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Physiological Peculiarities of Erythrocytes' Aggregation in Rats Of Elder Ages.

Medvedev I N*.

Russian State Social University, st. V. Pika, 4, Moscow, Russia, 129226

ABSTRACT

Erythrocytes is one of the most important elements of microcirculation. Changing their ability to aggregation they significantly determine hemodynamic and metabolic homeostasis of tissues and influence the level of many adaptive reactions of a body. Taking into account the importance of erythrocyte microrheological properties in development of various age-connected disturbances, the studying of dynamics of red corpuscles' aggregation in aged rats becomes necessary as they are often the objects of laboratory researches. In our research it was established that rats at the age between 18-30 months had increased quantity of acylhydroperoxides in plasma by 16.9% at weakening of its antioxidant protection by 25.0%. Between 18-30 months of life the rats were found to have strengthening of erythrocytes' aggregative activity with the increase of their summary involvement into aggregates by 32.5% and quantity of aggregates by 25.0% at number lowering of free erythrocytes by 24.7%. In the result of the research it became clear that healthy aged rats had gradual increase of erythrocytes' aggregative activity. It inevitably led to number increase of their circulating aggregates of different sizes. Given changes can essentially contribute to increasing while aging morbid aggravation and sensitivity rise of a body to negative impacts of the environment. Received data can serve the basis for consequent experimental search of approaches to optimization of erythrocytes' microrheological properties in the course of late ages with following cautious transfer of the received data into gerontological researches of a human being.

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

Corresponding author

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INTRODUCTION

Notwithstanding the great progress of biology the urgency of further studying age-specific aspects of a body's functional state of mammals and human beings [1,2]. It is clear that their realization has genetic [3] and environmental components [4,5] in its basis which allow the process of aging to touch all the systems of a body. It progressively worsens their functioning and makes the death of a body more and more possible [6]. The state of blood rheological properties and its regular elements(including the cases of clinical health) in conditions of different pathology and in the result of separate correction variants' impact on a body [7,8] is very significant for the development of aging processes.

Being one of the most important elements of microcirculation, erythrocytes through their ability to aggregation mostly determine hemodynamic and metabolic homeostasis of tissues and influence the realization of many adaptive reactions of a body [9,10]. At the same time, their rheological properties can change at physiological, in-between and pathological states [11]. Elder age is rather vulnerable in this respect as in case of pathology development in an aged body changes of rheological properties of regular blood elements negatively influence microcirculation thus aggravating the course of the disease [12].

At the same time, while searching variants of therapeutic impacts at many pathological states of animals and human beings [13,14] it's impossible to do without application of different experimental models which are mostly conducted on laboratory animals [15]. Taking into account the importance of erythrocyte microrheological properties in development of various disturbances [16], including age-connected thrombophilia, the studying of dynamics of red corpuscles' aggregation in aged rats becomes urgent as they are often the objects of laboratory researches. Received data can serve the basis for consequent experimental search of approaches to optimization of erythrocytes' microrheological properties in the course of late ages with following cautious transfer of the received data into gerontological researches of a human being [17]. That's why we put the following aim in our research – to determine age-specific dynamics of spontaneous erythrocytes' aggregation in aged rats.

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 [18].

For conducting biochemical and hematological researches blood was taken from the caudal vein. The intensity of plasma lipids' peroxidation (LPO) in the observed animals was estimated according to the concentration of thiobarbituric acid (TBA)-active products by a kit "Agat-Med", acylhydroperoxides (AHP) with value detection of antioxidant activity (AOA) of liquid part of blood [19].

The state of erythrocytes' aggregative activity was determined in the course of light microscopy in Gorjaev's box by registering the quantity of erythrocytes' aggregates, number of aggregated and non-aggregated erythrocytes [20]. Basing on the received data we conducted the calculation of the value of an aggregate's average size = the sum of erythrocytes which were in aggregates/the quantity of erythrocyte aggregates. We determined the value of aggregation index = (average size of an aggregate × quantity of erythrocyte aggregates + quantity of freely lying erythrocytes)/(quantity of erythrocyte aggregates + quantity of freely lying erythrocytes). The received data were processed by Student's t-criterion in the program StatSoft STATISTICA for Windows 6.0.

RESULTSANDDISCUSSION

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 examined animals against the background of aging were noted to have activity increase of freely radical oxidation in lipids of liquid part of blood (the levels of AHP and TBA-active products increased by 16.9% and by 12.6%, respectively) at AOA lowering by 16.3%. In comparison with the control group the quantity of AHP and TBA-active products in 30 months' animals increased by 29.9% and by 23.7%, respectively. At the same time, the value of AOA in them yielded to the same one in the group of comparison by 24.1% (Table).

The examined rats while aging were found to have strengthening of erythrocytes' aggregative activity. They had increase of erythrocytes' summary involvement into aggregates and quantity of erythrocytes' aggregates at number lowering of free erythrocytes (228.7±0.31). It was accompanied in aged rats by an upward trend of the value of an aggregate's average size (Table).

The state of structures and functions of a body which provide its vitality, depends on various external and internal factors. Hemostatic and rheological blood properties [21,22] occupy a special place among them. These indices determining the inflow volume of nutrients and oxygen to tissues, inevitably change in ontogenesis under the impact of lots of reasons [9]. The state of regular elements which are under control from the side of vascular wall [23] and LPO processes [11], plays a great role in the dynamics of microcirculation.

In the course of the conducted research it was found out that aged rats had progressively weakening plasma antioxidant activity what caused concentrations' increase of AHP and TBA-products in it. Active LPO in liquid part of blood damaged endothelium of vessels and receptors on outer membranes of regular blood elements, including their most numerous population – erythrocytes, negatively influencing their characteristics [24].

Found in aged rats strengthening of erythrocytes' aggregation was mostly provided by coming changes of their membranes' charge because of degradation of some glycoproteins on their surface under the impact of active LPO. Intensification of oxygen active forms' generation in given conditions provided oxidative alteration of membrane's structures in aged rats at simultaneous damage of plasma globular proteins possessing the ability to be connected like "bridges" between erythrocytes and to realize their aggregation. At the same time, the increase of LPO products in plasma and erythrocytes rose the threshold of their disaggregation because of stimulation of red corpuscles' linkage in aggregates and speed rise of the given process [25].

There is some basis to consider that found in aged rats strengthening of erythrocytes' aggregation is mostly provided by the impact of catecholamines. Their concentration can significantly rise at various disorders in a body, including aging. In these conditions α_1 and α_2-adrenoreceptors are activated on the surface of erythrocytes. Against the background of α_1-receptors' activation the system Ca^{2+}-calmodulin and the cascade of intracellular reactions of phosphatidyl inositol become the main mediator. Activation of α_2-adrenoreceptors leads to suppression of adenylatecyclase in the course of physiological impact from receptors

to Gi-proteins. Both processes cause lowering of cyclic adenosine monophosphate quantity in erythrocytes, stimulate Ca^{2+} inflow into them and provide strengthening of their aggregation [26].

Quantity increase of freely circulating erythrocyte aggregates in blood of aged rats causes damage of their vessels' endothelial lining what promotes uncovering of sub-endothelial structures, stimulation of hemostasis processes and worsening of blood rheology processes [27]. Increasing quantity of freely circulating erythrocyte aggregates can partly block vasa vasorum. It plays a great role in weakening of vascular hemostatic control and disaggregative impacts on erythrocytes in the result of production decrease of nitric oxide and prostacyclin [28] in endothelium.

Table: Consider the parameters in senescent rats

Registered parameters	Aging rats, n=95, M±m			Control, n=27, M±m
	age 18 months, n=32	age24months, n=29	age30months, n=34	
Bodyweight, g	332.6±9.11**	351.6±9.70**	378.4±11.25**	231.6±7.22
Sailing time, s	138.2±4.95*	115.3±5.28**	91.4±6.88**	160.1±5.20
AHP, D_{233} /1ml	1.60±0.024	1.82±0.033*	1.87±0.058**	1.44±0.007
TBA-compounds mcmol / l	3.80±0.016	4.22±0.042*	4.28±0.032**	3.46±0.016
AOA%	30.7±0.32	28.2±0.27	26.4±0.29*	34.8±0.010
sum of all the erythrocytes in an aggregate	32.9±0.15	37.8±0.13*	43.6±0.18**	30.1±0.09
quantity of aggregates	6.4±0.08	7.1±0.11*	8.0±0.09**	6.1±0.06
quantity of free erythrocytes	285.2±0.28	242.6±0.29*	228.7±0.31**	293.0±0.34
aggregation index	1.09±0.006	1.12±0.009	1.15±0.11*	1.08±0.005
aggregate's average size, erythrocytes	5.1±0.07	5.3±0.06	5.4±0.08	4.9±0.05

Legend: the significance of differences of indicators between control and senescent rats – *<0,05; ** – p<0,01

CONCLUSION

The process of aging leaves a mark on all the components of blood system. Aged rats are found to have progressive weakening of plasma antioxidant activity causing increase of LPO products' concentrations in it. Given situation leads to the damage of erythrocytes' outer structures and negatively influences their functions. Healthy aged rats have gradual rise of erythrocytes' aggregative activity which leads to number increase of their circulating aggregates of different sizes. It essentially contributes to increasing with aging morbid aggravation and sensitivity rise of a body to negative impacts of the environment.

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