12. S. E. Khokhlov, Arkh. Patol., No. 4, 36 (1980).
13. A. Buchner and L. Sreebny, Oral Surg., 34, 209 (1972).
14. A. R. Hand, Am. J. Anat., 135, 71 (1972).
15. Hosoi Kazuo, J. Biochem., 82, 351 (1977).
16. Kim Sun Kee, J. Dent. Res., 60, 738 (1981).
17. G. Krekeler, J. Eschler, and G. Ochs, Dtsch. Zahnärztl. Z., 24, 943 (1969).
18. M. Kumegawa and T. Tacuma, Am. J. Anat., 149, 111 (1977).
19. G. Seifert and K. Donath, Beitr. Pathol., 159, 1 (1976).
20. J. Scott, Arch. Oral Biol., 22, 221 (1977).

TIME COURSE OF CONTENT OF CATIONS AND ORGANOPHOSPHORUS COMPOUNDS IN ERYTHROCYTES AFTER LOW-TEMPERATURE PRESERVATION (—196°C) WITH 1,2-PROPANEDIOL AND GLYCEROL

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For long-term low-temperature preservation of blood cells in the USSR [1, 2] and elsewhere [8, 10] protective media containing glycerol are most frequently used. However, the need to remove the glycerol from the cells before they are transfused places limitations on the use of this method, and for that reason the search is in progress for less laborious and more effective methods of preservation. In this respect a method of low-temperature preservation of erythrocytes under protection of 1, 2-propanediol (1,2-PD), developed at the Institute for Problems in Cryobiology and Cryomedicine, Academy of Sciences of the Ukrainian SSR, which essentially simplifies the procedure of removal of the cryoprotector from the preserved cells before use, is interesting.

This paper gives the results of a comparative study of the time course of the concentrations of ATP, 2,3-disphosphoglycerate (2,3-DPG), and also of Na^+ and K^+ ions in erythrocytes after preservation for five days at 4°C, in suspending media 8b and 8c (Central Research Institute of Hematology and Blood Transfusion) after low-temperature preservation with 1,2-PD are described in this paper. It can be postulated that this protection is due to the fact that the 1,2-PD remaining after washing takes part in cell metabolism, for we know that this compound, which is converted into monohydroxyacetophosphate, can take part in the glycolytic cycle [7] and act later as a source of ATP. The greater fall in the ATP level during the first 36 h of storage of depreserved cells frozen with glycerol, compared with cells frozen with 1,2-PD, indicates that the residual amounts of these penetrating cryoprotectors are involved in erythrocyte metabolism by different mechanisms, and to some extent they confirm data [9] showing that the inhibitory action of glycerol on the ATP level is reversible.

Investigation of the time course of the 2,3-DPG content (Fig. 1D), just as in previous experiments, revealed higher values in control samples suspended in media 8c. This became particularly noticeable with an increase in the length of keeping. In experimental samples suspended after heating both in medium 8b and in medium 8c, the 2,3-DPG content also was lower after all periods of keeping than in control samples suspended in medium 8c. This difference was smaller at the earlier times of keeping.

Judging from the time course of the biochemical parameters, blood preserved with 1,2-PD is comparable in quality with that preserved with glycerol, and in the early periods of

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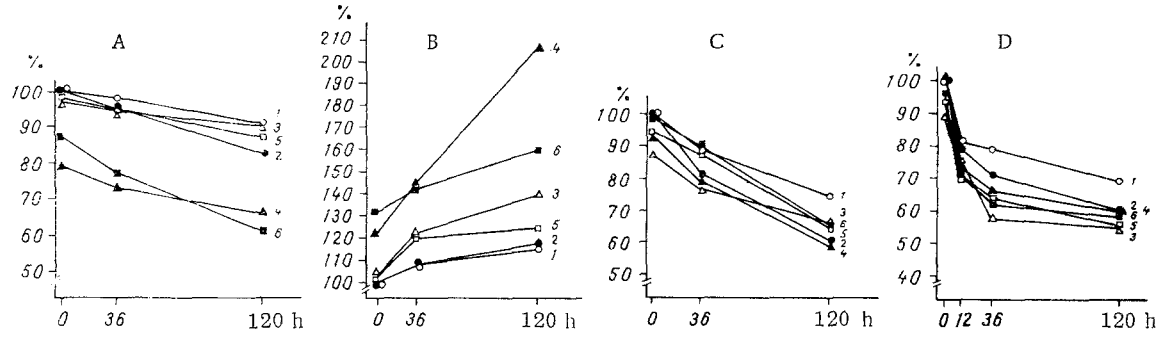

Fig. 1. Time course of content of intracellular K^+ (A), Na^+ (B), ATP (C), and 2,3-DPG (D) after freezing of erythrocytes with 1,2-PD and with glycerol and keeping for five days in media 8c and 8b at 4°C. A) Control level at point 0, corresponding to 103.4 ± 13.4 meq/liter of blood, taken as 100%; B) control level at point 0, corresponding to 10.67 ± 0.78 meq/liter of blood taken as 100%; C) control level at point 0, corresponding to 5.41 ± 0.38 μmoles/g Hb, taken as 100%; D) control level at point 0, corresponding to 12.2 ± 1.3 μmoles/g Hb taken as 100%. 1,2) control in medium 8c and 8b respectively; 3,4) after freezing with glycerol in medium 8c and 8b respectively; 5,6) after freezing with 1,2-PD in medium 8c and 8b respectively.

keeping at 4°C, it is a little better. Medium 8c can be used as resuspending medium for blood subjected to low-temperature preservation with 1,2-PD. The best times for transfusion are the first 24 h after warming the frozen erythrocyte suspension, although the difference is not significant ($P < 0.05$), but after 120 h of keeping a somewhat better result is obtained by analysis of blood samples subjected to low-temperature preservation with glycerol, although again the difference is not significant.

A similar picture also was observed when the inflow of Na^+ into the cells was studied. The permeability of erthrocytes frozen with glycerol for Na^+ at the same times of keeping was much higher when the depreserved cells were suspended in medium 8b (Fig. 1B). When the cells were transferred into medium 8c the Na^+ content in the cells preserved with glycerol and 1,2-PD was significantly higher ($P < 0.05$) than the control, starting from the 36th hour of keeping. On the 5th day of keeping with 1,2-PD, a lower Na^+ level could be maintained in the cells than with glycerol, and this was seen when the washed erythrocytes were resuspended both in medium 8c and in medium 8b. These results are evidence that 1,2-PD, like glycerol, although they exert a cryoprotective effect, cannot completely prevent damage to the plasma membrane of erythrocytes during low-temperature preservation. Accordingly further improvement and selection of resuspending media are necessary in order to prevent the development of latent injuries in the cell membrane after rewarming from the frozen state.

The fall in the ATP level during hypothermic storage of depreserved erythrocytes (Fig. 1C) can evidently be explained by the high permeability of the erythrocyte membrane for Na^+ and K^+ cations, which stimulates K^+, Na^+-ATPase [3]. The relatively high content of ATP (especially in the early times of keeping) in blood samples frozen with 1,2-PD and glycerol will be noted.

EXPERIMENTAL METHOD

Donor's blood stored at 4°C for not more than 24 h after removal, was used in the experiments. Low-temperature preservation of the blood with 1,2-PD and glycerol, including freezing to −196°C, heating on a water bath, and removal of the cryoprotectors, were carried out in accordance with the instructions described. The depreserved blood was resuspended in media 8b and 8c. After storage for 0, 12, 36, and 120 h at 2-4°C the ATP [6] and 2,3-DPG [4] levels in the cells were determined, and the intracellular concentrations of Na^+ and K^+ cations were estimated at the same periods of storage by flame photometry. These biochemical parameters are known to determine the structural integrity of erythrocytes and the oxygen-transporting function which they perform in the recipient's blood stream [5, 12]. 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 $MgCl_2$.

EXPERIMENTAL RESULTS

It is generally considered that loss of K^+ by cells and an inflow of Na^+ into them after low-temperature preservation are a reliable criterion of the presence of latent injuries to the plasma membrane [11]. As Fig. 1A shows, immediately after thawing and removal of the cryoprotectors, the outflow of K^+ from erythrocytes suspended in medium 8c was significantly less than from cells suspended in medium 8b. This pattern also was found in later observations, i.e., after keeping for 36 and 120 h at 4°C. When medium 8b was used, immediately after depreservation of the cells the intracellular K^+ concentration was significantly higher if they were frozen with 1,2-PD than if frozen with glycerol ($P < 0.05$). After keeping for 36 h, this difference [ceases to be significant].

LITERATURE CITED

1. F. R. Vinograd-Finkel', F. G. Ginzburg, L. I. Fedorova, et al., Probl. Gematol., No. 9, 3 (1973).
2. F. R. Vinograd-Finkel', L. I. Fedorova, S. N. Kudryashova, et al., Probl. Gematol., No. 9, 3 (1975).
3. V. K. Lishko, The Sodium Pump of Biological Membranes [in Russian], Kiev (1977).
4. N. P. Meshkova and N. V. Aleksakhina, Usp. Biol. Khimii, No. 2, 285 (1954).
5. R. Benesch and R. E. Benesch, Biochem. Biophys. Res. Commun., 26, 162 (1967).
6. E. Beutler, Red Cell Metabolism. A Manual of Biochemical Methods, New York (1975).
7. E. Huff and H. Rudney, J. Biol. Chem., 234, 1060 (1959).
8. H. T. Meryman and M. Hornblower, Transfusion, 17, 438 (1977).
9. G. Moroff and H. T. Meryman, Vox Sang., 36, 244 (1979).
10. C. R. Valeri and A. H. Runck, Bibl. Haematol. (Basel), 38, 249 (1971).
11. C. R. Valeri, Blood Banking and the Use of Frozen Blood Products, Cleveland (1976).
12. D. J. Valtis and A. C. Kennedy, Lancet, 266, 119 (1954).