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AGE-DEPENDENT INFLUENCE OF MINERAL HOMESOSTASIS ON THE ACTIVATION OF ALKALINE PHOSPHATASE IN RATS BLOOD

Alkaline phosphatase (AP) is contained in almost all human and animal body tissues. According to the organ specificity, five fractions of AF are identified. There is an opinion that the properties of the same enzyme from various factions may differ between themselves. In the case of AP this difference is connected with the character of activation by metal ions. As well-known, the active site of alkaline phosphatase contains zinc ions. Nuclear magnetic resonance prove for the existence of three closely-located metal-binding centers in each AP monomer. Two of them contain zinc ion, and the third one – magnesium. The depletion of metal ions leads to reversible loss of enzyme's activity, which may be restored by addition of corresponding ions. At this case the addition of magnesium ions is less effective for AP. The mechanism of AP activation by magnesium ions has not been studied completely, but there is the supposition that they simplify the access of the substrate to the active center by proper placement enzyme's subunits [1; 2].

The generalizing data on the age-related alterations of the content of macro- and micro elements in various organs and tissues of animals and humans remain still incomplete. It is known that at ontogenesis various organs and tissues are able to concentrate selectively one or another macro- and micro-elements. At account both of the important role of such inclusions in the enzymes' activation, and of the value of alkaline phosphatase in the exchange of phosphorus compounds, that are associated with energy generation, we focused on the study on the age-dependent character of the alterations of the influence of mineral homeostasis on the AP activity.

The studies were conducted in the laboratory of the Department of Biochemistry of the National University of Bioresources and Natural Resources of Ukraine. For the experiments three age groups of white non-breeding laboratory rats were used: 3-month old (young), 6-month (puberty period) and 18-month (old age) ones with 10 animals in each. The samples of the blood were taken immediately on decapitation and at preliminary preparation were used for determination of the content of macro- and micronutrients and of the level of AP activity. The content of sodium, potassium, calcium, magnesium, and phosphorus was determined in blood plasma on the

biochemical analyzer «Microlab – 200» (the Netherlands) according to the procedures specified in the instruction. The content in the blood of zinc, iron and copper ions were determined by spectrochemical method using the absorption regime on the atomic absorption spectrophotometer AAS-30 (Germany). Animal rearing and manipulation were carried out in accordance with the Council of Europe Convention for the protection of vertebrate animals used for scientific purposes. The results of the research were processed according to the generally accepted methods of variation statistics using the computer program MSSEcel using the t-criterion of the Student [3]. The results were considered to be reliable at $p < 0.05$.

The results of the studies indicate the age-related decline in AP activity in the blood of rats (Table). Thus, this indicator in the blood of rats at 18 months of age was significantly lower in 1.4 times than in 3 months animals. In the blood of rats in the period of puberty, the activity of AP varied at the level of the trend. Probably with age there is a decrease in mitochondria in the renal tubules, which ensure the normal functioning of the lysosomal hydrolases producing apparatus. In the study of sodium ions content in the plasma of the rats of different age groups, no significant changes were detected. Instead, the content of potassium significantly increased by 1.3 times in the blood of rats of compared with that of young animals.

It is known that the calcium content in the organism of animals is subject to age vibrations [4; 5]. The results of our studies found a reliable reduction in the calcium content of 1.3 times in rats of puberty compared to those in young animals.

Table

Indices of mineral homeostasis and activity of alkaline phosphatase in blood of rats of different age groupss (M ± m, n = 10)

Показники	Animals of different age groups		
	3-months of age	6-months of age	18-months of age
LF, mmol / h · l	242 + 15,4	208,5 + 12,2	175,2 + 10,7*
Sodium, mmol / l	144 + 10,0	145,0 + 10,2	148,5 + 10,5
Potassium, mmol / l	5,20 + 0,33	6,50 + 0,44*	5,34 + 0,40
Calcium, mmol / l	2,45 + 0,18	1,93 + 0,15*	2,20 + 0,15
Phosphorus, mmol / l	2,10 + 0,14	2,20 + 0,14	1,77 + 0,12*
Magnesium, mmol / l	1,85 + 0,12	1,77 + 0,13	1,42 + 0,10*
Zinc, mg / l	1,55 + 0,12	1,80 + 0,13*	1,34 + 0,08
Iron, mg / l	345,0 + 24,2	370,2 + 24,2	276,0 + 19,5*
Coper, mg / l	0,93 + 0,06	1,25 + 0,08*	0,89 + 0,05

Note: * – Changes are likely in relation to indices of rats of 3 months of age ($P < 0.05$).

The activity of AP is one of the indices of the state of phosphorous-calcium metabolism [7, 8]. This enzyme is involved in the transport of phosphorus through

cell membranes. Thus, the content of phosphorus decreased significantly in 1,2 times the blood of rats in the period of old age. It is probably due to the slow formation of energy in an organism with age due to transport of phosphorus through cell membranes.

It is known that with increased content of zinc ions the exchange of calcium, phosphorus and magnesium are disturbed. Calcium inhibits the absorption and removal of zinc [2; 5; 6]. We have established the age-related decrease in magnesium content. Thus, in the blood of old age rats, this index was probably reduced by 1.3 times compared with young ones. Zinc ions content in blood of puberty rats of was 1.2 times higher than that of young ones. Instead, in old age rats this index remained virtually unchanged, which may be due to its effect on the activity of ferromagnetic enzymes, as well as on the inhibition of free radical processes. Iron is constantly manifested in bone tissue, where they plays an important role in the processes of osteogenesis. This is manifested both in the competitive relations of calcium fermentation and with the direct influence on the system of cytochrome oxidases, that are involved in the metabolism of osteoblasts. The results of our studies showed a probable reduction in the content of iron ions in 1.3 times in the blood of 18 months rats at compare with that of 3-month ones. However, the contents of the copper revealed the opposite nature of the changes. In the blood of puberty period rats this index was increased reliably in 1.3 times, whereas in old age animals it remained at the level of young ones. Probably, copper ions, as well as iron ones, affect on phosphoric-calcium metabolism in bone tissue, as well as participate in the processes of mineralization the bones protein matrixes.

Thus, the results of our study prove for age-dependent decrease of AP activity in the blood of animals. The divergent nature of changes in the content of macro- and trace elements in the blood, as the most important activators of this enzyme, are due to the complex interactions between them and their direct participation in metabolic processes. The data presented by us on changes in the indexes of mineral homeostasis and their regulation in the change of AP activity are a small part of the research of compensatory-adaptation mechanisms and can serve as an integral index in assessing of their violation.

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