Changes in the State of Mitochondria and Intercellular Junctions of Hepatocytes for the Internal Application of Siliceous Water

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Structural-adaptive alterations in mitochondria and intercellular junctions are found in the liver against the background of elevated silicon when mineral siliceous water and silicon solution are applied internally. It is established that an increase of the number of tight and gap junctions takes place during the period of declining bioenergetic processes in hepatocytes. As the number and area of mitochondria increase, the extent of the intercellular junctions shrinks. These changes may be considered to be important components in the mechanism of action of siliceous water.

Key Words: *hepatic mitochondria; intercellular junctions; siliceous water*

The data thus far obtained attest to an active influence of water-soluble silicon compounds on the metabolic processes in tissues. When delivered to the organism in large amounts with food and water, silicon accumulates in various internal organs, particularly in the liver [3], while on the cellular level it is mainly concentrated in the nuclei and mitochondria [9]. In such cases changes are found in phosphorylation [1] and in the metabolism of calcium, phosphorus, proteins, and lipids [8]. Silicon is thought to be a constituent of the surface layer of the plasma membrane in cells and to influence their structure and permeability [4]. In this connection a study of the effect of mineral drinking water which contains high silicon concentrations (to 200-400 mg/liter) besides an ion-salt base is of practical interest.

The goal of the present investigation was to study the effect of mineral siliceous drinking water (MSW) and silicon solution (SS) on alterations in the liver mitochondria and intercellular contacts.

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MATERIALS AND METHODS

Experiments were carried out on 45 male albino rats weighing 200-250 g. Test animals were administered 3 ml sulfate-hydrocarbonate soda water with a silicon concentration of 200 mg/liter (the chemical formula of this water is $M_{7.3}$=HCO_3×64 SO_4×22/Na×88, pH 7.0) and sodium metasilicate aqueous solution with a silicon concentration of 200 mg/liter (pH 7.0). The daily dose of silicon was 3 mg/kg. The control animals received tap water. The course of treatment consisted of 24 procedures. The animals were decapitated after 15 or 24 procedures or on the 6th day after course completion. The silicon content in the liver was measured by a plasma spectrometry technique using a Jobin Yvon 70 Plus apparatus. For the light microscopic study the material was fixed in Carnoy fluid. Sections were stained with hematoxylin and eosin. For electron microscopy the pieces of liver were fixed in 3% glutaraldehyde, postfixed in 2% osmium tetroxide, dehydrated in ascending alcohol concentrations and propylene oxide, and embedded in an Epon-Araldite mixture. Ultrathin and semithin sections were obtained with an LKB-1 ultramicrotome and viewed in a JEM-100B electron microscope. For assessment of the state of mitochondria the stereometric method was used along with automatic morphometry with a Videoplan (Contron) apparatus. The indexes determined were as follows: the area, maximal diameter, the number of cristae, factor of ellipticity ($F_{ELL}=a/b$, where a is the lesser and b the greater diameter of mitochondria), and form factor (curvature of membrane) of mitochondria F_F=area/perimeter$^2\times4\pi$. The number of mitochondria was calculated per μ^2 of the section area. The coefficient of mitochondrial energization was computed as the product of the mitochondrial area times the number of cristae in the mitochondria [6]. The number and extent of intercellular junctions per arbitrary length of plasmalemma was measured [10]. Mathematical treatment of the data was performed as well as correlation-regression analysis.

RESULTS

As Fig. 1 shows, the silicon content was 4-5 times (p<0.05-0.01) increased in the liver after 15 procedures of MSW administration as compared to the control. By the 24th procedure of MSW drinking, silicon accumulation in the liver ceased, probably due to the ion-salt base in this water, while SS treatment resulted in further silicon accretion.

The revealed structural shifts on the whole show evidence of a similar nature of development, but with individual specificity in dependence on the type of siliceous water. An increase of hepatocyte size, which was particularly noticeable toward the end of the MSW procedures, was established in the microscopic study. In this case the area of the cells and nuclei increased by 12.9 and 10.9% (p<0.05), respectively, compared to the control. The mean number of nucleoli in the nucleus increased by 13-15% (p<0.05-0.01) at all times of study.

On the ultrastructural level marked changes were noted in the mitochondria and intercellular contacts, the interrelationships between which are reflected in Fig. 2. It was found that after 15 procedures of MSW administration the number and area of mitochondria was 45% (p<0.001) and 23.2% (p<0.01), respectively, lower than in the control (Fig. 2, b). By the end of the course the number of mitochondria rose markedly, but their size remained small. Toward the 6th day after completion of the course the indexes returned to the control level with the mitochondria becoming more oval and the membrane curvature decreasing (F_{ELL}: control 0.66±0.02, experiment 0.73±0.02, p<0.05; F_F: control 0.81±0.01, experiment 0.86±0.01, p<0.05). The correlation between these indexes was r=0.87±0.01 versus 0.78±0.01 in the control. Dystrophic alterations were not found in the mitochondria.

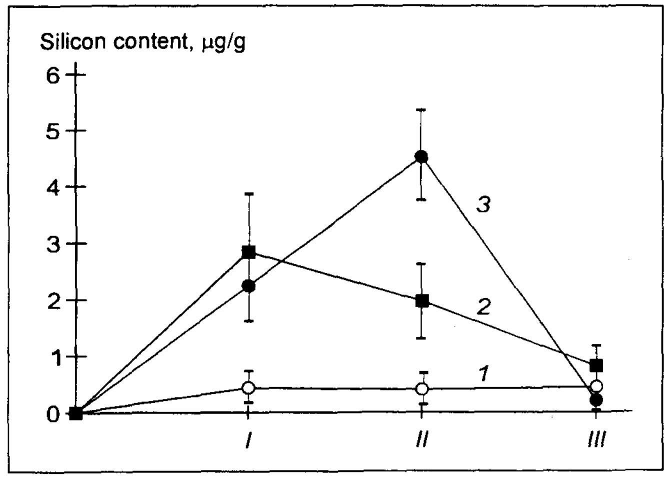

Fig. 1. Dynamics of silicon accumulation in the liver during internal application of siliceous water. *1*) control; *2*) mineral siliceous water; *3*) silicon solution. Here and in Figs. 2 and 3: times of the study are plotted on the abscissa: the 15th (*I*) and 24th (*II*) procedures, and the 6th day after the completion of procedures (*III*).

The specificity of SS action is depicted in Fig. 2, d. A reliable 28.8% (p<0.05) decrease of the number of cristae was noted toward the 15th procedure, while the diminution of the number and area of mitochondria was expressed to a lesser degree (28.3 and 18.9%, p<0.01, respectively). Recovery of the indexes occurred as soon as the course was over.

Thus, it may be assumed that, on the whole, these waters lowered the level of bioenergetic processes in the cell up to the 15th procedure and thereafter their values recovered and according to some indexes even exceeded the control.

Changes in the structure of the hepatocyte intercellular junctions mainly manifested themselves in a length increase. In Fig. 2, a it is shown that by the 15th procedure of MSW drinking, precisely during the period of the maximal decrease in the number and area of mitochondria, the extent of both tight and gap junctions was 2.5 times (p<0.001) increased. These shifts occurred due to an increase of the mean length of each contact and of their number. The peculiarity of SS action (Fig. 2, c) consisted in an increase of the length of tight junctions only, while the gap junctions did not change. At later times the length of the intercellular contacts under conditions of MSW and SS treatment gradually decreased, without, however, reaching the control level. Figure 3, a, b shows curves which reflect in general form the relation between the total length of both types of intercellular junctions and the coefficient of mitochondrial energization. It is seen that during the period when the bioenergetic processes are most decreased the extent of the intercellular junc-

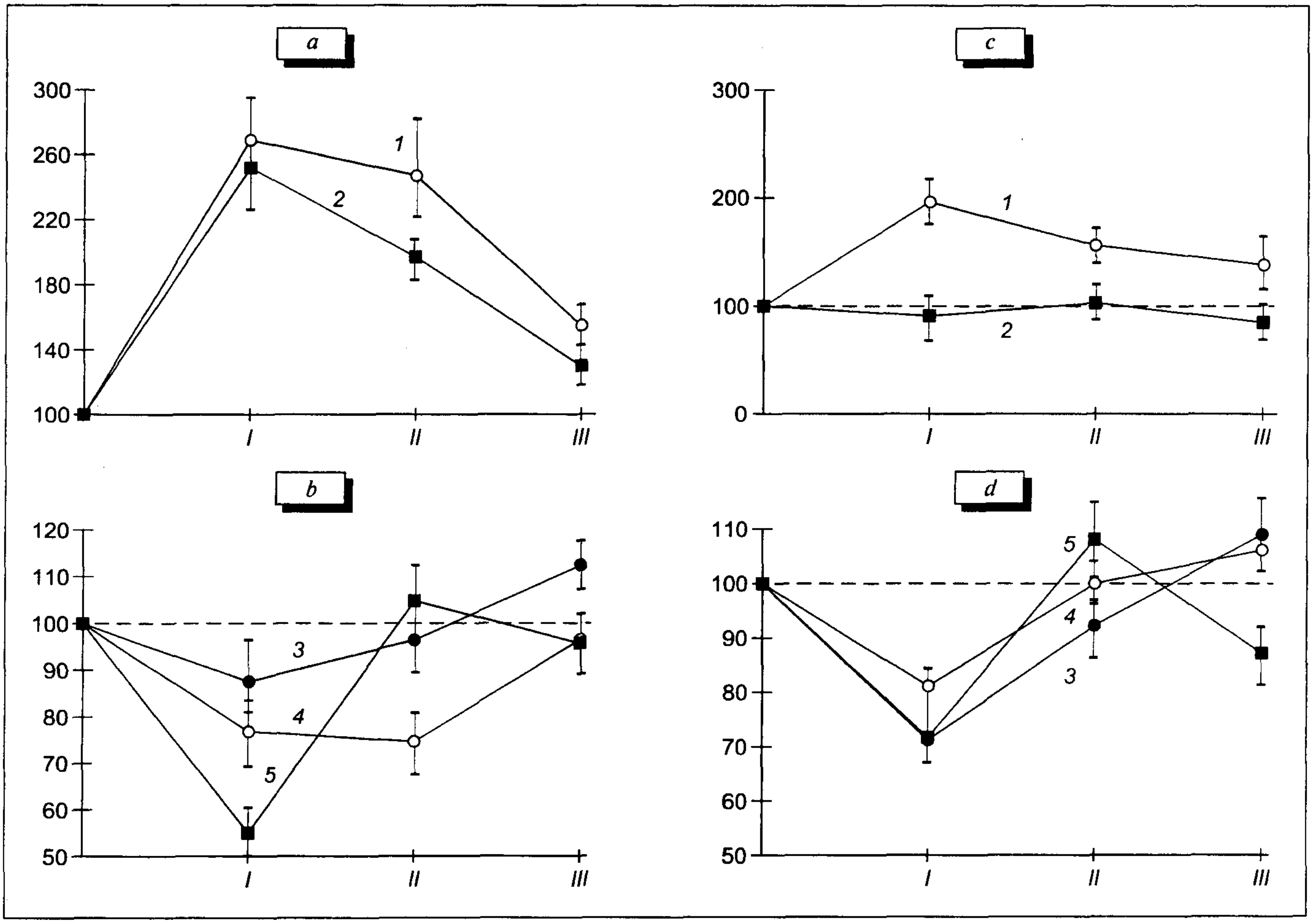

Fig. 2. Interrelationship of alterations between morphometric indexes of mitochondria and the extent of the intercellular contacts of hepatocytes for internal application of mineral siliceous water (*a*, *b*) and siliceous solution (*c*, *d*). Tight (*1*) and gap (*2*) types of intercellular contact; number of cristae in mitochondria (*3*); area (*4*) and the number (*5*) of mitochondria. Here and in Fig. 3: the ordinate shows the morphometric indexes (in percent of the control, 100%).

tions increases, whereas towards the end of the course and 6 days later these indexes approximate the control level.

Therefore, a correlation in the dynamics between the mitochondria and intercellular contacts in hepatocytes was revealed during the internal use of sili-

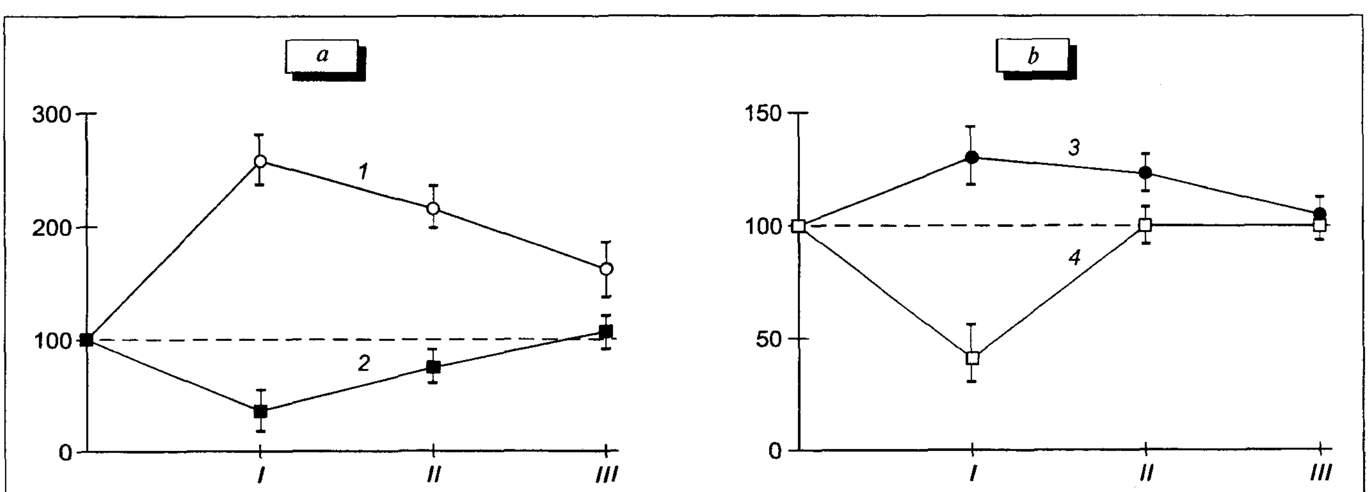

Fig. 3. Relationship between total length of intercellular contacts (*1*, *3*) and coefficient of mitochondrial energization (*2*, *4*) under conditions of internal application of mineral siliceous water (*a*) and siliceous solution (*b*).

ceous water against the background of silicon accumulation in the liver. These processes develop in two phases. In the first period (up to the 15th procedure) a decrease of the bioenergetic supply of hepatocytes was found as well as an increase of the number of intercellular contacts. The latter, as regulators of metabolic processes between cells [2,11], presumably assist the development of structural-adaptive reactions which form the basis of the second phase of shifts (after the 15th procedure). As they form, in particular, as the number and area of mitochondria increase, the need for intercellular junctions gradually lessens, which is expressed in a shrinking of their length. The return to the control level of bioenergetic indexes occurs more rapidly than in the case of the intercellular contacts.

Comparative analysis of the effect of both types of water showed that the silicon contained in it plays an important role in determining the nature and pathway of the main alterations, whereas the ion-salt base of MSW boosted the expression of a number of indexes and widened the range of action of the water. Thus, the involvement in the reaction of the gap junctions, which are usually unresponsive to silicon, may be interpreted as an influence of the MSW ion-salt base because it is this type of contact which regulates ion exchange between the cells [5,7]. On the whole, the changes found in the mitochondrial apparatus and intercellular junctions of hepatocytes suggest that they are important components in the mechanism of action of siliceous water.

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