at the same times of keeping, in the case when the cells after preservation were suspended in medium 8b. When the cells were transferred into medium 8c the $Na^+$ concentration in cells preserved with glycerol and 1,2-PD was significantly higher ($P < 0.05$) than the control, starting from 36 h of keeping. On the 5th day of keeping the use of 1,2-PG enabled a lower $Na^+$ level to be maintained in the cells than with glycerol, as was shown both when the washed erythrocytes were transferred to medium 8c and when medium 8b was used. The results are evidence that 1,2-PD like glycerol, although it gives a cryoprotective effect, cannot completely prevent damage to the plasma membrane of the erythrocytes during cryopreservation. Further improvement and selection of resuspending media are therefore necessary in order to prevent the development of latent injuries to the cell membrane after rewarming from the frozen state.

The fall in the ATP level during hypothermic preservation of previously preserved erythrocytes (Fig. 1c) can evidently be explained by the high permeability of the erythrocyte membrane

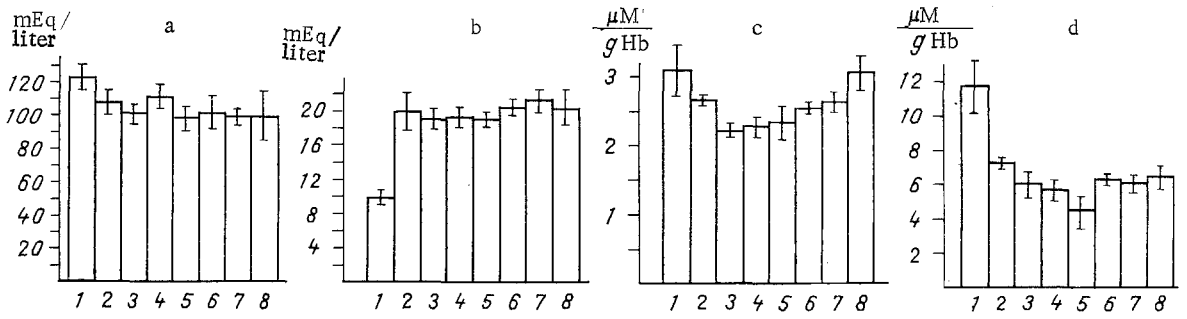


Fig. 2. Effect of residual concentration of cryoprotectors and of hypothermic preservation on intracellular concentrations of $K^+$ (a), $Na^+$ (b), ATP (c), and 2, 3-DPG (d) in erythrocytes. 1) Control in medium 8c (0 h); 2) control in medium 8c (120 h); 3) final concentration of glycerol 0.5% in medium 8c; 4) final concentration of glycerol 1.0% in medium 8c; 5) final concentration of glycerol 1.5% in medium 8c; 6) final concentration of 1,2-PD 0.5% in medium 8c; 7) final concentration of 1,2-PD 1.0% in medium 8c; 8) final concentration of 1,2-PD 1.5% in medium 8c.

for $Na^+$ and $K^+$ cations, which stimulates $K^+$,$Na^+$-ATPase [4]. The relatively high ATP concentration (especially at the early stages of keeping) in blood specimens cryopreserved under 1,2-PD protection will be noted. This was probably due to involvement of the 1,2-PD remaining behind after rinsing in cell metabolism, for this compound is known to be converted into monohydroxyacetone phosphate, to take part in the glycolytic cycle [10], and subsequently to act as a source of ATP. The more marked fall in the ATP level during the first 36 h of keeping of cells restored after preservation by freezing with glycerol compared with cells frozen with 1,2-PD is evidence of different mechanisms of involvement of residual quantities of these penetrating cryoprotectors in erythrocyte metabolism, and it confirms to some degree data [12] showing the reversible inhibitory action of glycerol on the ATP level.

Investigation of the time course of the 2,3-DPG concentration (Fig. 1d), just as in previous experiments, revealed higher values in the control samples suspended in medium 8c. This became particularly noticeable when the keeping time was increased. In experimental samples suspended after rewarming both in medium 8b and in medium 8c, the 2,3-DPG concentration also was lower at all times of keeping then in control samples suspended in medium 8c. This difference was less marked at early times of keeping.

The results show that residual quantities of glycerol and 1,2-PD evidently become involved in the metabolism of the cells restored after preservation. To make a penetrating study of this problem, in the next experiments the effect of increasing concentrations of glycerol and 1,2-PD on the parameters determined above was investigated immediately after rewarming and after keeping for 120 h under hypothermic conditions.

It will be clear from Fig. 2a that the $K^+$ level fell steadily to this time [2, 14], whereas the $Na^+$ level rose a little (Fig. 2b), but glycerol and 1,2-PD in the concentrations tested caused no significant change in the concentrations of these cations compared with the control.

In the next series of experiments the effect of 0.5 and 1.5% concentrations of cryoprotectors on the ATP and 2,3-DPG concentrations was studied during hypothermic keeping of the cells for 120 h. As will be clear from Fig. 2c, the ATP concentration toward the end of the chosen keeping time was significantly reduced, in good agreement with data in the literature [2, 14]. The presence of glycerol in the incubation medium, in all the concentrations studied, led to a statistically signficant decrease in this parameter. Meanwhile 1,2-PD, in concentrations of 0.5 and 1%, had no significant effect on the ATP level in erythrocytes restored after preservation, whereas in the presence of 1.5% 1,2-PD the ATP concentration was significantly higher than in the control. A similar picture was found during the study of the 2,3-DPG level. The experiments showed that an increase in the glycerol concentration in the keeping medium had an increasingly strong inhibitory action on the 2,3-DP concentration in the erythrocytes, whereas 1,2-PD did not affect 2,3-DPG metabolism in any concentration tested (Fig. 2d).

Blood preserved under protection by 1,2-PD is thus comparable in quality with blood preserved under protection by glycerol, and with regard to some parameters it may actually be

rather better than the latter. Medium 8c can be used as resuspending medium for blood preserved at a low temperature under protection by 1,2-PD. The most favorable times for transfusion are the first 24 h after rewarming of the frozen erythrocyte suspension. Glycerol and 1,2-PD in residual amounts, had no significant effect on $Na^+$ and $K^+$ metabolism in the course of hypothermic keeping for 5 days, whereas 1,2-PD has a more favorable action on ATP and 2,3-DPG metabolism than glycerol.

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# LACTATE DEHYDROGENASE ISOZYME SPECTRUM OF FAST AND SLOW MUSCLES DURING DISTURBANCES OF INNERVATION


R. A. Mavrina, Kh. Sh. Khamitov, and N. P. Rezvyakov



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KEY WORDS: skeletal muscle; neurotrophic control; colchicine.


The problem of neurotrophic control of skeletal muscle has attracted many experimental investigations [3, 8, 11]. The factors involved in its realization, of which the most important are acknowledged to be the flow of impulses along the nerve and the necessity for substances synthesized in the perikarya of the motoneurons and transported along axons to muscle fibers to be present [2], have been discussed. It has been shown that denervation, causing cessation of spike activity, and application of colchicine, blocking axoplasmic transport, are manifested as denervation-like changes in muscles. Studies of development of skeletal muscles have shown that early myogenesis takes place in the absence of nervous influences, but later differentation would be impossible without interaction between muscle tubules and motoneurons [10].

The question whether blockade of axoplasmic transport affects the differentiation of skeletal muscles still remains unstudied. The writers have investigated the effect of application of colchicine to the nerve and of denervation on differentiation of fast and slow muscles.

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Department of Normal Physiology and Department of Histology, Kazan' Medical Institute.


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