INVESTIGATION OF THE PROPERTIES OF THE GLITCEROL CRYOPROTECTANT ON BIOTECHNOLOGICAL OBJECTS

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Introduction. Widespread use of erythrocytes in modern human medicine and biotechnology makes the actual task of their long-term storage in conditions of low temperature cryopreservation (at -196°C).

Cycle of biotechnological process of low-temperature preservation biological material consists of several stages: the preparation of biological material to freezing (equilibration with cryoprotectant), freezing, heating, removal of cryoprotectants. Influence of cryo-damaging factors on the cells that act on the freezing stage, you can simulate using hypertonic shock, which consists in transferring cells to hypertensive one's medium at positive temperature values.

Recently, veterinary practice for the treatment of animals widely use hemotransfusion, so it is advisable to cryo-preservation of blood animals for the purpose of their long-term storage and the creation of donor stocks blood features of the composition of the plasma membrane of erythrocytes of various species mammals can determine the different cell response to the effect of cryoprotectants and others factors of cryopreservation. At present, attempts to cryoprotect animal red blood cells are single and crumpled, so the research carried out in this direction are relevant.

Aim. Investigation of the effect of cryoprotectant glycerol on stability biotechnological objects (for example, human red blood cells, bull and horse) in conditions of hypertonic shock.

Materials and methods. For the study, red blood cells were used, derived from human donor blood (Nomo sapiens), bull (Bos taurus), horse (Equus caballus), prepared on the hemoconstrictor "Gluciation". Output erythrocyte suspension was obtained by adding cell sieve to physiological solution in a ratio of 1:10.

Hypertonic shock of mammalian erythrocytes was carried out by transferring 50μl the initial cell suspension in 1.0 ml of NaCl solution (4.0 mol/l) at a temperature of 37 or 0°C, followed by incubation for 5 minutes. End hematocrit - 0.4%. Erythrocytes with glycerol were incubated for 2 minutes, after which they were exposed hypertonic shock at 0 or 37°C. The accuracy of the temperature measurement is ± 0.5°C.

The content of hemoglobin released into the supernatant was determined spectrophotometric method on a spectrophotometer SF-4A with running fluid a cuvette at a wavelength of 543 nm and expressed as a percentage relative to 100% hemolysis of red blood cells. For 100%, they took absorption of the sample into which Triton X-100 was added at a concentration of 0.1%.

Results and discussion. The simulation of one of the factors is carried out cryopreservation caused by freezing of free water in the process freezing of red blood cells, with hypertonic shock. The stability of mammalian erythrocytes to hypertonic shock is shown essentially depends on the type of mammal and temperature. At 37°C the most stable to the action of hypertonic shock (at a level of hemolysis in 4,0 mol/l NaCl) are erythrocytes horse, at 0°C - bull cells. For erythrocytes of all mammals level hypertonic hemolysis at 0 ° C lower than at 37°C, especially this temperature-dependent decrease in hemolytic damage is expressed for bulls cells.

It was found that glycerin reduces the level of hypertonic hemolysis erythrocytes of man at 37°C and 0°C, bulls at 37°C. Using glycerol with hypertonic shock of the red blood cells of the horse at both temperatures regimes turned out to be ineffective.

Conclusions. Based on the analysis of experimentally obtained results on the study of the influence of cryoprotectant glycerol on the stability of biotechnology objects (on an example of human red blood cells, bull and horse) with hypertonic action shock shows the high efficiency of this cryoprotectant in relation to erythrocytes of a person in contrast to animal cells.