

### Гіпотермічне зберігання еритроцитів барана

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### Hypothermic Storage of Ovine Erythrocytes

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Erythrocytes undergo numerous morphological and biochemical changes, reducing their deformability and viability, that leads to hemolysis during hypothermic storage. Extracellular hemoglobin is thought to be the cause of pulmonary vasoconstriction that occurs in sheep and humans when the preserved erythrocytes are transfused [Muenster, 2016]. Ovine red blood cells have been widely used for transfusion in veterinary medicine [Sousa, 2014; Kumar, 2017], by researchers in immunological studies [McAllister, 2020] as well as for transfusion modeling [Simonova, 2014; Fung, 2016]. Sheep erythrocytes are known to be one of the smallest among mammalian erythrocytes, they do not aggregate and deform as readily as erythrocytes of other species [Adili, 2014]. They have a high sensitivity to changes in osmolarity and pH and low mechanical stability [Jikuya, 1998].

The aim of this study was to compare the resistance of ovine and human erythrocytes to a prolonged hypothermic storage.

Ovine and human erythrocytes were washed three times by centrifugation at 800g for 10 min in a 10× volume of isotonic NaCl solution containing 0.01 M Tris buffer (pH 7.2). The Alsever's solution (20.5 g / l glucose, 8 g / l sodium citrate, 0.552 g / l citric acid, 4.2 g / l NaCl) and the mannitol medium (50 g / l mannitol, 0.9 g / l NaCl) were used. Antibiotic Cifran (0.01 mg / ml) was added to all cell suspensions, and they were stored in refrigerator at 2...4°C. The level of erythrocyte hemolysis was spectrophotometrically determined at a 543 nm wavelength. Osmotic erythrocyte fragility was determined by placing the cells in sodium chloride solutions with different tonicity (from 0 to 150 mm) and by measuring hemolysis. The erythrocyte distribution density by the sphericity index was determined by the low-angle light scattering [Gordiyenko, 2004].

Erythrocyte hemolysis was found to be significantly lower when stored in mannitol medium if compared with Olsver medium and not significantly different for human and ovine erythrocytes. When stored in the Alsever's solution for 6 and 12 weeks, the hemolysis of ovine erythrocytes was significantly lower than for human erythrocytes. The osmotic fragility of ovine erythrocytes is much higher than human erythrocytes, but changes much less during prolonged hypothermic storage. Hypothermic storage of human erythrocytes in Alsever's solution for 8 weeks leads to a shift in the peak density of distribution in the direction of lower sphericity indices and in mannitol medium the number of flattened forms of discocytes increases. Hypothermic storage of ovine erythrocytes results in a shift of distribution density curves to a range of higher indices.

### Фізико-хімічні властивості та кріозахисна дія розчинів полівінілового спирту, гліцерину та середовищ на основі їх комбінацій при заморожуванні еритроцитів

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### Physicochemical Properties and Cryoprotective Action of Solutions of Polyvinyl Alcohol, Glycerol and Media Based on Their Combinations During Freezing of Red Blood Cells

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The development of new effective cryoprotectants for cryopreservation of red blood cells is an important task of modern cryobiology. One of the directions to solve it is the development of cryopreservatives with their enhanced effect on the formation and growth of ice crystals, ice re-crystallization during heating to improve the cryopreservation of red blood cells.

In particular, a topical approach is to use synthetic compounds in the media, having the anti-recrystallization activity. Synthetic polymer, namely, the polyvinyl alcohol deserves a special attention in the list of these compounds.

The research material was erythroconcentrate obtained from donor blood of human group A (II) procured with the Glucir blood preservative at Kharkiv Regional Center of Blood Transfusion, and stored for a maximum of 48 hours at (4 ± 2)°C. Erythroconcentrate was obtained by centrifugation of preserved donor's blood at 1250 g for 25 min.

Glycerol concentrations from 20 to 40%, polyvinyl alcohol in different concentrations (m. m. of 9 and 31 kDa) were used as cryoprotectant. PVA was prepared by weight method on the base of 0.1 M FSB (pH 7.4), glycerol by 0.9% NaCl solution, and was expressed as a mass percentage (wt.%). Used after 24 hours at a temperature of 20°C. The retention of erythrocytes after freeze-heating was evaluated by hemolysis, osmotic fragility, hematocrit, free and total hemoglobin. The retention of erythrocytes after freeze-heating was evaluated by hemolysis, osmotic fragility, hematocrit, free and total hemoglobin.

Physicochemical properties have been studied to provide a glimpse of hydrophilic-hydrophobic interactions in cryobiological systems, as well as freezing and melting temperatures, the micellization of solutions of individual compounds and combined media. The combination of PVA polymer solutions with glycerol in media was established to change not only the studied parameters, but also the regularity of these changes. So, if in some solutions of PVA and glycerol, the rise in the concentration of compounds increases the dynamic viscosity, but decreases the surface tension, then in the combined media the values of all the studied parameters enhance.

Investigation of the cryoprotective effect of the media of different composition during freezing of erythrocytes showed a tendency to possible increase of the preservation rate of erythrocytes when using the combined media as compared to individual glycerol and PVA solutions.

The findings indicate the prospect of the studies to develop the combined media based on glycerol and PVA of 9 kDa and 31 kDa for freezing the human red blood cells.

