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ОЦЕНКА УРОВНЯ СВОБОДНЫХ РАДИКАЛОВ В ПЕЧЕНИ И ПОЧКАХ ЛАБОРАТОРНЫХ МЫШЕЙ ПРИ КРАТКОВРЕМЕННОМ ПЕРЕОХЛАЖДЕНИИ

ASSESSMENT OF THE LEVEL OF FREE RADICALS IN THE LIVER AND KIDNEYS OF LABORATORY MICE WITH SHORT-TERM HYPOTHERMIA

АННОТАЦИЯ: В данной работе изучено действие кратковременного переохлаждения на организм лабораторных животных. Проведена оценка уровня свободных радикалов в гомогенатах печени и почек методом биохемилюминесценции. По результатам исследования выяснено, что уровень свободно-радикальных метаболитов кислорода при кратковременном действии холодового стресса на организм лабораторных мышей увеличивается.

ABSTRACT: In this paper, the effect of short-term supercooling on the organism of laboratory animals has been studied. The level of free radicals in liver and kidney homogenates was estimated by the method of biochemiluminescence. According to the results of the study, it was found that the level of free-radical metabolites of oxygen in the short-term effect of cold stress on the body of laboratory mice is increasing.

КЛЮЧЕВЫЕ СЛОВА: биохемилюминесценция, люминол, гомогенаты, переохлаждение, стресс.

KEY WORDS: biochemiluminescence, luminol, homogenates, hypothermia, stress.

Any living organism in the process of vital activity is exposed to various unfavorable factors from the external environment, which causes the formation of stress reactions [1]. One of the most important stress factors is cold [2]. The supercooling of the organism and the formation of its resistance to the action of low temperatures remains one of the most important and urgent problems at the present time. Overcooling leads to the formation of a large number of free radicals in the body [3].

What are free radicals? A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic

orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants [4]. Free radicals play a dual role as both toxic and beneficial compounds, since they can be either harmful or helpful to the body [5]. When exposed to various stress factors, in particular low temperatures, a large amount of free radicals are formed in the body [3]. With such excess concentrations, they are factors of disorganization of all cellular structures, which, in the final analysis, will lead to cell death [6].

The formation of free radicals is accompanied by chemiluminescence [7]. Any cells and tissues of animals emit light in the course of their vital activity, that is, they have a so-called intrinsic chemiluminescence. This emitting was so weak that it could not be detected for a long time, in this regard it was called "superweak luminescence". However, such a low intensity of luminescence is the main obstacle for research. That's why, the measurement of biochemiluminescence is carried out in the presence of special compounds – activators, among which luminol is actively used. Activators dramatically increase the intensity of luminescence associated with the formation of free radicals [8].

The purpose of our study was to evaluate the intensity of the biochemiluminescence of tissue homogenates of the examined internal organs in laboratory mice exposed to short-term supercooling and in animals that were not subjected to stressful effects.

Materials and methods of research. This research was conducted on white mongrel mice males weighing from 22 to 40 gram. Animals were kept in standard conditions of vivarium and on a normal diet. In the course of the experiment, the mice were divided into two groups: a control group and an experimental group. We modeled short-term cold stress by placing the animals in a cooler at a temperature of plus 4 degrees Celsius. After one hour of exposure to the cold, the animals were decapitated and the material for examination was extracted: liver and kidneys. And homogenates were subsequently prepared. After that, 10% homogenates were prepared (in the

calculation of 1 g of tissue per 9 ml of medium). A 0.9% aqueous solution of sodium chloride (NaCl) was used as the medium.

The device, which determines the intensity of biochemiluminescence of homogenates is "Biochemiluminometer – 3606M" (Russia). As an activator the above-mentioned luminal was used.

The technique of biochemiluminescent analysis is as follows. 40 μl of blood is brought in the cuvette with 2 ml of physiological solution NaCl 0.9% and a few crystals of luminal are added. After, the cuvette placed in biochemiluminometer cuvette drum. The measured luminance is not true, because each element of the luminometer has its own spectral sensitivity. Therefore, in order to obtain the true level of illumination, it is necessary to introduce changes to sensitivity of the instrument. There is the most simple method of obtaining the spectral sensitivity curve of the device. Measuring the luminescence of the substances is already known as the true level of illumination. For this, a reference solution is used, which is placed in the first cell of the cell holder of the drum. Light chemiluminescence is measured with a helped of a sensitive device. An electrical signal is amplified by an amplifier and then it is processed and analyzed by the computer. The computer displays information in the dependence graph, which shows the luminescence intensity, which depend on at time. On this graph the luminescence light sum is calculated during 10 minutes [9, 10, 11].

Results of the study. The obtained results are presented in table 1. The data here show a significant, which increase of the level of homogenates studied biochemiluminescence tissues of animals under cold stress in compared these animals, which were not under stress.

Table 1

Parameters of biochemiluminescence of homogenates of internal organs, c. u.*10^3 (M ± m)

Group of mice	Liver	Kidneys
I – control	52,873±1,092	56,655±2,126

(n=10)		
II – cold stress (n=10)	64,494±2,062***	66,289±1,871***

Note: *** - P<0,001 - the statistical significance of the differences compared to the control; n is the number of mice in the sample.

Figure 1 shows an example of graphs showing the intensity of the glow. The biochemiluminescent method does not detect the concentration of the substance, this method makes it possible to determine the reaction rate in which free-radical oxygen metabolites are formed. The intensity of biochemiluminescence is proportional to the rate of radical formation, and on the basis of this fact we can assess the level of free radicals formed in the system [12, 13].

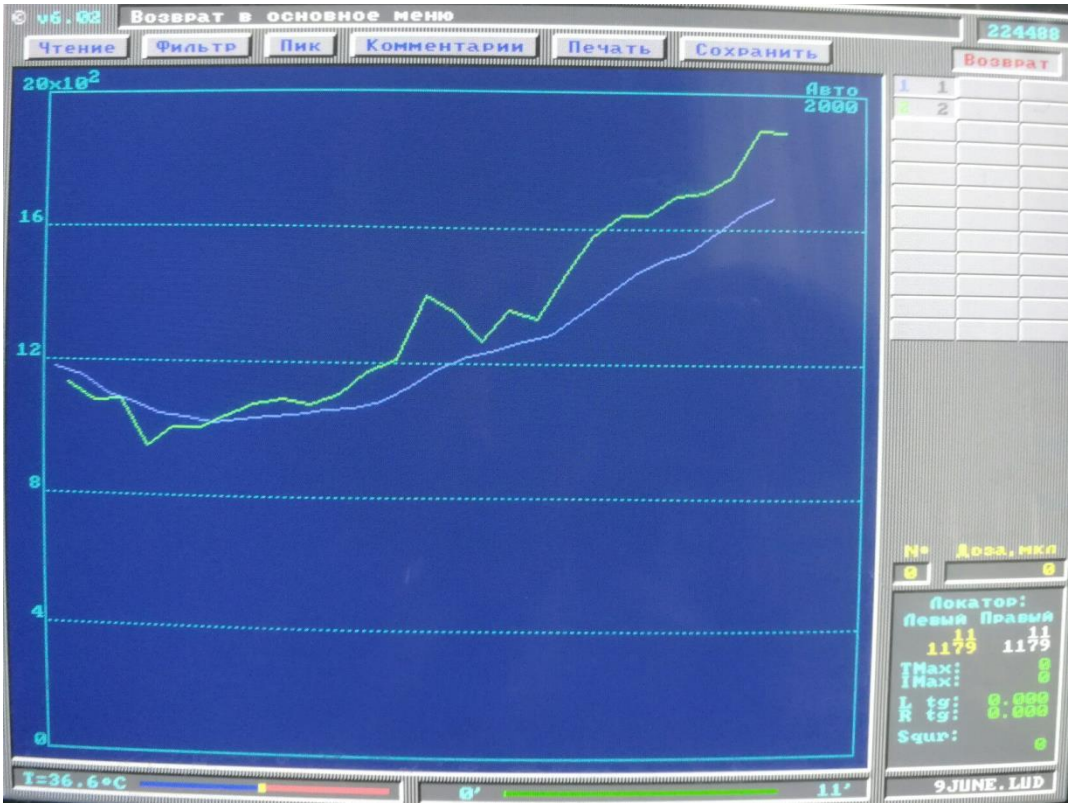

Fig. 1. Intensity of glow of tissue homogenates.

Conclusions. Method of biochemiluminescence integrally characterizes the ratio of prooxidantand and antioxidant capacity of the organism [14]. Under a short-

term cold stress free radical oxidation is increased, resulting in significantly increased levels of free radical metabolites of oxygen. The obtained results agree with available literature data about the strengthening of the free radical oxidation and the increase of free radicals under the stress factors on the body [3, 15, 16].

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