JAP&T TPA-Timişoara

Available online at www.japt.tpa.usab-tm.ro

Journal of Agroalimentary Processes and Technologies 2008, 14(2), 361-366

Journal of Agroalimentary Processes and Technologies

Peculiarities of hepatic metallograms in rabbits after nitrates administration

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Abstract

The impact of the administration of sodium nitrate on hepatic metallograms of rabbits was evaluated using the atomic absorption spectrophotometry technique. The experimental model created in order to evaluate the effects of nitrates on the biochemical homeostasis, regarding especially the variations of some macro- and trace elements in liver, was based on the administration of sodium nitrate by drinking water in high concentrations related to the established value of maximum contaminant level (MCL). The investigation followed the effects of sodium nitrate (NaNO3) administration on two groups of rabbits in concentrations of 20 x MCL and 40 x MCL. The analytical determinations underline the effects of nitrates on biochemical homeostasis of macroelements (Na, K, Ca, Mg) and trace elements (Zn, Fe, Cu, Mn) by determination of their concentrations in thes liver of rabbits.

Keywords: hepatic metallograms – rabbits.

1. Introdution

Biomineral compounds present in the organism are involved in the morphogenetic processes that occur at the level of the connective tissues and especially in support tissues - cartilage and bones. They are found as bioconstituents and also as biochemical effectors in the structure of some enzymes (functioning as activators or inhibitors. The metallic ions have an important role in the maintenance of the osmotic pressure of liquids environment in physiological parameters; maintenance of the normal physico-chemical status of the body's colloid and of colloid oncotic (colloid-osmotic) pressure; in genesis of the buffer system from liquid environment (especially blood); in the function of transmembranal transit at the level of cellular membranes (e.g.: Na-K pomp), etc. (Ghergariu, 1980; Grecu et al., 1982; Denac et al., 2000).

In this paper the homeostatic variations of some macro elements (Na, K, Ca, Mg) and some trace elements (Zn, Fe, Cu, Mn) in rabbits liver was followed.

2. Materials and methods

Experimental model. In the tap water of rabbits sodium nitrate ($NaNO_3$) was added. The effect of this substance, at "ad libitum" consumption, on the mineral metabolism of rabbits was studied. For this experiment it was taken as a reference value, the maximum contaminant level (MCL) admitted in drinking water, a value established by the Environment Protection Agency (EPA) from

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United States Department of Agriculture (USDA) to 10 mg/L nitrogen nitrate (usually noted as N-NO_3) and 1 mg/L nitrogen nitrite (usually noted N-NO_2) – see Ghibu et al., 2007.

In this study rabbits (Oryctolagus cuniculus) with the age of 4 weeks and the average weight of 700 ± 25 g were used. The rabbits were included in 2 groups. Each experimental group (E_A and E_B) comprised 5 animals (3 males and 2 females) and were fed with VivaBio - a granulated fodder for rabbits produced by Freeman S.R.L (content: 14.94% protein, 2.86% fat and 8.51% cellulose and necesary minerals). Animals from E_A group received a sodium nitrate solution with a concentration equivalent with 20 x MCL. The E_B group received a sodium nitrate solution with a concentration equivalent with 40 x MCL established for nitrite in drinking water.

Before the beginning of the experiment, a quarantine period of 10 days was kept. In this period it was observed the health status and the rabbits were accommodated with the experimental laboratory environment and with the diet made of granulated fodder based on alfalfa. After the quarantine, the experiment has started and lasted 20 days.

In order to collect liver samples, the subjects were killed. The procedure was made according to O.G. nr. 37/2002 using ketamin administrated intravenously. After killing the rabbits, liver samples were taken according to the techniques of laboratory animals necropsy and biological samples were collected. The obtained liver samples were put in 25 ml glass bottles and placed in a refrigerator until the biochemical investigations were performed.

Biochemical investigations. On the liver samples of rabbits from experimental groups (E_A şi E_B), the determination of macroelements – Na, K, Ca, Mg – and trace elements – Cu, Zn, Mn, Fe – were made.

The investigations were performed in the "Laboratory of molecular and atomic spectroscopy" of the Faculty of Food Products Technology from Timişoara.

Each liver samples were weighted and then were calcinated at 700 ºC for a 3 hours time, with a preseted period of 30 minutes in which the temperature in the calcinator rises gradually from the room temperature to 700 ºC. The obtained ash was the mineralized with nitric acid (0,5 N) and brought in gradated flasks of 50 ml in order to be analyzed using a spectrophotometer with continuous atomic absorption. The model of the spectrophotometer was Analytik Jena ContrAA 300. The obtained results were given in micrograms/grams (µg/g).

Statistical analysis. Mean values ($\overline{X}$) and standard deviation (SD) were determined for each parameter obtained.

3. Results and discussions

Liver macro elements investigated in this study were: Na, K, Ca and Mg. The results obtained are presented in table 1.

The obtained analytical data revealed that sodium nitrate determines homeostatic changes in liver metallograms. A direct proportional increase of sodium, potassium and magnesium concentration with the increase of ammonium nitrates dose was observed while, in case of calcium concentration a reverse proportionality was revealed.

Decrease in calcium concentration and increase of sodium concentration might be explained by the fact that Na and Ca have extracellular location, predilectly. Their concentration may maintain osmotic and colloid osmotic balance.

The statistical evaluation did not follow the determination of the Student test because the evaluation of homeostatic variations of macro- and trace elements had in view only the effects of various concentrations of $NaNO_3$ on similar experimental groups.

Tabel 1. Concentrations of macro elements in rabbit liver

Group	UM	n	Na	K	Ca	Mg
			$\overline{X} \pm SD$	$\overline{X} \pm SD$	$\overline{X} \pm SD$	$\overline{X} \pm SD$
E_A	μg/g	5	1069.18 ± 25.21	1767.21 ± 26.80	124.49 ± 46.37	245.68 ± 22.57
E_B	μg/g	5	1145.60 ± 00.00	1831.45 ± 17.90	103.67 ± 7.25	259.64 ± 27.53
$\Delta X = X_{E_B} - X_{E_A}$			+ 76.42	+ 64.24	- 20.82	+ 13.96

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The main macro elements in the organism are Na, K, Ca and Mg. It is known the fact that K and Mg are found especially inside the cells (intracellular compartment) while Na and Ca are in higher concentration in the various biological fluids of the body (extracellular compartment).

Studies concerning the action of nitrates on biological systems are of interest for xenobiochemistry (Garban, 2005; Ghibu et al., 2007).

Macro elements are important bioconstituents in the organism, with structural and functional role in the skeletal system, the nervous system, the cardiovascular system, etc.

Macro elements are involved also in the transmembranal transport due to the Na-K and Ca-Mg pumps. In the process of ions transport across the membrane it is discussed the voltage gated ion channels. Voltage gated sodium channels are crucial for the propagation of action potentials in excitable membranes. They cause the cell membrane to depolarize by allowing the influx of sodium ions into the cell (Denac et al., 2000). Potassium channels are essential in excitable membranes. They are responsible for re-polarizing the cell membrane after an action potential has passed (Doyle et al., 1998). Voltage gated calcium channels regulate the flow of Ca^{2+} in excitable membranes (Zhorov and Ananthanarayanan, 1997). Magnesium was shown to play an important role in the inactivation of voltage gated Ca channels. By blocking N-type Ca^{2+} channels at nerve endings, Mg^{2+} inhibits norepinephrine release, which decreases blood pressure independent of its direct vasodilating action (Shimosawa et al., 2004).

Sodium, in ionic form (Na^+) is important in the repartition of water and takes part in: the regulation of osmotic pressure, of acid-base balance, in the phenomenon of polarization-depolarization of cell membranes, etc.

Potassium is a macro element that has mostly a intracellular distribution. It ensures the osmolarity of the intracellular media; increases the neuro-muscular excitability; play an important role in the maintenance of the cardiovascular automatism, it is a component of the pancreatic and intestinal juice; it participates directly in the process of energogenesis.

Calcium is the most abundant macro element in the organism, especially due to the high quantity found in the skeleton. Of the total amount of Ca in the organism almost 99% is concentrated in the bones as calcium carbonates and phosphates. The other 1% of Ca plays also an important role in biological processes like coupling of excitation whit the muscular contraction. Also, Ca mediates neurotransmition, cellular growth, the activity of some enzymatic systems and the process of blood coagulation. Calcium concentration is regulated by the action of parathormone, calcitonin and calcipherols (vitamin D). In blood, almost half of de Ca amount is found in its ionic form (Ca^{2+}). The ionic form of Ca is responsible for most of its physiological effects. The other half of Ca amount from blood is bind to albumin and is very sensible to pH variations. The decrease of pH value triggers the release of Ca in his ionic form while the increase of pH value activates the binding of Ca^{2+} to albumins.

Magnesium is found mostly in the skeleton (~60%) and inside the cells or in the extracellular space. Magnesium has multiple physiological functions: intervenes in the carbohydrates, lipids and protides metabolism; it ensures the resistance of the bone structure together with calcium and phosphorus; reduces the neuro-muscular excitability of myocardic fibers; alongside of Ca^{2+}, Na^{+}, K^{+}, the Mg^{2+} ions are involved in the heart electric conductibility and in the contractibility of myocardic fibers; mediates the breaking of macroergic phosphate bonds from ATP, AGP, ACP, etc., thus ensuring the oxidative phosphorilations necessary in the biosynthesis processes (e.g.: nucleic acids synthesis, protein synthesis, a.o.); intervenes in the transmission of nervous influx and in transmembranary transport processes; it reduces the accumulation of cholesterol on the level of vascular walls in the process of atherogenesis; activates the immunoglobin synthesis, etc.

The normal quantum of the macro elements in human body is evaluated at: 90g for sodium, 140g for potassium. 1000-1500g for calcium and 25-30 mg for magnesium.

The investigated trace elements in rabbits' liver were Zn, Fe, Cu and Mn. In table 2 there are given the obtained results.

Data referring to trace elements concentration revealed an increase only in the case of zinc with the increasing dose of $NaNO_3$ administered in water. The concentration of other trace metals (Fe, Cu, Mn) decreased with the increasing dose, i.e. there is a reverse proportionality between their concentration in the liver and the $NaNO_3$ in water. Increase of natrium concentration (see table 1) produces serious damage in traffication of trace elements and especially of their elimination from the liver tissue.

The trace elements are present in living organism mostly in the structure of metalloenzymes where they play the role of coenzymes, in the structure of some hormones, some metalloproteins with transporter functions and in deposit substances that have the role of keeping the normal physiological concentration.

Among the most frequent met metalloenzymes are the superoxide dismutases (enzymes that generally contain Cu and Zn but can also contain Mn or Fe). Metallic ions from this category are found also in the structure of some important hormones (e.g.: Zn in insulin). Some trace elements can be found in the structure of metalloproteins involved in the oxygen transport. Such an example is given by iron found in the structure of hemoglobin, myoglobin, hemerythrin and copper found in the structure of hemocyanin from the inferior organisms.

Zinc (Zn) is a trace element present in a large number of enzymes with various functions in the organism: carbonic anhydrase, alkaline phosphatase, carboxypeptidases, dehydrogenases, mannosidase, DNA-polymerase, RNA-polymerase, Zn-Cu superoxid dismutases (McCall et al., 2000). Zinc is also necessary for the activation of some enzymatic systems as: thymidinkynase, phytase, arginase, enolasea, aldolase.

Tabel 2. Concentrations of trace elements in rabbit liver

Group	UM	n	Zn	Fe	Cu	Mn
			$\overline{X} \pm SD$	$\overline{X} \pm SD$	$\overline{X} \pm SD$	$\overline{X} \pm SD$
E_A	μg/g	5	23.70 ± 1.35	59.63 ± 00.00	3.71 ± 0,00	1.13 ± 0.23
E_B	μg/g	5	25.14 ± 1.41	54.40 ± 00.00	2.66 ± 0,00	1.09 ± 0.09
$\Delta X = X_{E_B} - X_{E_A}$			+ 1.44	- 5.23	- 1.05	- 0.04

Zinc intervenes in the action of some hormones, being essential for the insulin synthesis (insulin contains cca. 0.153% Zn) and for the synthesis of hypophysar gonadotrop hormones. Zinc deficiency can severely affect the gastrointestinal system, the central nervous system, the immune system and the reproductive system (Hambridge, 2000).

Iron (Fe) is an indispensable constituent of the organism, important especially through its presence in the structure of chromoproteids (hemoglobin), of some enzymes (catalases, peroxydases) and also through its implication in various physiological processes. In the organism a large amount of iron is found as hemoglobin, which is the mobile form of iron but also there is an important amount of iron that is deposited in liver, spleen and bone marrow as ferritin and hemosiderin. Therefore the iron from the organism can be either heminic (iron from hemoglobin, myoglobin, cellular ferments) or nonheminic (iron from ferritin and hemosiderin which have are the deposit forms of iron – and iron from transferring – the transporter form of iron.

Copper (Cu) is a trace element with complex functions in the organism through various enzymes in which structure it takes part (cytochromeoxydase, lisyloxidase, monoaminoxidase, ceruloplasmin, superoxid dismutase, ascorbic-oxidase, tyrosinase). Copper also has an important role in the synthesis of complex proteins from the connective tissues of the skeleton and sanguine vessels and in the synthesis of some neuroactive compounds.

Manganese (Mn) is involved in: reproductive function, development of skeletal system, the good functioning of nervous system, metabolisms of carbohydrates, lipids, etc. The implications of manganese and its biological functions are a consequence of the fact that it enters as a constituent in the structure of some enzymes as: piruvat carboxylase, Mn superoxid dismutase, diaminoxidase, glutamine synthetase or because it acts as an enzymatic activator for alkaline phosphatase, acid phosphatase, galactotransferase, arginase.

In the liver of laboratory animals it was observed a lower accumulation tendency for Mn as compared with Fe and Cu. High concentrations of manganese in blood neurological symptoms like facial paralysis and headaches may develop (O'Donnell and Radigan, 2003).

Between trace elements synergistic or antagonistic effects may appear as the presence of one element may potentate or inhibit the action of other elements. Thus trace elements may present synergism, antagonism and anergism (when different trace elements are present without any influence on each other). The synergic or antagonistic effects are usually based on the property of trace elements to compete and to inhibit or stimulate the absorption of others elements. For example a Ni deficiency has been shown to disturb the Ca metabolism and to affect the skeletal system due to Ca depletion. The decrease of Ca in Ni deficiency is followed by an increase of Mg which substitutes the Ca depleted from bones (Kenney and McCoy, 1992). Also it can be mentioned the antagonism between Fe and Zn or Cu.

The antagonism between Fe and Zn is better evidenced when they are both administrated in solutions or in small portions dispersed in solid foods (Sandströrm, 1985).

Acknowledgements

The authors express their gratitude to Prof. Dr. Marian Bura and Assoc. Prof. Dr. Ana Driha for their help in the preliminary stages of experiments. Also, they express their gratitude to Prof. Dr. Iosif Gergen, Assoc. Prof. Dr. Simona Drăgan and Drd. Monica Hărmănescu for their aid in analytical stages of studies on metallograms.

Conclusions

1. The resulted data of this study regarding macro elements revealed the increase of sodium, potassium and magnesium and the decrease of calcium concentration.
2. As to the trace elements concentration, the data showed a direct proportional increase of zinc concentration after the administration of $NaNO_3$ in high dose. The quantum of iron, copper and manganese was reverse proportionally with the dose of $NaNO_3$ administered in water.

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