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MORPHOMETRIC CHARACTERISTICS OF HEPATOCYTE MITOCHONDRIA FOLLOWING PERORAL ADMINISTRATION OF WATER CONTAINING BORON

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Key Words: liver mitochondria; boron-containing waters; morphometric analysis

Boron compounds are widely distributed in nature, and they invariably occur in the composition of both ordinary drinking waters and of mineral waters; they may reach high concentrations, moreover, in the latter [4, 8]. If boron-containing waters are taken internally, they can modify coupling of oxidation and phosphorylation [5] and can intensify processes of preparative regeneration [2]. Meanwhile many aspects of the biological and therapeutic action of boron-containing waters remain to be studied.

The aim of this investigation was to study changes in hepatocyte mitochondria and the boron content in the liver of animals taking internally the natural Semigor'e and artificially prepared boron-containing waters (NW and AW respectively), containing equal concentrations of boron, but differing in their ionic salt basis.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 200-250 g. The experimental animals were given perorally 3 ml of sodium chloride-hydrocarbonate NW with a boron concentration of 250 mg/liter (chemical composition of the water: $M_{10.6} = (HCO_3 \cdot 63Cl \cdot 34)/Na \cdot 96$ (pH 8.0) and AW in the form of an aqueous solution of sodium tetraborate with a boron concentration of 250 mg/liter (pH 8.0). The daily dose of boron was 3.75 mg/kg. Animals taking piped water served as the control. The course consisted of 24 procedures. The animals were killed by decapitation after 15 and 24 procedures, and also on the 6th day after the end of the procedures. The boron content in the liver was determined by a spectrophotometric method with H-resorcin [6]. Pieces of liver tissue for electron microscopic investigation were fixed in 3% glutaraldehyde, made up in phosphate buffer (pH 7.37), and then post-fixed in OsO_4. The material was dehydrated in alcohols of increasing concentrations and propylene oxide, and embedded in a mixture of Epon and Araldite. Ultrathin sections were examined in the IEM-100C electron microscope. For quantitative estimation of the state of the mitochondria we used stereometric system analysis [1] and automated morphometry, with the aid of an IBAS-2 television image analyzer ("Opton," West Germany). Measurements were made automatically under IBAS-2 control, using a program developed in the Laboratory of Cytology, Research Institute of Physicochemical

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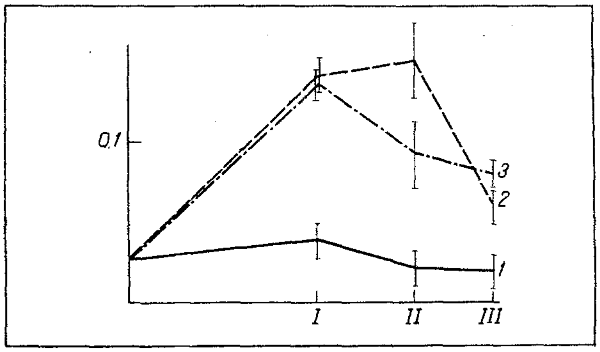

Fig. 1. Time course of boron accumulation in liver following internal administration of boron-containing waters. Abscissa, here and in Fig. 2, times of investigation: I) 15th procedure, II) 24th procedure; III) 6th day after end of procedures. 1) Piped water (control), 2) AW, 3) NW. Ordinate, boron concentration (in μg/g).

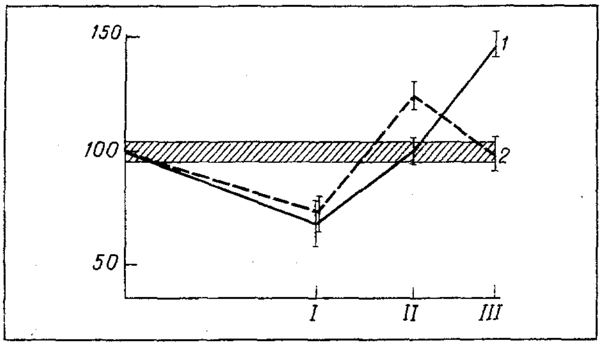

Fig. 2. Dynamics of area of mitochondria during internal administration of boron-containing waters. Ordinate, area of mitochondria (in % of control, taken as 100%). 1) AW, 2) NW.

Medicine, Ministry of Health of the RSFSR. The following parameters were measured: the area, the ellipticity factor ($F_{ell} = a/b$), and the form factor (curvature of the membranes) of the mitochondria ($FF =$ (area/perimeter^2) 4π). The results were subjected to statistical analysis, with plotting of histograms and correlation-regression analysis. The number of mitochondria was counted in 1 μ^2 area of section. To represent the changes in area of the mitochondria graphically, normalization was carried out to control values. The representativeness of the quantitative data was ensured by following the recommendations in [1].

EXPERIMENTAL RESULTS

After internal administration of the boron-containing waters boron accumulated in the liver, and the process depended on the type of water consumed (Fig. 1). A noteworthy feature of the distribution of boron was that its concentration fell actually during the intake of NW, evidently on account of the high degree of mineralization of this water.

Microscopic investigation of the liver after 15 procedures of administration of NW and AW to the animals revealed basically similar changes. These included a marked decrease in size of the hepatocytes, the formation of translucent structureless zones in the cytoplasm, and the appearance of small mitochondria, usually with an electron-dense matrix. The area of the mitochondria was reduced by 27-30% ($p < 0.01$) compared with the control (Fig. 2), but their mean number in the cell was unchanged.

Later during the investigation differences in the action of the boron-containing waters were manifested more clearly. Toward the end of the course of NW the dimensions of the hepatocytes and their nuclei were increased by 18% ($p < 0.05$) and by 15% ($p = 0.05$) respectively. The number of mitochondria was reduced ($p < 0.05$) but their area was increased, to exceed the

TABLE 1. Morphometric Characteristics of Liver Mitochondria (in relative units; $M \pm m$)

Water	Ellipticity factor	Form factor	Ellipticity factor	Form factor
	24th procedure		6th day after end of procedure	
Containing boron (control)	0,68±0,01	0,78±0,01	0,68±0,01	0,79±0,01
NW	0,55±0,02**	0,66±0,02***	0,68±0,01	0,78±0,02
AW	0,60±0,01*	0,74±0,01*	0,63±0,01*	0,74±0,01

Legend. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with control.

control value by 26% ($p < 0.01$). In many hepatocytes, well-marked polymorphism of the mitochondria also was found. Reduction of the values of their ellipticity and degree of curvature of the membranes also was determined morphometrically in this series: by 19% ($p < 0.01$) and by 15% ($p < 0.05$; Table 1) respectively, evidence of an increase in the number of more elliptical mitochondria and in the number of those with altered shape. Analysis of the histograms representing these parameters also enabled these mitochondria to be isolated into a separate group, not occurring in the control. Correlation-regression analysis revealed strong correlation between the ellipticity and degree of curvature of the membranes ($r = 0.94 \pm 0.01$ compared with $r = 0.81 \pm 0.01$ in the control), evidence that with the assumption of the elliptical form the mitochondrial membranes at the same time became more curved. These parameters returned closer to the control values 6 days after the end of the course of procedures with NW.

The particular features of the action of AW were as follows. After the course of taking the waters, the size of the cells and area of the mitochondria returned to the control levels, but their mean number was unchanged. The ellipticity of the mitochondria and the degree of curvature of their membranes were reduced, although by a lesser degree than in animals taking NW. Correlation between these parameters was weaker ($r = 0.78 \pm 0.01$; in the control $r = 0.81 \pm 0.01$), and identification of a separate group of mitochondria was not observed on the histograms. Toward the 6th day after the end of the course changes in the mitochondria became more marked: they were swollen, their matrix became translucent, and their cristae were disorganized. The area of the mitochondria increased by 48% ($p < 0.001$) compared with the control, and by 78% ($p < 0.001$) compared with that at the 15th administration of the waters. The number of mitochondria, on the other hand, fell significantly ($p < 0.05$). The ellipticity and curvature of the membranes still remained below the control levels. Analysis of the histograms showed that mitochondria with a change only in the value of their ellipticity constituted a separate group.

Thus changes in the hepatocyte mitochondria were linked with changes in the boron content and were adaptive in character. It was shown that, irrespective of the type of boron-containing waters, after the first 15 procedures, i.e., in the period of boron accumulation in the liver, a decrease in area of the mitochondria was observed. Conversely, in the period of a relatively stable boron content, characteristic of AW, and in particular, during elimination of boron from the body, which took place at different times of the experiment, and was related to the type of boron-containing water, an increase in the area of the mitochondria and moderate translucency of their matrix were observed. However, within this uniform type of reaction of the mitochondria, they showed differences in their ellipticity and in the degree of curvature of their membranes. Consequently, ellipticity and curvature of the membranes, and determination of correlation between them, provide a basis for distinguishing characteristic structural differences in the nonspecific reaction of the mitochondria, so that, in conjunction with visual analysis, a more profound assessment of the state of these organelles can be made.

Comparison of the results indicates that boron itself plays the role of basic active factor, and that the ionic-salt composition of the boron-containing water has a substantial influence on the mechanism of its action. It can be postulated that the changes discovered are based on changes in membrane permeability of the structures and changes in ionic equilibrium in the liver tissues, for boron can modify the exchange of several chemical elements and, in particular, of calcium [2].

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MORPHOLOGICAL AND HISTOCHEMICAL ANALYSIS OF THE CENTRAL EFFECT OF ANGIOTENSIN II ON RATS PREVIOUSLY EXPOSED TO EMOTIONAL STRESS

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Previous exposure to emotional stress significantly modifies drinking behavior induced by central administration of angiotensin II (A-II). The latent period of the drinking response was considered shortened and the response itself was intensified. Other forms of behavior accompanying the drinking reflex, namely grooming, moving around the cage, eating food, and so on, were not present in the animals subjected to previous emotional stress. It has been suggested that the changes mentioned above in drinking behavior are due to stress-induced structural and neurochemical damage.

This paper presents morphological and histochemical data on the state of several brain formations, responsible for the drinking reflex following intracentral administration of A-II. Comparison of intact animals and animals previously exposed to emotional stress can be used to identify the role of central structures of feeding behavior in stress-induced changes in the drinking reflex in rats.

EXPERIMENTAL RESULTS

Experiments were carried out on male Wistar rats weighing 250-300 g. Three of the five groups of animals (1st, 3rd, and 5th) were chosen for morphological and histochemical analysis. Rats of group 1 received an injection of A-II ("Serva") in a dose of 100 ng in 5 μl of physiological saline into the lateral ventricle. Animals of group 3 received an injection of A-II, after previous exposure to immobilization stress. Rats of group 5 were immobilized after having previously been scalped. Animals kept under the same conditions in the animal house served as the control. Five animals were studied in each group. After decapitation the brain was removed in the cold and fixed in Carnoy's mixture. Serial paraffin sections were stained by Einarson's method for determination of total nucleic acids (NA) [9]. Quantitative estimation of NA was carried out on the LYUMAN I-3 microscope with FMÉL-1 photometric attachment, and filter with wavelength of 570 nm. The density of the photic flux was measured in the cytoplasm and nucleoli of the neurons in the test structures, after which the optical density of basophilic granules in the cells was determined by the equation:

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