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Physiological Dynamics of Erythrocytes' Cytoarchitecture In Aged Rats.

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ABSTRACT

The state of erythrocytes' cytoarchitecture is mostly determined by hemodynamic and metabolic tissue homeostasis on the level of capillaries. It can have special significance for an aged body as the development of changes of their surface geometry can negatively influence microcirculation on the whole and aggravate the common state. At the same time, physiological dynamics of erythrocytes' cytoarchitecture on the late stages of ontogenesis is studied rather poorly. Taking into account the great significance of this index and high frequency of experimental researches with the usage of laboratory rats, there appears the necessity of studying the dynamics of erythrocytes' cytoarchitecture in this specie of laboratory animals. In the conducted research the aged rats were noted to have gradual strengthening of lipids' peroxidation in erythrocytes: the content of acylhydroperoxides in them increased between 18-30 months of life by 27.2%. It was accompanied in them by gradual activity lowering of erythrocyte catalase and superoxide dismutase by 18.4% and 11.9%, respectively. Between 18-30 months of life the blood of rats was noted to have quantity lowering of erythrocytes-discocytes by 18.1%. At the same time, there was noted the quantity increase of their reversibly and irreversibly modified forms in the rats' blood in the given terms by 48.9% and in 2.07 times, respectively. So, healthy aged rats have gradual increase of erythrocytes' cytoarchitectural changeability what promotes worsening of microcirculation in their tissues. It significantly contributes to increasing while aging morbid aggravation and sensitivity rise of a body to negative impacts of the environment. In this respect the state of erythrocytes' cytoarchitecture can be considered in future researches as one more point of geroprotectors' optimizing impact and received results – as control values.

Keywords: aging, rats, erythrocytes, cytoarchitecture, lipids' peroxidation.

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INTRODUCTION

The process of mammals' ontogenesis is accompanied by the dynamics of morpho-functional indices of all the tissues and organs [1]. Many aspects of the given changes are not yet fully studied [2]. Modern science recognizes that age-specific dynamics of a body's structure and functions is connected with constant interaction of genetic mechanisms [3] and environmental impacts [4,5]. The researchers study the process of aging now from these very positions as it touches all the systems of a body, progressively worsens their functioning and makes its death more and more possible [6]. There is some basis to consider that age-specific changes of blood rheological properties and its regular elements [7] are very important for the realization of aging. These disturbances are admitted to play a great role in the formation of various pathology in an aged body and in the process' activation of a body's death [8,9].

Erythrocytes is an important component of microcirculation. Peculiarities of their cytoarchitecture mostly determine hemodynamic and metabolic homeostasis of tissues on the level of capillaries [10,11]. It's noted that cytoarchitecture of some erythrocytes can be changed at physiological, border-line and pathological states [12]. It has special significance for an aged body as the development of changes of regular blood elements' rheological properties can negatively influence the common state [13,14]. At the same time, physiological dynamics of erythrocytes' cytoarchitecture on the late stages of ontogenesis is studied rather poorly. Taking into account the great significance of this index and high frequency of experimental researches with the usage of laboratory rats, studying the dynamics of erythrocytes' cytoarchitecture in them on the late stages of ontogenesis seems to be very important. Received data can become widely essential in the course of future experimental researches devoted to optimization of microcirculation in late ages with consequent cautious transfer of the received data into gerontological researches on human beings [2].

The aim of the research is to determine age-specific dynamics of erythrocytes' cytoarchitectural peculiarities in healthy rats in the course of aging.

MATERIALS AND METHODS

All the investigations in the present work were conducted in full correspondence with ethical norms and recommendations on humanization of work with laboratory animals containing "The European Convent on the protection of vertebrate animals used for experiments or in other scientific purposes" (Strasbourg, 1986).

There were observed 95 healthy male-rats of Vistar line, including 32 rats at the age of 18 months, 29 animals at the age of 24 months and 34 rats at the age of 30 months. Before this research the rats had participated in no experiments and had suffered no diseases. The control group was composed of 27 healthy male-rats of Vistar line at the age of 6 months. All the rats were taken out of the laboratory animals' hatchery of the Russian Academy of Sciences (Russia, Moscow region, town Puschino) at the age of 2 months. The animals were kept in the vivarium in spacious cages (the area of floor in a cage on 1 animal was equal to 200 cm^2). There was used natural lighting; the temperature was kept at 18-22ºC; relative air humidity was equal to 50-65%. The rats received full-ration combined feed for laboratory animals PK-120 (Laboratorkorm, Russia). Water was in free access for rats.

We estimated the common state of animals. Their body mass was registered with the help of electronic balance VM1502M-II (Vesta, Russia). The level of endurance in rats was estimated with the help of swimming test with additional load (10% from the animal's body mass) which was tied to the tail's base. The test was conducted in the aquarium with water depth 0.8-0.9m and water temperature 24-26ºC. We determined the duration of swimming till the appearance of complete fatigue which manifested itself by interruption of swimming movements and 10-seconds' immersion of the animal under water [15].

For conducting biochemical and hematological researches blood was taken from the caudal vein.We estimated the levels of lipids' peroxidation (LPO) products in their erythrocytes – malondialdehyde (MDA) and acylhydroperoxides (AHP) [16] with activity determination of catalase and superoxide dismutase (SOD) [17].

Erythrocytes were subdivided into discocytes, reversibly and irreversibly modified forms with the help of light phase-contrast microscope Olympus CX-41 (Olympus, Japan) with magnification 1200. The following

calculation was made on the basis of these indices: values of transformation index= (reversibly modified erythrocytes + irreversibly modified erythrocytes)/discocytes; reversible transformation index = reversibly modified erythrocytes/discocytes; irreversible transformation index = irreversibly modified erythrocytes/discocytes and reversibility index = reversibly modified erythrocytes/irreversibly modified erythrocytes [18].

Received data were processed by Student's t-criterion in the program StatSoft STATISTICA for Windows 6.0.

RESULTS AND DISCUSSION

While aging the observed rats were found to have strengthening of typical external evidences of this process – dull fur, its thinning out, lowering of activity and appetite in animals, absence of interest to the environment, paleness of visible mucous membranes. While aging the examined rats were noted to have physiological increase of body mass reaching in 30 months' animals 378.4±11.25 gr. It was accompanied by gradual lowering of their endurance in the test of forced swimming with some load by 33.9% in 30 months' rats in comparison with 18months' rats and by 42.9% in comparison with the control values.

The experimental animals against the background of their chronological age rise were noted to have the increase of AHP and MDA content in erythrocytes' membranes. Their levels in 30-months' animals surpassed the same ones in 18-months' rats by 27.2% and 26.1%, respectively, and prevailed over the control values by 38.4% and 37.7%, respectively. At the same time, the activity of erythrocyte catalase and SOD in the experimental rats summarily lowered while aging by 37.4% and 35.3%, respectively. At that, their activity in 30-months' animals yielded to the control values by 38.4 and 37.7%, respectively (Table).

While aging the blood of the experimental animals was noted to have gradual lowering of erythrocytes-discocytes' quantity till 70.1±0.19% in 30-months' rats. It was accompanied by gradual quantity increase of erythrocytes' reversibly and irreversibly modified forms (in 30-months' rats in comparison with the control values by 53.7% and in 2.22 times, respectively). Found dynamics of erythrocytes' modified forms was accompanied in rats by reliable increase of the transformation index, the index of reversible transformation and the index of irreversible transformation, and also by lowering of the reversibility index.

The common vitality of a body mostly depends on its genetics [19] and the character of influencing it various environmental factors [20,21]. Great significance in this respect belongs to the state of blood rheological properties [22,23] strongly determining trophic conditions in all the tissues [24]. Taking into account that the processes of metabolism take place on the microcirculatory level, special attention of many researchers is devoted to erythrocytes' rheological properties [25]. At the same time, peculiarities of their changes in aged mammals are still studied rather poorly. It served the cause for the conduction of the given research.

Found lowering of erythrocytes' antioxidant protectability in aged rats leads to activation of lipids' peroxidation in them and causes changes in structural-functional properties of their membranes and protein cytoskeleton. Coming against the background of strengthened LPO weakening of ATP synthesis in aged rats' erythrocytes leads to activity lowering of ion pumps which (because of energy deficiency) gradually lose the ability to manage with displacement of increasing Ca^{2+} and Na^{+} inflow into erythrocytes and maintenance of decreasing (with age) level of intraerythrocyte K^{+} [26]. In the result of Ca^{2+} and Na^{+} content increase in erythrocytes and K^{+} quantity lowering the quantity of water decreases and hemoglobin concentration increases a bit what leads to changes of the form of some of them.

It's possible that disturbances of erythrocytes' cytoarchitecture in aged rats also happen because of changes in the structure of spectrin net in the result of coming against the background of active LPO change of cytoskeletal proteins and distance decrease between spectrin molecules. It promotes the decrease of surface area of lipid bilayer's internal part with the formation of erythrocytes-echinocytes. In these conditions there also take place some changes in phosphor-lipid complex of the membrane with the formation of protein-free sites which are easily joined. Forming Ca^{2+} surplus in erythrocytes of aged rats is linked with the main acid-phosphate polar groups of lipid bilayer and causes the formation of their structural bonds. In aged rats it finally

leads to the decrease of the internal part of erythrocytes' lipid bilayer and appearance of erythrocytes-sphere-echinocytes in blood [27].

In aged rats given situation promotes gradual number increase of erythrocytes which have lost their biconcave form. So, the rats by 30 months have significantly increased quantity of erythrocytes undergone the processes of echinocytosis till the state of spheres with the formation of acanthus of various forms on their membranes and through mechanisms of stomacytosis till unilaterally arched disks. Consequent transformation of these erythrocytes goes fast till sphere-echinocyte, sphere-stomatocyte, leading finally to the formation of spherecytewhich is soon lysed [28]. Coming changes of erythrocytes complicate the processes of their circulation along vessels of the least caliber [29]. Worsening of microcirculation is connected with the fact that going along capillaries modified erythrocytes lengthen worse and take not an ellipsoid form but anugly one. As it is known, this ability is maximally expressed in discocytes–they lengthen maximally from 8 till 17 mcm. In the same conditions spherecytes lengthen just a bit – from 5.5 till 8 mcm, and sphere-stomatocytes – from 5.5 till 7 mcm [26]. Besides, spherecytes constantly rotate in bloodstream and behave as hard particles what makes them the worst variant of erythrocytes in rheological respect [18].

CONCLUSION

While aging the rats were found to have progressive weakening of erythrocytes' antioxidant protection what is accompanied by the increase of LPO products in them. Given situation inevitably leads to damage of erythrocytes' structures and to negative dynamics of their functions. The rats between 18-30 months of life were found to have gradual degree rise of erythrocytes' cytoarchitecture changing what inevitably worsens their microrheological properties. It can essentially contribute to the weakening of anabolic processes in all the tissues of aged rats and to sensitivity rise of their bodies to negative impacts of the environment. Received data can become widely essential in the course of future experimental researches devoted to optimization of microcirculation in late ages with consequent cautious transfer of the received data into gerontological researches on human beings.

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