ROLE OF TRANSMEMBRANE POTENTIAL IN DISTURBANCE OF THE BARRIER PROPERTIES OF ERYTHROCYTE MEMBRANES DURING CRYOPRESERVATION

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In the course of cryopreservation cells are placed under extremely unfavorable external environment conditions [4]. This happens first at the stage of equilibration with the cryoprotective media, which contain, as their main component, cryoprotectors, which are diphilic compounds capable of modifying the structure and surface properties of biomembranes substantially as a result both of changes in the properties of the solution [5] and of direct binding with the membrane [5, 6]. In the case of nonpenetrating cryoprotectors (sucrose, polyvinylpyrrolidone, PÉO-1500, etc.) to maintain osmotic equilibrium the cryoprotective media do not contain salts or contain them only in exceedingly small amounts. To judge from the available data [3, 8], this may lead to the development of a diffusion transmembrane potential (TMP), and even of electrical failure of the membranes. Subsequent freezing worsens the dielectric properties of the biomembranes, which facilitates the development of the above-mentioned process even more.

In this investigation, to optimize conditions of cryopreservation, the possibility of development of a diffusion TMP in cryoprotective media prepared on the basis of nonpenetrating cryoprotectors, and the creation of conditions preventing this phenomenon were studied.

EXPERIMENTAL METHOD

Experiments were carried out on erythrocytes from fresh (stored 1-3 days) donors' blood. Sucrose and PÉO-1500 were used as cryoprotectors. Erythrocytes were washed by centrifugation 3 times in physiological saline to remove blood plasma. The pH of the medium was carefully maintained at 7.4 during this procedure. The packed cells (0.2 ml) were transferred to a cuvette with continuously mixed medium containing 10 ml of a mixture of unbuffered solutions of 0.5 M sucrose or 0.13 M PÉO-1500, pH 7.4, containing different quantities of NaCl. The kinetics of the change in pH of the medium was recorded by an ESL-41G-04 electrode on a KSP-4 automatic writer [3]. The dynamics of the outflow of intracellular K^+ into the external medium was investigated in these same blood samples by flame photometry, as described in [1]. The erythrocytes were rapidly frozen by immersing ampuls containg 1.5-2 ml of blood in liquid nitrogen. The rate of freezing under these circumstances was 200-400° C/min. The cells were thawed on a water bath at 37°C.

EXPERIMENTAL RESULTS

When the erythrocytes were transferred into solutions of nonpenetrating cryoprotectors of closely similar osmolarity (0.5 M sucrose or 0.13 M PÉO-1500 [2]) with a reduced NaCl concentration, acidification took place (Fig. 1). The value of the initial change in pH (Δf), i.e., the change in TMP [7], was proportional, as might be expected, to log C_0/C, where C_0 = 150 mM NaCl, and C is the NaCl concentration in the given sample. When the critical value (60-70 mV) was exceeded, acidification was replaced by alkalification. It has been suggested that this is connected with electrical failure of the membranes [8].

Comparison of the degree of acidification of the medium and the corresponding TMP in solutions of cryoprotectors shows that values of these parameters were lower in PÉO-1500. According to data in the literature [2] this is due to a change in the electrical properties of the membranes during binding of cryoprotector molecules.

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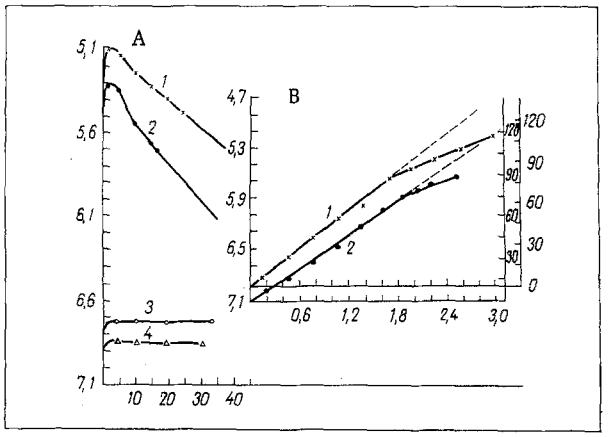

Fig. 1. Changes in pH of cryoprotective medium on addition of erythrocytes to it, depending on NaCl concentration. Abscissa: A) time (in min), B) log C_i/C_o; ordinate: A) pH, B) pH (on left) and Δf (on right). A: 1) 0.5 M sucrose + 0.14 nM NaCl; 2) 0.13 M PÉO-1500 + 0.28 mM NaCl; 3) 0.5 M sucrose + 0.15 M NaCl; 4) 0.13 M PÉO-1500 + 0.15 M NaCl; B: 1) 0.5 M sucrose; 2) 0.13 M PÉO-1500.

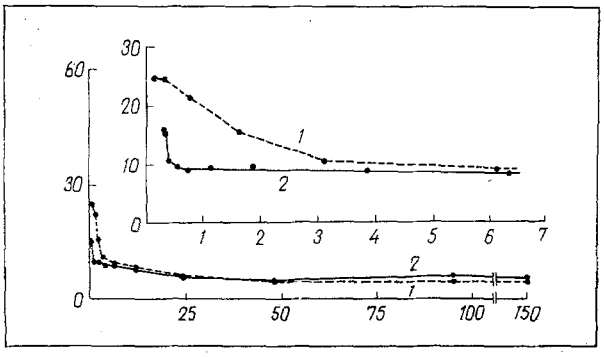

Fig. 2. Outflow of intracellular K^+ into medium of nonpenetrating cryoprotector, containing different NaCl concentrations. Abscissa, NaCl concentration (in mM); ordinate, K^+ concentration (in mg-eq/liter). 1) 0.5 M sucrose, 2) 0.13 M PÉO-1500.

It was shown previously [8] that one manifestation of disturbance of the barrier function of membranes when TMP reaches a critical level is an increase in the outflow of intracellular K^+. Under the experimental conditons used a decrease in the NaCl concentration below 3 mM in solutions of 0.13 M PÉO-1500 and, by a greater degree, in 0.5 M sucrose also caused an increased outflow of K^+ from erythrocytes into medium (Fig. 2). The effect of the NaCl concentration in the cryoprotective medium on preservation of the erythrocytes after freezing and thawing was studied next. Examination of the data in Fig. 3 shows that in the region of high NaCl concentration (48-150 mM) sucrose had a more marked cryoprotective effect, but if the salt concentration was low, better results were observed in solutions of PÉO-1500, which is in good agreement with the value of TMP recorded during equilibration (Fig. 3).

The results as a whole are thus evidence that a considerable TMP may develop in solutions of nonpenetrating cryoprotectors if the NaCl concentration is below 40-50 mM, and this leads to a disturbance of the barrier properties of the biomembranes and to an increase in sensitivity of the cells to freezing and thawing.

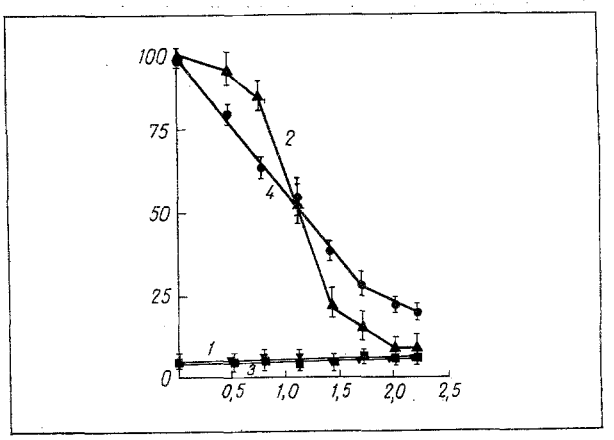

Fig. 3. Hemolysis of erythrocytes during freezing and thawing in medium of nonpenetrating cryoprotector, containing different NaCl concentrations. Abscissa, log of NaCl concentration (in mM); ordinate, hemolysis of erythrocytes (in per cent). 1) Equilibration with 0.5 M sucrose; 2) equilibration with 0.5 M sucrose + freezing and thawing; 3) equilibration with 0.13 M PÉO-1500; 4) equilibration + freezing and thawing with 0.13 M PÉO-1500.

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