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Physiological Features of Erythrocytes in the Aging Organism

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Abstract

The progression of aging affects various blood parameters, which can weaken the viability of the body. Clarification of various aspects of this process requires additional observations, especially with the use of animals. The study was conducted on 43 healthy outbred male rats aged 24 months. Also, 32 healthy outbred male rats 3 months old, which formed the control group, were taken under observation. The examined 24-month-old rats showed standard aging phenomena. In their erythrocytes, there was an increase in lipid peroxidation caused by a weakening of the activity of their antioxidant defense. In the blood of two-year-old rats, there was a decrease in the level of erythrocyte-discocytes. This was accompanied in them by an excess content of erythrocytes in the blood, which had a changed shape to varying degrees. In rats of the older group, erythrocyte aggregation was increased, as indicated by an increase in the numberof erythrocytes that entered the aggregates in the blood and an increase in the number of most erythrocyte aggregates with a decrease in the level of free erythrocytes. For two-year-old rats, low content of erythrocytes-disconcytes in the blood and an excess number of erythrocytes with a modified shape, as well as an increase in their aggregation, are characteristics. An important reason for these changes is the weakening of the antioxidant protection of erythrocytes, leading to a pronounced activation of lipid peroxidation in them.

Keywords: Erythrocytes, Aging, Rats, Rheology, Physiology, Hematology

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Introduction

Science recognizes aging as one of the most common processes in nature. It affects all structures of the body of living beings (Dontsov *et al*., 2010). The continuation of the development of biological science requires a detailed elucidation of the manifestations and mechanisms of the appearance and development of signs of aging, which is especially convenient to do during the experiment (Vatnikov *et al*., 2018). This is recognized as necessary for the further development of gerontology and biology (Kishkun, 2008).

The ongoing scientific research is increasingly aimed at studying in detail the main stages of the implementation of aging and the processes of formation of age-related pathology with a deep consideration of the mechanisms of gerontogenesis on various models (Klebanov *et al*., 2019). Modern researchers attach particular importance to monitoring various parameters of blood cells (Kulikov *et al*., 2020) and especially the most numerous group - erythrocytes (Zavalishina *et al*., 2018) in different conditions of the body (Zavalishina *et al*., 2019) and of course in the course of aging (Vakhtin, 2009). The significance of these studies is that aging affects the state of all cells in the body, as a rule, leading to a weakening of their work, with the occurrence of dysfunctions, and sometimes pathological processes.

Under conditions of aging, especially in the presence of obvious pathological manifestations, there is an excessive activity of platelets, which worsens many hematological parameters (Usha *et al*., 2019). The emerging situation weakens the implementation of microcirculation and lowers the level of metabolism in all organs (Bespalov *et al*., 2018; Vorobyeva *et al*., 2018). At the same time, the state of microrheological parameters of erythrocytes under aging conditions cannot be considered fully elucidated.

Rats remain one of the leading objects of research in gerontology. They test various hypotheses and carry out experimental effects on the body with different goals (Dontsov & Krutko, 2019). As a rule, various changes in morphology and physiology, including those associated with the development of aging, are monitored in rats. In this regard, the work aims to determine the degree of physiologically acceptable changes in the microrheological characteristics of erythrocytes in aging rats.

Materials and Methods

This work was carried out in accordance with ethical standards and fundamentals of humane work with laboratory animals, which are formulated in the European Convention for the Protection of Vertebrate Animals used in any experiments.

43 healthy outbred male rats at the age of 24 months were taken into work. During their lives, these animals did not get sick and did not take part in any acute or chronic experiments. 32 outbred healthy male rats, aged 3 months, were also taken into the study. They formed a control group. All rats were examined once.

The level of plasma lipid peroxidation was assessed in the course of determining the active substances contained in plasma using a kit manufactured by Agat-Med (Russia) and by the content of acyl hydroperoxides in plasma (Ashtiani *et al*., 2021; Ong *et al*., 2021). In all cases, the level of plasma antioxidant activity was determined (Abdaltif *et al*., 2021). The level of lipid peroxidation processes in erythrocyte structures was recorded by the content of malondialdehyde and acyl hydroperoxides in them. In rat erythrocytes, using the enzymatic method using a kit manufactured by Vitaldiagnostikum (Russia), the cholesterol content was determined, and the total content of phospholipids was determined by the amount of phosphorus in them. The ratio of cholesterol content in erythrocytes to phospholipids was calculated. The activity of erythrocyte catalase and superoxide dismutase was determined.

Surface features of red blood cells were determined by phase contrast microscopy. According to the shape of erythrocytes, they were divided into three groups: discocytes, reversibly altered erythrocytes, and irreversibly altered erythrocytes. The severity of erythrocyte aggregation was determined using a light microscope and a Goryaev camera. The number of erythrocyte aggregates, the number of erythrocytes that entered into aggregation, and the erythrocytes that remained free were assessed.

The results found during the study were processed statistically by calculating the Student's t-test and indicators of systemic multivariate analysis.

Results and Discussion

When examining aging rats, standard manifestations of aging were found. They consisted of a dulling of the coat, a weakening of the severity of appetite, and a decrease in interest in the outside world. In the control group, the appearance of the animals had no features.

The rats of the older group had high plasma levels of molecules of acyl hydroperoxides and molecules of substances capable of combining with thiobarbituric acid, which exceeded the control levels by 24.3% and 16.9%, respectively. The found increase in lipid peroxidation in aging rats occurred due to the weakening of the plasma antioxidant system to a level of 24.3 ± 0.47%, yielding to the level of the control group by 19.7% **(Table 1)**.

Table 1. Dynamics of hematological parameters in observed rats

Registered indicators	A group of aging rats, M±m, n=43	Control group, M±m, n=32
Level of acyl hydroperoxides in plasma, D_{233}/1ml	1.94±0.032 $p<0.01$	1.56±0.023
The content of thiobarbituric acid-active products in plasma, μmol/l	4.29±0.039 $p<0.01$	3.67±0.028
Plasma antioxidant potential, %	24.3±0.47 $p<0.01$	29.1±0.42
Erythrocyte cholesterol level, μmol/10^{12} erythrocytes	1.03±0.029 $p<0.05$	0.91±0.038
The level of total erythrocyte phospholipids, μmol/10^{12} erythrocytes	0.65±0.029	0.69±0.020
Cholesterol/total erythrocyte phospholipids ratio	1.58±0.036 $p<0.01$	1.32±0.032
Number of erythrocyte acyl hydroperoxides, D_{233}/10^{12} erythrocytes	3.43±0.028 $p<0.01$	2.71±0.041
The amount of malonic dialdehyde in erythrocytes, nmol/10^{12} erythrocytes	1.14±0.036 $p<0.01$	0.88±0.027
Erythrocyte catalase activity, IU/10^{12} erythrocytes	7940.0±15.7 $p<0.01$	9920.0±9.5
Erythrocyte superoxide dismutase activity, IU/10^{12} erythrocytes	1660.0±6.11 $p<0.05$	1870.0±7.6

The content of erythrocytes-discocytes, %	73.0±0.52 $p<0.05$	85.1±0.53
Number of reversibly altered erythrocytes, %	17.9±0.41 $p<0.01$	9.3±0.26
Number of irreversibly altered erythrocytes, %	9.1±0.24 $p<0.01$	5.6±0.17
The total number of all erythrocytes in the aggregate, cells	47.9±0.12 $p<0.01$	36.5±0.09
Number of aggregates, units	12.3±0.10 $p<0.01$	8.5±0.10
The number of free erythrocytes, cells	222.0±0.69 $p<0.05$	251.3±0.32

Symbols: p – reliability of differences between the recorded indicators and the control group.

In rats of the older group, the amount of cholesterol in the composition of erythrocytes was increased with a slight decrease in the content of total phospholipids in them. This led to an increase in the ratio of cholesterol to total phospholipids in erythrocytes.

In older rats in the structures of erythrocytes, activation of lipid peroxidation was noted, which was caused by a decrease in the biological capabilities of their antioxidant system (Ahmed & Althanoon, 2022). The revealed changes in the levels of products resulting from lipid peroxidation in the composition of erythrocytes in rats of the older group were carried out as a result of a decrease in their antioxidant protection, and primarily due to a weakening of catalase and superoxide dismutase.

In rats of the older group, there was a significant decrease in the amount of disk-shaped erythrocytes in the blood (Abdaltif *et al*., 2021; Ahmed, 2021). At the same time, in the blood of these rats, there was an excess of erythrocytes having a changed shape. In rats of the older group, an increase in the number of erythrocytes included in the aggregates and an increase in the number of these aggregates in their blood was noted, with a simultaneous decrease in the number of erythrocytes that did not involved in aggregation.

The processing of the data found in the work by systemic multivariate analysis in older rats made it possible to calculate the value of the pro-aggregation potential of erythrocytes and the size of the disaggregation potential of erythrocytes in them, to determine the contribution of all indicators recorded in the work, and also to calculate the value of the erythrocyte total aggregation potential.

When calculating the pro-aggregation potential of erythrocytes in rats of the older age group, the average size of the erythrocyte aggregate (Pi=579.3), the content of erythrocyte aggregates in the blood (Pi=481.3), the number of erythrocytes that have an irreversibly changed shape (Pi=433.6) and the total number of erythrocytes included in the aggregates (Pi=411.6). The size of the weighted average pro-aggregation potential of erythrocytes, which comprehensively characterizes erythrocyte aggregation in rats of the older age group, was 0.109.

The most significant in the disaggregation potential of erythrocytes in rats of the older age group were the activity of erythrocyte superoxide dismutase (Pi = 526.4), the content of erythrocytes in their blood with a discoid shape (Pi = 524.3) and the biological capabilities of catalase (Pi = 518.7). The weighted average of the disaggregation potential of erythrocytes in aging rats, which generally characterized the functioning of the mechanisms that inhibit aggregation of erythrocytes, reached a value of 0.086, while the total aggregation potential of erythrocytes in these animals was 0.023.

In the pro-aggregation potential of erythrocytes in rats of the control group, the average aggregate size (Pi=452.6), the number of erythrocyte aggregates (Pi=437.0), the level of irreversibly changed erythrocytes (Pi=373.0) and the total number of erythrocytes that entered into the aggregate (Pi=362.6). The value of the weighted average of the pro-aggregation potential of erythrocytes in the control reached 0.095.

The most important in the disaggregation potential of erythrocytes in control rats was the level in their blood of erythrocytes with a discoid shape (Pi=617.5), the severity of the enzymatic capabilities of erythrocyte catalase (Pi=598.5) and superoxide dismutase (Pi=598.2). The value of the weighted average disaggregation potential of erythrocytes in control rats reached 0.098, while their total aggregation potential of erythrocytes was -0.003.

The development of all phenomena of aging can be considered a consequence of the complex simultaneous interaction of the hereditary program and the range of environmental effects on the body (Dyleva *et al*., 2023). From this point of view, one can also look at age-related changes in the rheological parameters of erythrocytes. They are very convenient to study in rats, which are a recognized physiological model. The life span of rats is short, and the development of the aging process in them is similar to that in other mammals, therefore the mechanisms and manifestations of aging revealed in this living object can be transferred to other mammals with some caution.

Aging under standard conditions in the observed rats was manifested by the appearance of some hematological disorders, which were established in our work at the age of 24 months of life. The authors consider their presence to be a consequence of the development of natural aging. In many ways, they are associated with the formation of membranopathies in blood cells caused by the aging process and external influences on the body.

With aging, the observed rats had a weakening of the antioxidant capacity of the blood, which led to an increase in the content of acyl hydroperoxides and substances capable of reacting with thiobarbituric acid, which inhibits metabolism in tissues. The resulting increase in the phenomena of lipid peroxidation in the liquid part of the blood leads to increased alteration of the vascular endothelium and outer membranes of blood cells (Kotova *et al*., 2017). This fully applies to their most numerous population, erythrocytes, which may exhibit various dysfunctions in the course of aging (Tabeeva, 2019). This situation can be significantly aggravated due to the development of hypoxia, which forms during aging, primarily against the background of the appearance of membranopathy in erythrocytes caused by an increase in cholesterol in them, a decrease in phospholipids and activation of lipid peroxidation due to a decrease in the antioxidant defense of erythrocytes (Dorontsev *et al*., 2022).

The current situation leads to the loss of some of the erythrocytes of their discoid shape, worsening the entire microcirculation in the capillary zone. Developing changes in the erythrocyte structure leads to an increase in their level in the blood with the reversible and irreversible transformation of the form (Yashkichev, 2018). In rats with signs of aging, the blood level of erythrocytes with signs of echinocytosis and stomatocytosis significantly exceeded that in the control group. In the future, their transformation inevitably leads to an increase in the number of erythrocytes in the blood, which are spheroechinocytes, spherostomatocytes, and spherocytes, which do not participate in gas exchange, which weakens metabolism (Karpov *et al*., 2020).

It can be thought that shape disturbances in a significant part of the erythrocyte population in the blood can be caused by damage to the intraerythrocytespectrin network, leading to changes in many erythrocytes of the optimal distance between adjacent spectrin strands.

The increase in erythrocyte aggregation found in older rats is caused by the development of changes in the charge of their surface with age due to degradation under the influence of lipid peroxidation activation of some of the glycoproteins located on it, which have a negative charge (Karpov *et al*., 2021). An increase in the synthesis of ionized oxygen species in the blood and, especially, in the erythrocytes of aging rats, even those kept under optimal conditions, is inevitable. This is accompanied by oxidative alteration of many membrane lociand changes in the optimal tertiary structure of various proteins in blood plasma. These changes stimulate the interaction of damaged blood proteins with free erythrocytes, activating the phenomena of their aggregation. The excess lipid peroxidation products present in this case accelerate the onset of erythrocyte aggregation as a result of the development of multiple oxidative damages to their membranes and inhibition of erythrocyte disaggregation processes by increasing the strength of the bond between erythrocytes in aggregates of any size (Mikhaylova *et al*., 2021).

The activation of erythrocyte aggregation phenomena in aging rats is also largely due to the increased action of catecholamines on their surface, the concentration of which in the blood increases very often during aging. This can be realized during the activation of erythrocyte α1-receptors with subsequent stimulation of the Ca^{2+}-calmodulin system and involvement of erythrocyte phosphatidylinositols in the activation processes. Also, aggregation of erythrocytes increases in aging rats due to an increase in the functionality of α_2-adrenergic receptors of erythrocytes. This leads to depression of the adenylate cyclase enzyme in them and an increase in the amount and activity of cytoplasmic G_i proteins. This situation contributes to a decrease in the amount of cytoplasmic cyclic adenosine monophosphate in erythrocytes and an increase in the flow of Ca^{2+} ions into them (Zavalishina *et al*., 2021). All this greatly enhances the aggregation of red blood cells directly in the bloodstream, causing several undesirable consequences.

An increase in the content of small erythrocyte aggregates in the blood of aging rats leads to an increase in the number of injuries to the internal endothelial lining of blood vessels. This increases the degree of expression of subendothelial structures in the blood, thereby stimulating the mechanisms of hemostasis and inhibiting hemocirculation in general (Zavalishina, 2020). An increase in the number of erythrocyte aggregates suspended in the blood impairs blood perfusion through the existing vasavasorum. This leads to a clear weakening of all types of metabolism in the walls of blood vessels, causing a significant decrease in the level of generation in endotheliocytes of substances that have disaggregation activity (Zdravomyslova, 2022) and are extremely important for hemocirculation (nitric oxide and prostacyclin).

Conclusion

In aging rats in the blood, there is a low level of erythrocytes having a discoid shapeand an increase in the number of their varieties with an altered surface. Also, their erythrocytes are characterized by an increased tendency to aggregate. This is largely due to an increase in the content of the ratio of cholesterol and phospholipids in the structures of erythrocytes and a weakening of the antioxidant capabilities of their structures, leading to the intensification of lipid peroxidation processes. This situation in the body of aging rats creates conditions for increased injury to the vascular endothelial layer, which makes elements of their subendothelium available to them and enhances the work of hemostasis. violations. They can exacerbate the manifestations of aging, closing the vicious circle: "senile changes - microrheological disorders."

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