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Effects of Succinate-Containing Preparation on Characteristics of Rat Erythrocytes in Exhaustive Swimming with a Load

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We studied the dynamics of the red blood cell composition of Wistar male rats at rest and when swimming with a load (4% body weight) before and after administration of a succinate-containing preparation (meso-2,3-dimercaptosuccinic acid). In rats receiving the succinate-containing preparation, a decrease in the number of red blood cells and an increase in their volume and absolute and relative number of reticulocytes were observed at rest in comparison with vivarium control. In rats exposed to exhaustive swimming after preliminary administration of the test preparation (12 h before the test), we observed a decrease in hematocrit and erythrocyte diameter in comparison with the corresponding parameters in rats not treated with the preparation; the level of hemoglobin did not change. The pattern of changes in the cellular composition of red blood in rats at rest and during swimming against the background of treatment with the succinate-containing preparation in comparison with vivarium control is considered as a result of its effect on physical exercise under conditions of stabilization of hemoglobin and hematocrit levels, activation of proliferative activity of red bone marrow, and an increase in time of swimming to exhaustion by 2.8 times.

Key Words: *rats; red blood cells; swimming load; succinate-containing drug*

Physical exercise induces morphological modification of the erythron [11], affects erythrocyte and hemoglobin content in the blood [2], and improves stress resistance [5]. The effect of succinic acid and its derivatives used for increasing the efficiency of physical activity is due to improvement of oxygen release [6], osmotic stability of cells [12], their sorption capacity [13], and decrease of blood lactate concentration [14], which delays fatigue. The mechanism of fatigue development is not fully understood; therefore, it is necessary to develop new approaches to identify the adaptive capabilities in humans and animals.

We studied the pattern of changes in morphofunctional parameters of the red blood in rats treated with a succinate-containing agent at rest and during exhaustive physical exercise.

MATERIALS AND METHODS

The study was carried out on mature male Wistar rats (n=40) weighing 250-300 g. The animals were kept in cages (4 rats per cage) on a standard diet with free access to water at 21±1°C and 12-h illumination cycle. The experimental protocol was approved by the Local Bioethics Committee of the Institute of Physiology of the Komi Research Center.

The succinate-containing agent, meso-2,3-dimercaptosuccinic acid (Suc) was kindly provided by Acad. O. N. Chupakhin (I. Ya. Postovsky Institute of Organic Synthesis); the substance was administered *per os*. The optimal dose of the agent (50 mg/kg body weight) was calculated based on published data on maximum daily doses of known succinate-containing drugs for humans and the formula for the bioequivalent dose of the sub-

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stance given the metabolic coefficient of this species of laboratory animal. The time of Suc administration determined experimentally in a series of preliminary studies was at least 12 h before the start of exhaustive swimming.

The animals were divided into control and experimental groups. Animals kept on a standard diet in a vivarium (*n*=10) and animals kept under similar conditions, but receiving Suc 12 h before the examination (*n*=10) served as the control. The experimental groups included rats subjected to exhaustive swimming with a load equivalent to 4% body weight (*n*=8) and rats additionally receiving Suc 12 h before exhaustive exercise (*n*=12). The animals of the experimental groups were preliminarily adapted to water [9] followed by a 14-day recovery period.

For evaluation of physical endurance, the animals were forced to swim until exhaustion in an opaque container (diameter 45 cm, height 60 cm) with the temperature of desaturated water 28°C and room temperature 21-23°C. The distance from the water surface to the edge of the container was at least 15 cm. A metal weight was attached to the base of the tail with elastic non-traumatic tape. The stopwatch was activated at the moment the animal was put in water, and the time until the development of obvious symptoms of fatigue was recorded. The criteria for the manifestation of complete fatigue included three unsuccessful attempts to come to the water surface or immersion in water accompanied by sinking to the bottom for a period of more than 10 sec. Then the animal was removed from the water, dried rapidly, and decapitated.

The blood was stabilized with heparin (5000 U/ml, AKOS) and the following parameters were measured: hemoglobin (Sahli's method; GS-3), hematocrit (MPW-310 centrifuge; Mechanika Precyzyina), erythrocyte count (Goryaev's chamber), reticulocyte count (after 12-15 min incubation of the blood with 1% brilliant cresyl blue; Diachim-GemiStein-RTTs). The diameter of 100 erythrocytes was measured on Romanowsky-stained smears (Diachim-GemiStein-R (classic); ABRIS+) using a PZO microscope with oil immersion objective (lens ×100 with calibrated scale ×12) [3].

The significance of the difference between the groups was assessed by the nonparametric Kruskal—Wallis test using Microsoft Excel and Statistica 6.0 (StatSoft, Inc.) software. The data are presented as *M*±*m*. The difference was significant at $p<0.05$.

RESULTS

The morphological and functional composition of red blood in rats of the vivarium control group corresponded to the known values [7].

In comparison with the vivarium control group, control rats receiving Suc showed lower erythrocyte count and erythrocytes to hematocrit ratio and higher volume of erythrocytes and reticulocyte count (Table 1).

In experimental group rats receiving Suc, hematocrit and erythrocyte diameter decreased and erythrocyte to hematocrit ratio increased in comparison with the corresponding parameters in rats receiving no Suc; swimming duration increased by 2.8 times.

At constant hemoglobin concentration in the control group receiving Suc and in both experimental groups, the decrease in erythrocyte count can be associated with blood redistribution to the vessels of working organs [4]. Selective hemolysis due to different osmotic resistance of erythrocytes cannot be excluded,

TABLE 1. Effect of a Succinate-Containing Agent (Suc) on Cell Composition of the Blood in Rats at Rest and during Exhaustive Swimming with a Load Equivalent to 4% Body Weight (*M*±*m*)

Parameter	Vivarium control	Vivarium control+Suc	Swimming	Swimming+Suc
Swimming duration	0	0	1 h 30 min	4 h 16 min
Hemoglobin, g/liter	144.90±1.22	145.51±1.89	148.13±2.24	146.53±1.89
Hematocrit, %	44.84±0.33	43.93±0.31^{+++}	49.49±0.81**	46.48±0.81$^{+\circ\circ}$
Erythrocytes, 10^{12}/liter	8.16±0.16	7.09±0.06***	7.29±0.19**	7.34±0.09***$^{\circ}$
Hemoglobin concentration in erythrocyte, %	32.23±0.35	32.85±0.26^{+++}	30.59±0.33**	31.34±0.23
Erythrocyte to hematocrit ratio	0.181±0.004	0.165±0.004*$^{++}$	0.147±0.004**	0.159±0.004**$^{+}$
Erythrocyte volume, μm^3	55.08±0.97	62.04±0.59**	63.57±2.60**	63.31±0.89***
Erythrocyte diameter, μm	6.18±0.02	6.24±0.04^{++}	6.41±0.03***	6.21±0.04^{+++}
Reticulocytes, 10^{12}/liter	0.103±0.005	0.23±0.03**	0.16±0.02**	0.17±0.01***
Reticulocytes, ‰	12.61±0.65	29.62±3.17***$^{+}$	19.52±2.93*	22.58±1.02***$^{\circ}$

Note. *$p<0.05$, **$p<0.01$, ***$p<0.001$ in comparison with vivarium control; $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ in comparison with swimming group; $^{\circ}p<0.05$, $^{\circ}p<0.01$ in comparison with vivarium control+Suc group.

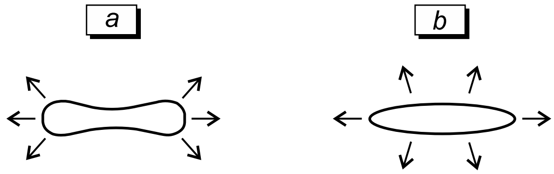

Fig. 1. Scheme of presumptive distribution of electronegativity on the erythrocyte surface (concave cell shape, *a*) and its modification due to swelling (shown by arrows, *b*).

because the mean volume of cells increased. Similar changes at the cellular level in rats were consistent with the results obtained in experiments with 30-day administration of multicomponent biostimulants [1].

At the same time, the observed discrepancy between hematocrit and decreased number of erythrocytes can be attributed to changes in erythrocyte shape and distribution of the negative charge on their surface caused by cell swelling [8] (Fig. 1).

The shift of the electronegativity of the field to the central zone of the erythrocyte can probably resist cell squeezing during centrifugation. This value presumably corresponds to the erythrocytes to hematocrit ratio, which was maximum in animals of the vivarium control group (0.181); after swimming, this parameter decreased to 0.147 ($p<0.01$), while administration of Suc in the control and experimental groups increased it to 0.165 and 0.159, respectively. The erythrocyte swelling rate is believed to be affected by the osmotic stability of membranes [12], which, along with the maintenance of the internal charge of erythrocytes, is directly related to energy supply [10].

The significant increase in the absolute and relative numbers of reticulocytes in the control group after administration of Suc is associated with not only proliferative activity of red bone marrow, which is characteristic of myogenic reticulocytosis, but probably with intensified erythropoietic function of the myeloid tissue under the effect of nonspecific action of Suc as a signaling molecule. Unidirectional changes in the erythrocyte composition of the blood in rats of the control and experimental groups receiving the succinate-containing agent implies transition of the functional state of the body to a level corresponding to effective implementation of physical activity.

The decrease in hemoglobin concentration in erythrocytes of rats of the experimental group during exercise ($p<0.01$) can be associated with increased oxygen demand [5]. The use of the succinate-containing drug before swimming caused a tendency towards an increase in hemoglobin concentration in erythrocytes, which probably indicates well-maintained level of oxygen supply to the muscle tissue. The changes observed in the erythrocyte composition of the blood in rats do not fully explain significant increase in the performance of the animals, because specific effect of succinate-containing agents is manifested in direct interaction with the respiratory chain [11].

Thus, the morphofunctional changes in blood erythrocytes within the normal range observed in control animals receiving Suc are associated with proliferative activity of the myeloid tissue and qualitatively new properties of erythrocytes: increase in their volume without changes in hematocrit.

A significant increase in the swimming time in rats receiving Suc was not associated with changes in erythrocyte volume or absolute concentration of reticulocytes. Hematocrit and mean erythrocyte diameter decreased and approached the values typical for intact animals.

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