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THE INFLUENCE OF OXYETHYLIZED ALKYLPHENOLS ON IONIC EXCHANGE IN CONDITION OF SUBACUTE INFLUENCE ON THE WHITE RATS

The influence of the oxyethylized alkylphenols on ionic exchange in condition of the subacute influence on the white rats has been studied. We established membrane pathology and changes of ionic exchange, which are the base of inhibition of bioenergetic processes and tissue respiration.

Key words: oxyethylized alkylphenols, neonols, ionic exchange.

Due to the continuous development of various industries, the number of new chemical substances with surface-active properties, is growing all the time. These substances are currently ranks the first in the world by volume and range of products based on them [1, 2] and are the most common pollutants of the human environment. Numerous literature data indicates that, in spite of its low toxicity in an acute experiment, detergents can have complex influence on the body. Having properties of the surface-active agents (surfactants), they stimulate the resorption of some chemical compounds in the gastrointestinal tract, increasing the amount of cholesterol in the blood, violate the excretory function of the liver, change water and electrolyte balance in the body. [3] Acting on the permeability of biological membranes and disrupting the resorption of different substances in the body, surfactants create real prerequisites of the potentiation strengthening (which has toxic effect) of a mixture of substances, even in cases when these compounds enter the body in a noneffective dose.

At present the problem of environmental pollution by detergents has gone beyond individual states and acquired the status of a global environmental problem. To solve it, the efforts of many experts are needed - ecologists, hygienists, biologists,

pathophysiology and etc. At the same time, there are numerous scientific data showing that activation of the free radical processes (PSA), lipid peroxidation (LPO) and the inhibition of the antioxidant system (AOS) are the leading pathogenetic mechanisms of structural and metabolic abnormalities under the influence on the body by the detergents [1, 2, 3]. According to many authors, quite informative indices in the development of pathological conditions are dynamic changes in the content of metal ions [1, 2, 3]. So far it was found that there are a lot of evidences that metabolic micro - and macro- elements related both to their lack of exposure in the body and in the violation of the biochemical processes which accompany many diseases [1, 4].

In the formation of the violations of the leading functional systems there is a special emphasis on the trace elements that provide the catalytic activity of many enzymes [1, 2, 3]. These include sodium, potassium, calcium, magnesium, zinc, copper, iron, manganese and other. The metal ions play an important role in metabolism and energy, providing the vital processes of organisms. Their content inside and outside the cells is not a permanent feature, it dynamically changes according to the change in the intensity of metabolism and functional activity of the tissues [1, 2, 3, 5, 6]. Metal ions provide a wide range of different functions in the body: structural, transport, hormonal, energetic-transforming, cofactoring, regulatory, detoxification, chemiosmotic and others [11, 12, 13, 14, 15, 16].

Metal ions are the key biochemical metabolic system - enzymes, hormones, vitamins, receptors, nucleic acids, ribosomes, chromatin supramolecular complex. This defines their unique role in various physiological and biochemical processes - fertilization, mitosis, cell maturation, the transition from proliferation to quiescent state, amino acid transport through the membranes of nerve impulses, and others [7, 8, 9, 10]. Studies have shown that the lacks of metal ions, as well as their excessive intake in the body, lead to the metabolic disorders and the development of pathological conditions. Taking everything into consideration, it was topically to study the actual ion exchange in white rats in a subchronic toxicology experiment under the influence of a new group of detergents and to have a substantiation report of their prognostic role in the development of metabolic disorders.

The materials and approaches of research.

The objects of research were a new group of detergents based on the ethoxylated alkylphenols, which has industrial called "Neonols" of the AF grades 9-6, AF 9-10, AF 9-12 with regulated physical and chemical properties. According to the acute oral toxicity Neonols are moderately toxic compounds (hazard class 3), having strongly marked cumulative properties [1, 2]. The exchange of metal ions has been studied in a sub-acute experience on some mature white rats population WAG (weighing 185-210g.). Daily, before feeding the animals, we had been intraperitoneally injecting them through a metal probe with some aqueous solutions of the "neonol" in doses of 1/10, 1/100, 1/1000 DL_{50} for 45 days. Some average lethal doses (DL_{50}) for the "neonol" AF 9-6, 9-10 and AF 9-12 were respectively 4.2, 4.3, and 3.4 g / kg of the animal's body weight. The control group of the animals received the appropriate volume of water. In each of the groups (experimental and control) there were 8-10 animals. All in all in the subacute experiment we used 80 white rats in compliance with the Law of Ukraine "on the protection of animals from cruel treatment" from 21.02.06 № 3477-IV and "The common moral principles of animal experiments " approved by the First National Congress on Bioethics (Kiev, 2001).

The research program was aimed to identify the determination of the content in the serum and red (blood) cells of the potassium, sodium, calcium, magnesium, zinc, copper, iron, phosphorus and manganese by atomic absorption method [17]. For the analysis, the samples were subjected to a preliminary sample digestion and extraction by E.A. Loyko [17] and G.O. Babenko [18]. The extracts were submitted after the incineration in the device, and they measured the absorption of light by atoms of the analyzed element in the gaseous state. Evaporation of the analyzed ions was carried in the flame of a gas burner. According to the degree of the light absorption (with a certain wavelength) by the analyzed elements we recorded the presence of metal ions, which were compared with the standard samples. Statistical analysis of the results of the research was carried out with the help of the

methods of variation statistics and evaluation of significant differences by Student-Fischer.

Results and discussions.

The results showed that the "Neonols" in a sub-acute experiment under the influence of 1/10 DL₅₀ had significantly changed the metal content in the blood serum of the white rats. The dose of 1/100 DL₅₀ caused the minor abnormalities in the metabolic metal ions, and 1/1000 DL₅₀ was inoperative on the indices of ion exchange. Dynamic orientation of ion exchange in the blood serum was characterized by decreasing Na⁺, Ca²⁺ and increasing K⁺, Mg²⁺, P⁵⁺, Fe²⁺, Zn²⁺, Cu²⁺, Mn²⁺. Thus, the studying "Neonols" in a dose 1/10 DL₅₀ led to a reduction of Na⁺ in the serum to 16.8%; 18.6% and 22.3%, calcium to 33.4%, 23.9% and 38 1%, respectively, when under the influence of AF 9-6, 9-10 and AF 9-12 (Table 1). With the reduction of Na⁺ and Ca²⁺, there was the increase of the concentration in the serum of potassium to 65.6%, 50.1% and 72.2%, magnesium - to 197.8%, 208.1% and 240.4%, phosphorus - to 15.7%, 31.5% and 42.1%, iron - to 100.4%, 57.8% and 109.8%, zinc - to 44.4%, 28.9% and 53.6 %, copper - to 121.2%, 89.1% and 118.1% of manganese - to 86.3%, 73.9% and 117.8%, respectively, under the influence of "neonol" AF 9-6, AF AF 9-10 and 9-12.

The highest levels in the serum were characteristic to the ions of magnesium, iron, copper and manganese. It should be noted that an increase of the metal ions in the blood serum might be explained, firstly, by the violation of the structural and functional organization of the connective tissue and its matrix, cytoplasmic membranes of cells, intracellular organelles, metabolic complex supramolecular complexes (energy and synthetic), ribosomes, chromatin , DNA, RNA, enzymes, etc., and secondly, by the reduction of processes associated with the usage of them for the needs of cellular synthetic apparatus [1,2,9,10].

Table 1. The content of metal ions in the blood serum of the white rats influenced by 1/10 DL₅₀ in subacute experiment

The biogenic elements	The Monitoring Group, M ± m			
	The control	AF 9-6	AF 9-10	AF 9-12
K ⁺ (millimole /l)	3.2 ± 0.21	5.3 ± 0.6 *	4.8 ± 0.7 *	5.6 ± 0.4 *
Na ⁺ (millimole /l)	157.4 ± 4.3	130.7 ± 3.5 *	128.2 ± 4.7 *	122.4 ± 5.4 *
Ca ²⁺ (millimole /l)	2.1 ± 0.16	1.4 ± 0.12 *	1.6 ± 0.13*	1.3 ± 0.15 *
Mg ²⁺ (millimole /l)	0.94 ± 0.07	2.8 ± 0.17 *	2.9 ± 0.20 *	3.2 ± 0.25 *
P ⁵⁺ (millimole /l)	1.9 ± 0.17	2.2 ± 0.18 *	2.5 ± 0.22 *	2.7 ± 0.23 *
Fe ²⁺ (millimole /l)	22.3 ± 1.4	44.7 ± 3.2 *	35.2 ± 3.4 *	46.8 ± 3.5 *
Zn ²⁺ (millimole /l)	25.2 ± 1.8	36.4 ± 2.4 *	32.5 ± 1.7 *	38.7 ± 4.2 *
Cu ²⁺ (millimole /l)	17.4 ± 1.3	38.5 ± 3.7 *	32.9 ± 2.6 *	37.6 ± 3.1 *
Mn ²⁺ (millimole /l)	14.6 ± 1.1	27.2 ± 1.8 *	25.4 ± 2.1 *	31.8 ± 2.9 *

Note: * - the differences are significant, p <0.05.

Some researches have convincingly shown that the reduction of the concentration of ions in the sodium and calcium in the blood serum may be found due to the increase of the process of removing them from the body and increase of the need for the use of them for the anabolic purposes [1, 14, 15, 16]. Considering, firstly, cofactor, regulatory, structural, transport and energy function of ions of Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe²⁺, Zn²⁺, Cu²⁺, P⁵⁺ and Mn²⁺, as well as establishing the dynamics of their content in the blood serum, it can be assumed that under the influence of the "neonol" in the forefront there will be the structural changes of different membranes, the violation of redox processes, bioenergy and tissue respiration. There was revealed relatively high levels of K⁺, Mg²⁺, Fe²⁺, Cu²⁺, Zn²⁺ against the background of the decrease in Na⁺ and Ca²⁺, which also may indicate the violations of acid-base balance in the rats under the influence of the "neonol."

The determination of the metal ions in red blood cells revealed in the majority of cases the opposite direction of the indicators compared with the blood serum. The dynamics of ions was characterized by the increase of sodium in the red blood cells and the reduction of potassium, calcium, magnesium, phosphorus, iron, zinc, copper and manganese (Table 2). The survey showed the decrease under the influence of the neonol AF 9-6, AF 9-10, AF 9-12/10 DL₅₀ potassium - to 38.1%, 32.2% and 48.7%, calcium – to 42.9% , 33.4% and 48.8%, magnesium - to 36.3%, 29.3% and 35.4%, phosphorus - to 45.6%, 31.1% and 48.9%, iron - to 35.1%, 28.5% and 39.8%, zinc - to 23.5%, 13.4% and 25.3%, copper - to 53.5%, 36.9% and 57.8% , manganese - to 35.8%, 12.9% and 42.5%, respectively.

It should be noted that all Neonols had significantly increased the level of sodium in the red blood cells, "neonol" AF 6.9 increased Na⁺ in erythrocytes to 173.2%, "neonol" AF 9-10 – to 250% and AF 9-12 – to 217% compared to the control group. A significant increase of Na⁺ by the "neonol" against the background of the decrease of K⁺ in red blood cells might indicate a problem of structural and metabolic activity of the plasma membrane Na⁺, K⁺ [9, 13, 15, 16]. The reduction of the concentration of Ca²⁺, Mg²⁺, P⁵⁺, Fe²⁺, Zn²⁺, Cu²⁺, K⁺, and Mn²⁺ in red blood cells may indicate that in these enucleated cells were inhibited the oxidation-reduction processes, which accompany the suppression of bioenergy recovery synthesis, disruption of transport functions of substrates through plasma membrane of cells and the ability of the cells to deliver oxygen to the peripheral organs and tissues. Dysfunction of the exchange of ions in Na⁺, K⁺, Zn²⁺, can lead to acid-base balance that against the background of the membrane pathology and reduction of iron, copper in red blood cells may be accompanied by the hypochromic anemia [1, 10, 11]. We should pay some special to the content results of ion Na⁺ and K⁺ in erythrocytes. Many authors have convincingly shown that in some diseases there is the intracellular increase of Na⁺ ions and the reduction of K⁺ ions, besides even a slight increase in the concentration of Na⁺ intensifies the synthesis of DNA, RNA and proteins [15, 16].

Table 2. The content of metal ions in erythrocytes of the white rats influenced by the “Neonol”

The biogenic elements	The Monitoring Group, M ± m			
	The control	AF 9-6	AF 9-10	AF 9-12
K ⁺ (millimole /l)	90.7 ± 4.5	56.2 ± 4.8 *	61.5 ± 3.7 *	53.8 ± 2.9 *
Na ⁺ (millimole /l)	5.6 ± 0.7	15.3 ± 1.4 *	19.6 ± 1.2 *	17.8 ± 1.6 *
Ca ²⁺ (millimole /l)	0,84 ± 0.06	0.48 ± 0.03 *	0.56 ± 0.04*	0.43 ± 0.05 *
Mg ²⁺ (millimole /l)	3.25 ± 0.18	2.05 ± 0.13 *	2.30 ± 0.06 *	2.10 ± 0.14 *
P ⁵⁺ (millimole /l)	39.7 ± 2.4	21.6 ± 1.5 *	27.4 ± 1.8*	20.3 ± 1.4 *
Fe ²⁺ (millimole /l)	238.2 ± 15.3	154.5 ± 7.8 *	170.4 ± 10.3 *	143.6 ± 6.5 *
Zn ²⁺ (millimole /l)	158.6 ± 7.2	121.3 ± 6.1 *	137.4 ± 7.2 *	118.6 ± 5.8 *
Cu ²⁺ (millimole /l)	43.4 ± 3.7	20.2 ± 1.8 *	27.4 ± 2.3 *	18.3 ± 1.6 *
Mn ²⁺ (millimole /l)	17.9 ± 1.3	11.5 ± 1.2 *	15.6 ± 1.4 *	10.3 ± 1.1 *

Note: * - the differences are significant, p <0.05.

It was also found that the direct blocking of Na - channels in the outer membranes and stopping the flow of Na + ions in cells lead to a suppression of the initiation of DNA, RNA and proteins synthesis. The results of recently published researches clearly show that there are loci on chromosomes, activation and repression of which, occur in strictly defined proportions of monovalent cations Na +, K +, and are caused by the change of this ratio in the nuclei [6, 12].

The results of the sodium-potassium exchange showed the increase in K + but the decrease in Na + in the serum, while in the erythrocyte the orientation of their exchange was the opposite – there was the decrease of K + in erythrocytes, and the increase of Na +. Dynamics of monovalent cations may indicate the membrane

pathology and inhibition of the enzyme activity of $\text{Na}^+ / \text{K}^+ - \text{ATPase}$, which provides the ion concentration gradients across the membrane of red blood cells and other cells. The researchers had found that the ions Ca^{2+} and Na^+ launch early metabolic changes in the cell, control the level of biosynthesis and activity of enzymes, proteins, the processes of fertilization and early embryogenesis, provide active transport of substances, tissue respiration, the reduction of muscle tissue, are involved in the function of coagulation and anticoagulation system of blood, have regulatory, hormonal and structural functions [12, 17, 18].

CONCLUSIONS.

Therefore, the results of the study have shown that in a sub-chronic exposure of the "neonol", we are able to observe the membrane pathology and profound changes of the ion exchange, which can be the basis of the inhibition of bioenergy and tissue respiration processes. This fact requires the need for the membrane-protective correction and normalization of ion homeostasis. The violations of ion exchange of the monovalent cations Na^+ and K^+ , create favorable conditions for the metabolic induction of tumor growth and may be a prognostic indicator of the growing process of differentiation and proliferation of tissues at a delay of Na^+ cations in the body and increase of the excretion of K^+ with urine. In all cases, the "Neonols" in 1/1000 DL_{50} had no effect on the ion exchange.

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