

The Dynamics of the Functional Characteristics of Red Blood Cells under Regular Physical Exertion

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The presence of unclear moments in the dynamics of erythrocyte catalase activity under physical exertion in early postnatal ontogenesis prompted the conduct of this study. A total of 32 rats of thirteen days old were examined (immediately after opening the eyes). Every day from 13 to 60 days of life they were subjected to stressful effects in the form of forced swimming, at a water temperature of about 32° C. 30 intact pups of the same age served as control. Blood samples from animals were taken from the tail for 13, 15, 21, 30, and 60 days of life. Erythrocyte count and catalase activity were evaluated, which were expressed by the value of the catalase index. Under stressful conditions in animals in early postnatal ontogenesis, a gradual increase in the concentration of red blood cells was observed with a decrease in the catalase index. Increased erythropoiesis was observed in experimental animals, starting from day 30, was associated with the maturation of their hypothalamic-pituitary-adrenal system and the beginning of its antistress work. A decrease in catalase activity against the background of an increase in the number of red blood cells due to physical activity in the second month of life of rats should be considered as a marker of adaptation of their body to physical activity.

Keywords: Red blood cells, Physical activity, Rats, Postnatal ontogenesis.

Red blood cells are the main formed elements of the blood, providing gas exchange in the body and rheological properties of blood in vessels of any caliber¹. In the process of postnatal ontogenesis in erythrocytes significant functional changes occur. Largely thanks to them, the organism adapts to changes in environmental conditions, especially during the transition from antenatal to postnatal existence². It is known that erythrocytes at the beginning of ontogenesis possess high functional stability, which gradually

weakens during the growth of an organism^{3,4}. It has been established that in addition to the red bone marrow, the nervous system, endocrine glands, liver, spleen, kidney and gastrointestinal tract are involved in the regulation of erythropoiesis⁵. They synthesize its stimulant - erythropoietin and inhibitors of this process.

Until now, many issues of postnatal restructuring of the functional state of red blood cells have not been sufficiently studied. In particular, the dynamics of the activity of the

enzyme catalase in erythrocytes functioning in vivo in early postnatal ontogenesis has not been fully clarified. Of particular interest is the “safety margin” of the enzymatic activity of this protein under stress conditions, which is a physiological test of the biological strength of any systems in a living organism. In particular, the role of the catalase enzyme in the functioning of red blood cells during exercise in early postnatal ontogenesis is far from fully understood. It is known that this enzyme decomposes hydrogen peroxide, the accumulation of which leads to the oxidation of cell structures and a decrease in the activity of the key enzymes glycolysis and pentose phosphate shunt, which weakens ATP synthesis, leading to hemolytic transformation of red blood cells⁶. Under stress, the amount of hydrogen peroxide in cells, including erythrocytes, often increases, posing a threat to their structures and raising questions about the dynamics of catalase activity in them. Unfortunately, to date, the available information on the mechanisms of the restructuring of the functional state of red blood cells in postnatal ontogenesis is fragmentary⁷. This is largely due to the fact that the available work on this issue was not performed using identical methods on different biological objects, in groups of different ages and incomparable with each other and not experiencing a state of stress. In order to partially close the existing gap in the system of scientific views, it seemed important to study the dynamics of the functional state of red blood cells in postnatal ontogenesis under stress exposure.

The aim of the study was to study the dynamics of the functional state of red blood cells in postnatal ontogenesis in a model of stress-induced changes in the hormonal status of rats.

MATERIALS AND METHODS

The study was conducted in strict accordance with the ethical principles established by the European Convention for the Protection of Vertebrates Used for Experimental and Other Scientific Purposes (adopted in Strasbourg on March 18, 1986 and confirmed in Strasbourg on June 15, 2006).

Healthy white Wistar rats from the age of thirteen to the sixtieth day of animal life were used in the experiments. To obtain them, females

of approximately the same body weight of 180–200 g were selected, which were kept in cells of 8 individuals each. After preliminary getting used to the females, 3-4 males, about the same mass, were planted for one day for mating. Fertilized females (this was determined by the presence of sperm in their vaginal smears) were placed in individual cells and kept with unlimited food and drink. From the first day after birth, the pups were distributed 8 cubs per lactating female. Male rats were weaned on the thirtieth day with further separate keeping of males and females of 8 individuals.

The study evaluated the effect of physical activity on the quantitative characteristics and catalase activity of the red blood system in rat postnatal ontogenesis. For this, 32 animals from the age of thirteen days old (immediately after opening the eyes) were subjected to stressful exposure daily in the form of forced swimming, at a water temperature of about 32° C. The first 5 days of exposure lasted 3-4 minutes, then by 21 days of life, adding 1-1.5 minutes daily, bringing to 10 minutes per day, by 30 days of age of rats to 30 minutes per day, and then to 60 days of age, swimming loads were 40 minutes per day. As a control, 30 intact pups were used.

To study the tested parameters in animals, 1.0 ml of blood was taken from the tail for 13, 15, 21, 30, and 60 days of life.

The number of red blood cells was counted in the Goryaev's cell. Catalase activity was determined by the standard method⁸. The principle of this method is based on the determination of undecomposed hydrogen peroxide with a solution of permanganate. The difference in the amount of permanganate spent on titration of hydrogen peroxide between the control and the experiment was expressed as a catalase number. The catalase index was determined by the ratio of the catalase number to the number of red blood cells.

Statistical data processing was carried out by the method of variation statistics using Student's criterion.

RESEARCH RESULTS

The neonatal period is characterized by significant changes in the morphofunctional state of the organism as a whole and especially the red blood system. The concentration of red blood cells

(Table 1), starting from the 13th to the 15th day of the postnatal life of rat pups, increased significantly in the control and experimental groups, then by 21 days there was a significant increase in red blood cells relative to 15 days of the life of animals in both observed groups, and a tendency to an increase in the concentration of red blood cells in the experimental group relative to the control. By 30 days of the study, there was a significant increase in red blood cells, in both groups relative to 21 days, and in the experimental group, an increase in the concentration of red blood cells led to significantly higher values relative to the control values. By the 60th day of observations, a significant increase in the concentration of red blood cells was observed relative to the 30th day of the life of animals in both groups, and the concentration of red blood cells in the experimental group was significantly higher compared to the control.

In postnatal ontogenesis in rats, significant changes were noted for the catalase index of red blood cells. On the thirteenth day of the life of rat pups, he was quite high in experience and control. By 15 days of life, this indicator significantly decreased in both groups. Further, throughout the entire observation period, the catalase index remained practically unchanged, equal in value to the index of sixty-day animals, with the exception of the thirtieth day in the control group. Towards the end of the observations, the catalase index of red blood cells in the experimental group

significantly decreased relative to the control values. The results obtained suggested that at the beginning of rat ontogenesis in the control and experimental groups, the concentration of red blood cells gradually increased with a decrease in the catalase index.

DISCUSSION

The results obtained in the study contradict the prevailing opinion that erythropoiesis in rats in early postnatal ontogenesis is reactive⁹. This is explained by the fact that in this age period the amount of erythropoietin in the blood is very high and, as a result, erythropoiesis is at the peak of activity¹⁰. Previous experiments by researchers with the introduction of plasma animals with a high concentration of erythropoietin showed no increase in the rate of incorporation of radioactive iron into red blood cells¹¹. Given these changes, we exclude that in our study, the ongoing increase in the number of red blood cells is associated with an increase in the level of endogenous erythropoietin caused by physical exertion¹².

Considering that in rats the consolidation of the hypothalamic-pituitary-adrenal system occurs by the end of the second week of postnatal life¹³, we can talk about the reaction to stress in the form of forced swimming was carried out with the participation of glucocorticoid hormones¹⁴. It is known that with an increase in the concentration of

Table 1. The effect of daily forced swimming on red blood cells in rats at the beginning of ontogenesis

Age of animals	Groups of animals	The concentration of red blood cells in 1 ml ³ , M±m	Red blood cell catalase index, M±m
13 days	Control, n=30	3.39±0.20	0.671±0.029
	Experienced, n=32	3.41±0.15	0.683±0.043
15 days	Control, n=30	3.89±0.23*	0.549±0.025*
	Experienced, n=32	4.07±0.23*	0.548±0.037*
21 days	Control, n=30	4.45±0.05*	0.556±0.021
	Experienced, n=32	4.51±0.13*	0.533±0.019
30 days	Control, n=30	5.22±0.17*	0.699±0.039*
	Experienced, n=32	5.93±0.31* p<0.05	0.521±0.035 p<0.05
60 days	Control, n=30	8.01±0.29*	0.565±0.027*
	Experienced, n=32	8.81±0.43* p<0.05	0.498±0.015 p<0.05

* - the difference is significant relative to the previous observation period in the control and experimental group. p - the difference is significant between groups at the same observation period.

glucocorticoids in the blood of animals, an increase in T-lymphocytes of regulators expressing lyt-1 + and lyt-2 + antigens on their surface in bone marrow tissue occurs, followed by activation of erythropoiesis and an increase in the concentration of red blood cells in peripheral blood after 6-9 days¹⁵. T-lymphocytes-regulators in bone marrow tissue interact with monocytes / macrophages, as a result of which lymphokines are actively released from the latter¹⁶. They, increasing the sensitivity of early erythroid committed precursors to erythropoietin, strongly stimulate its proliferation and differentiation¹⁷. Taking this into account, it becomes clear that erythropoietin under the action of glucocorticoids is the resultant factor in the activation of erythropoiesis. It is very important for understanding the mechanism of this process that the administration of anti-erythropoietin serum¹⁸, adrenalectomy and thimectomy¹⁵ do not contribute to an increase in the rate of erythropoiesis.

It can be thought that from the thirteenth day of life in experimental animals exposed to stress, in the form of forced swimming in the first week of the experiment, it did not cause activation of erythropoiesis due to the absence of consolidation of the hypothalamic-pituitary-adrenal system in animals during these periods. Subsequently, as it ripens and continues physical exertion by the 30th day of life, erythropoiesis intensifies, which manifests itself in an increase in the concentration of red blood cells in the experimental group of growing animals.

Explaining the dynamics of the catalase activity of erythrocytes, we consider it appropriate to proceed from the known data¹⁹ on the functional inequality of erythrocyte populations in different organisms. They found that the catalase index of red blood cells of the peripheral blood of healthy people and animals is in negative correlation with their number^{20,21}. A decrease in catalase activity against the background of an increase in the number of red blood cells during our studies in both groups of rats confirms the presence of this phenomenon²². A significant decrease in the catalase index of erythrocytes and an increase in their concentration in the experimental group on the 30 and 60 day of the animals' life may indicate the adaptation of the growing organism to physical activity based on aerobic-anaerobic adaptive processes^{23,24}.

There is reason to believe that the results

obtained in this study have a great biological meaning and are important for understanding the physiology of hematopoiesis and adaptation of the organism in early ontogenesis. Stress factors begin to act on the body from the very beginning and weaken the level of its antioxidant defense, especially in the blood system²⁵. Physical activity is the most frequent and necessary for optimal existence factor of the external environment, without adaptation to which normal life activity is not possible²⁶, nor the process of rehabilitation after an illness^{27,28}. In this regard, in mammals and in humans, it will be justified to conduct additional studies in the future in order to further clarify the dynamics of the activity of erythrocyte catalase and consider it as a marker of adaptation of a young organism to physical stress. It can find its practical application primarily in rehabilitation^{29,30} and in junior sports³¹.

CONCLUSION

The persistence of ambiguity in the dynamics of erythrocyte catalase activity under physical exertion in early postnatal ontogenesis prompted the present study. Under stressful conditions in animals in early postnatal ontogenesis, a gradual increase in the concentration of red blood cells was observed with a decrease in the catalase index. Increased erythropoiesis was observed in experimental animals, starting from day 30, was associated with the maturation of their hypothalamic-pituitary-adrenal system and the beginning of its antistress work. A decrease in catalase activity against the background of an increase in the number of erythrocytes under physical exertion in the second month of life of rats should be considered as a marker of their organism adaptation to physical exertion. In this regard, one should think about the possibility of considering the dynamics of erythrocyte catalase activity in young athletes as an indicator of their organism for physical training.

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