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Стан еритроцитів при гіпертонічному та постгіпертонічному шоці

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The State of Erythrocytes During Hypertonic and Posthypertonic Shock

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The model experiments are used to study the impact of cryodamage factors to the erythrocytes. Hypertonic shock (HS) is applied to explore the effect of damage factors on cells, acting at the freezing stage, and a post-hypertonic shock (PHS) is used for those factors, the action of which is realized during thawing. The processes associated with transmembrane pore formation underlie the cell damage under exposure to temperature-osmotic shock, therefore the use of membrane-tropic substances is expedient.

The research aim was to study the development of hypertonic and posthypertonic hemolysis of human erythrocytes at 37 and 0°C in the presence of chlorpromazine (CPR) and trifluoperazine (TFP). Hypertonic shock was performed by transferring erythrocytes into 4.00 mol/L NaCl, while posthypertonic shock was initiated by transferring cells from dehydration medium (1.65 mol/L NaCl) to rehydration one (0.15 mol/L NaCl) at 37 and 0°C. The erythrocyte hemolysis rate was determined spectrophotometrically ($\lambda = 543$ nm).

The erythrocytes are damaged under HS and PHS conditions. The erythrocyte hemolysis rate depends on temperature, while at 0°C it was lower than at 37°C. The use of CPR and TFP results in a decreased rate of erythrocyte hypertonic hemolysis at 37 and 0°C, moreover an antihemolytic (AH) activity of both amphiphilic compounds is about 2 times higher at 37°C. In contrast to the above, the efficacy of amphiphiles under PHS of erythrocytes is observed at 0°C only. A comparative analysis of CPR and TFP efficiency showed higher AH activity of CPR (70%) compared to TFP (60%) under PHS of erythrocytes, and, conversely, under HS conditions (0°C) the values of AH activity for TFP (80%) exceeded similar indices of CPR (70%). But for both shock types, the effective concentrations of CPR are higher than those for TFP.

It was found that under HS of erythrocytes, the effective concentrations of amphiphiles were significantly lower as compared to the values obtained under PHS. The fact that a larger amount of substance is needed to implement a protective effect of amphiphiles under erythrocyte PHS may suggest a different nature of hemolytic pores formation when exposed to the factors specific to different stages of erythrocyte cryopreservation.

Since a protective effect of amphiphiles is realized under erythrocyte PHS at low temperature only, and under HS the compounds are more efficient at 37°C than at 0°C, the action mechanism of amphiphiles under HS may be assumed as associated with perturbation of erythrocyte membrane, while in case of PHS this can be due to its stabilization.

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Встановлення першорядних факторів для успішного біобанкінгу мідій

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Establishing Key Factors for Successful Mussel Biobanking

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The Mediterranean mussel (*Mytilus galloprovincialis*) is a species with an economic interest in Spain and worldwide. In order to improve cryopreservation, how novel cryoprotective agents (CPAs) affect egg and larval viability was tested, and how cryopreservation affects pre-selected families by large size (PB); second generation large mussels; or extreme temperature shock compared to environmental control (EC).

Embryos were incubated at 18ºC for 72h to obtain D-larvae. These larvae were cryopreserved using $MeSO_2$ 15% added in 1 step, incubated for 60 minutes and charged in 0.25 ml straws. For cryopreservation Heres *et al.* (2021) protocol was followed. D-larvae were transferred to 1.5 L plastic bottles, incubated for 2 weeks, and then moved to 10L boxes for settlement.

Toxicity tests were carried out by using from 0.5 up to 3M of N,N-dimetilformamide; methanol, and CryoStor®. CPAs were added in 1:1 ratio in 1 step and equilibrated for 15 minutes; gametes were washed out, transferred to filtered sea water, fertilized and incubated for 72h to see the percentage of D-larvae.

After 2 weeks post-thaw survival drops in all the preselected mussel families compared to the controls of every family. The best results were obtained with the EC (5.3%), but survival was significantly lower than the controls of the same family (20.8%). After 1 month, the best settlement percentages of the cryopreserved D-larvae were obtained in the PB. Toxicity tests have shown that when using 3M of N,N-dimetilformamide, the development to D-larvae reaches up to 75%. Concentrations of methanol greater than 1.5M cause a reduction of development from 70 to 25% at 3M. CryoStor® did not cause a big development decrease since 75% normal D-larvae was reached when using it in eggs prior to fertilization.

Mussel cryopreservation has been done successfully using D-larvae stages, but is affecting survival after 2 weeks post-thaw. Gamete quality also affects cryopreservation results, as we observed the best gamete quality on the EC and it had the highest survival 2 weeks post-thaw compared to the control. The use of these new cryoprotectants does not seem to greatly affect the development of D-larvae, but it remains to be seen how they behave in cryopreservation, if they are capable of protecting the cells and that they develop after thawing.