

## Вплив торпору на електрофоретичний спектр імуноглобулінів крові кажанів

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## Effect of Torpor on Electrophoretic Spectrum of Bat Blood Immunoglobulins

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During hibernation, the bats go into a torpor state, being a complex of adaptive mechanisms for survival. In this state, they demonstrate a suppressed metabolism, reduced circulating leukocytes, cytokine production and lymphocyte proliferation [Petit, 2018].

Biochemical studies in bat blood have some limitations. The most important among them is that most bat species are referred to the Endangered Species List. Therefore, according to the international conventions, they can not be excluded from natural conditions without a permit. We managed to solve this task by using the animals, wintered under artificial conditions at the Bat Rehabilitation Center of Feldman Ecopark. The studies were performed in noctule bats (*Nyctalus noctula*) [Schreber, 1774] within the following periods: before hibernation (September-October 2018), during hibernation (January-February 2019), and after spring arousal (March-April 2019). Plasma was isolated from heparinized blood with MicroV centrifuge (FicherScientific, Inc., USA) at 1000 G for 10 min. The spectrum of molecular weights of plasma proteins was determined by electrophoresis under denaturing conditions in a polyacrylamide gel. [Laemli, 1970]. Recombinant proteins (Sib Enzyme M35) with a mass within the range of 10–250 kDa served as the markers of electrophoretic mobility. The total protein concentration was determined by the Bradford protein assay with Kumasi G-250 dye [Bradford, 1976].

According to [Baker, 2018, Rižner, 2014], there were identified the IgA (fraction above 250 kDa), IgE (200 kDa fraction), IgD (180 kDa fraction) and IgG (120 kDa fraction). The redistribution of immunoglobulin fractions prior to torpor was as follows: IgA – 21.0% of all the immunoglobulin fractions, IgE – 47.2%, IgD – 7.7% and IgG – 24.0%, respectively. During torpor, the IgA fraction almost disappeared (up to 0.06%), the IgE and IgD content decreased (down to 23.5 and 1.2% respectively). This redistribution of fractions during torpor is stipulated by a difficulty of synthesizing the heavy compounds with a limited amount of substances and low metabolism. An increased content of IgG (up to 75.3%) against the background of a decrease in other immunoglobulin fractions may have a compensatory immunological effect. During arousal, the spectrum of immunoglobulins changes oppositely, but the IgA content increases more intensely (from 0.06 up to 31.5%) than IgE (from 23.5 up to 49.9%) and IgD (from 1.2 up to 16.8%). Herewith, the IgG fraction nearly disappears (up to 1.7%). The content of low molecular weight fractions of blood proteins (below 100 kDa) in a torpor state and after arousal should be noted to remain virtually unchanged.

Thus, the torpor causes a significant redistribution of immunoglobulin fractions in bat blood, which reflects the possibility of their synthesis under limited metabolic potential.

## Фазові переходи в багатокомпонентних середовищах за низьких температур

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## Phase Transitions in Multicomponent Media at Low Temperatures

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In cryobiological techniques for microalgal cryopreservation, the culture media are most commonly used as the basic solutions. The processes, occurring in these solutions when temperature decreases significantly contribute into successful cryopreservation and the culture preservation after freeze-thawing. However, the reported data on the kinetics of phase transitions and eutectic temperatures for multicomponent media with different degrees of mineralization, used in microalgal cultivation, are insufficient.

The research aim was to conduct a thermographic study of the media, traditionally used for microalgal cultivation and to determine the post-cryo microalgae survival depending on final temperature of cooling.

A thermographic examination of freezing of the samples, containing the media with different concentration of sodium chloride for freshwater microalgae cultivation within the temperature range from  $(20 \pm 2)^\circ\text{C}$  up to  $40^\circ\text{C}$  with 1 deg/min cooling rate, have been performed.

During experiment, there were obtained the data about the degree of subcooling, crystallization onset temperature and that of eutectics for traditional algological multicomponent media BG-11 [Ilavarasi, 2011], with different sodium chloride concentration within it. The cooling thermograms of the freshwater microalgae *Chlorococcum dissectum* cell suspensions in the studied culture media were analyzed as well. The dependence of cell viability on the medium composition and final cooling temperature was established. The maximum viability was in the NaCl-free samples or in those supplemented with 0.06 M NaCl (91%) when cooled down to  $-40^\circ\text{C}$ , while after freezing down to  $-196^\circ\text{C}$  and further warming the maximum viability did not exceed 3%.

Our findings will be used for scientifically grounded development of the novel protocols for freshwater microalgae cryopreservation and optimization of the existing ones using various cryoprotectants and their mixtures.

