THE EFFECT OF THE ZEOLITE CLINOPTILOLITE ON SERUM CHEMISTRY AND HEMATOPOIESIS IN MICE

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***Abbreviations**:* *CEC – cation exchange capacity, DPD – 3,5-dichlorophenyldiazonium tetrafluoroborate, EDTA – ethylenediaminetetraacetic acid, GM – granulocyte-macrophage, GM-CFU – granulocyte-macrophage colony forming unit, Hb – hemoglobin, IMDM – Iscove's modified Dulbecco's medium, L – liter, MCV – mean cell volume, MCH – mean cell hemoglobin, MCHC – mean cell hemoglobin concentration, MPV – mean platelet volume, MTCp – mechanically treated clinoptilolite, NGCp – normally ground clinoptilolite, Plt – platelets, RBC – red blood cells, WBC – white blood cells, UV – ultraviolet*

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ABSTRACT

Zeolites are natural or synthetic crystalline alumosilicates with ion exchanging properties. Supplied in fodder, they promote biomass production and animal health. Our aim was to asses the effects of the natural zeolite, clinoptilolite on hematopoiesis, serum electrolytes, and essential biochemical indicators of kidney and liver function in mice. Two preparations differing in particle size were tested: a powderized form obtained by countercurrent mechanical treatment of the clinoptilolite (MTCp) and normally ground clinoptilolite (NGCp). Young adult mice were supplied with food containing 12.5%, 25% or 50% clinoptilolite powder. Control animals received the same food ration without the clinoptilolite. After 10, 20, 30 and 40 days, six animals from each group were exsanguinated as to obtain blood for hematological and serum for biochemical measurements as well as to collect femoral bone marrow for determination of hematopoietic activity. Clinoptilolite ingestion was well tolerated, as judged by comparable body masses of treated and control animals. A 20% increase of the potassium level was detected in mice receiving the zeolite-rich diet, without other changes in serum chemistry. Erythrocyte, hemoglobin and platelet levels in peripheral blood were not materially affected. NGCp caused leukocytosis, with concomitant decline of the GM-CFU content in the bone marrow. That was attributed to intestinal irritation by rough zeolite particles. The mechanically treated clinoptilolite preparation caused similar, albeit less pronounced changes. In a limited experiment mice having transplanted mammary carcinoma in the terminal stage showed increased potassium and decreased sodium and chloride levels, severe anemia and leukocytosis, decreased bone marrow cellularity and diminished content of hematopoietic progenitor cells in the marrow. The clinoptilolite preparations ameliorated the sodium and chloride decline, whereas the effects on hematopoiesis were erratic.

INTRODUCTION

Zeolites are crystalline, hydrated aluminosilicates having a fine network of structural cavities. They selectively adsorb water and exchange cations (Nagy *et al.* 1998; Mumpton, 1999). Zeolites have found multifarious applications as adsorbents, ion exchangers, and catalysts in industry, agriculture, veterinary medicine, sanitation, and environmental protection. Biological applications include the removal of ammonia from wastewater and animal manure (Bernal *et al.* 1993) air filtration and deodoration (Miner, 1980), soil amelioration and fertilization (Mumpton, 1999).

Animal fodder containing zeolites has been shown to increase biomass production in fisheries, to promote weight gain of chicken (Fethiere *et al.* 1994), swine (Ward *et al.* 1991) and sheep (Eady *et al.* 1980), to improve the quality of animal products such as eggs (Keshavarz and McCormick, 1991) or wool (Eady *et al.* 1990), to reduce bacterial contamination of the gut (Roman *et al.* 1987) and to counteract harmful effects of ingested toxic substances (Ward *et al.* 1993). In human medicine zeolites have been applied as antidiarrheal remedies (Rodriquez-Fuentes *et al.* 1997), for the external treatment of wounds and athlete foot, and for the removal of ammonia ions from kidney dialysates (Mumpton, 1999). Beneficial effects of zeolites in various disease states, including tumors have been described (Momčilović 1999; Pavelić *et al.* 2000).

There are not many data showing systemic effects of zeolites on hematopoiesis. Vrzgula *et al*. (1982) supplied pigs with food containing 5% zeolite clinopotilolite and observed a favorable effect on the weight gain and diarrhea, without concomitant changes in red or white blood elements. Dwyer *et al*. (1997) and Kececi *et al*. (1991) studied protective effects of clinoptilolite and of a synthetic zeolite against hepatotoxic and hematotoxic effects of alimentary mycotoxins (cyclopiazonic acid and aflatoxin, respectively), and as a corollary provided data showing no effects of peroral zeolite supply on the blood cell counts, hemoglobin concentration and hematocrit in control, non-intoxicated chicks. Kartashev and Baskurin (1995) described stimulation of erythropoiesis in young mice receiving a natural zeolite in food, but exhaustion in the senescent period after the prolonged supply. In vitro, clinoptilolite particles were found to cause hemolysis and macrophage toxicity (Korkina *et al.* 1984).

As regards serum chemistry, Ward *et al.* (1993) reported no effect of zeolite A on calcium and phosphorus concentration in chicken plasma, Dwyer *et al.* (1997) found no effect of clinoptilolite or clay on phosphorus concentration in sera of broilers, and Pond and Yen (1983) found no effect of zeolite A on plasma potassium, sodium and magnesium levels in swine. In swine supplied with zeolite A, however, Ward *et al.* (1991) observed a decrease of serum calcium and phosphorus concentrations, without their changes in the bone.

The aim of this study was to explore whether powdered natural zeolitic tuff, mainly clinoptilolite, added in food in a high percentage (12.5% up to 50%) would affect serum electrolytes, basic liver and kidney tests, and body weight of normal laboratory mice and in mice having transplanted mammary carcinoma. Theoretically, the following actions of ingested zeolite may be expected. First, the ion-exchange properties of the zeolite could alter the pH and the ionic composition (including trace elements) of gastrointestinal fluids, thereby changing the enzymatic activity of gastrointestinal secretions. Second, the zeolite might adsorb small amounts of low molecular weight substances produced by digestive processes (e.g., glucose, amino acids) or by the activity of intestinal bacteria (e.g., ammonia). Inasmuch as any zeolite in the gastrointestinal tract would be expected to be fully hydrated, the low molecular weight substances would probably be adsorbed on particle surfaces, but not enter its cavities; ammonia would be exchanged as the ammonium ion. Third, reactive (poly)silicate anions may be liberated from the zeolite, but that possibility is probably of minor importance in view of the low solubility of clinoptilolite under physiological conditions. As a result of these reactions, systemic changes of the electrolyte turnover in the body and of the liver or kidney functions might take place. Hematological changes might also take place. In view of data suggesting favourable effects of clinoptilolite as an adjuvant in antineoplastic treatment (Pavelić *et al.* 2000), mice having transplanted tumors were included into the study.

Two clinoptilolite preparations, differing in the particle size and uniformity were compared. The material prepared by a special countercollision process was expected to be more efficient, because of its finer particle size.

MATERIAL AND METHODS

Clinoptilolite

The natural clinoptilolite-rich tuff used in this study originated from a deposit in the vicinity of Vranje, southern Serbia. From the powder X-ray diffractometry, the sample consisted of about 85% clinoptilolite and 15% mordenite, montmorillonite, and silica minerals. Chemical composition, determined by atomic absorption spectroscopy, is shown in Table 1. The maximum amount of water desorption, determined by thermogravimetric analysis, occurred at 50 ^{o}C, and no phase transformations were observed < 800 ^{o}C. The total water content was 15%, of which 2% was loosely contained moisture and the rest, zeolitic water. The NH_4 cation-exchange capacity (NH_4-CEC) was about 1.5 meq/g (Subotić B., Ruđer Bošković Institute, personal communication, August 2000). The material was supplied in two forms. A fine powder produced by countercurrent particle collision yielded uniform size particles having an average diameter of 2.68 μm (98% of the particles <4.3 μm and 90% <2.67 μm) and a specific surface of 1.35 m^2/g (Pavelić *et al.* 2000). Normally ground material consisted of particles that were about eight times larger and less uniform in size. The particle distributions were determined by a laser light-scattering analyser. These materials will henceforth be designated MTCp (mechanically treated clinoptilolite) and NGCp (normally ground clinoptilolite).

Animals and feeding

Female CBA/Zgr mice entered the experiment at age 3 months (11 – 14 weeks). A total of 96 normal mice were divided into four groups of 24, assigned to four food regimens: normal food (controls), food supplemented with 12.5% or 25% (w/w) mechanically treated and finely pulverized clinoptilolite-rich tuff (MTCp), and food supplemented with 25% normally ground clinoptilolite-rich tuff (NGCp). Mice with tumors received 25% or 50% zeolite in either form. The animals were kept three per cage, with free access to food and water.

The clinoptilolite-containing preparations were added to the mouse ration, which was produced by the Animal Nutrition Institute (Inštitut za živinorejo), Domžale, Slovenia. The ration was procured in non-pelleted form, enabling the addition and uniform dry mixing of the zeolite-rich powder. The mixture was made into dough by the addition of tap water, pelleted, and offered to the mice *ad libitum* from the cage coverlids. Control mice received the ration without zeolite, processed and supplied in the same manner. Fresh food was prepared daily, except for the weekends, when the animals received the ration for 2 days prepared in advance. The daily allowance was 7 g of pure ration per mouse (*plus* 12.5, 25%, or 50% zeolite powder). That amount was almost completely consumed. Thus, the average daily supply of zeolite was 0.875, 1.75, or 3.5 g per mouse. Tap water was supplied *ad libitum*.

Six animals from each food regimen were sacrificed 10, 20, 30 or 40 days after the onset of feeding.

Tumor model, mice with transplanted mammary carcinoma

A transplantable mammary carcinoma was used. Injections of 10^5 tumor cells (Poljak Blaži *et al.* 1981) suspended in 0.2 mL of Hanks solution were given intramuscularly into the right thighs of 30 CBA female mice 8 weeks old. Food containing 25% or 50% MTCp or NGCp was supplied 1 day after the tumor inoculation and until sacrifice 30 days later. At that time, the tumor volumes were 700 – 1,200 mm^3 (about 0.7 – 1.2 g). At the beginning of the experiment, there were 6 mice in each group. Several mice died immediately before sacrifice, so that the experimental groups were reduced.

Blood samples, hematology and serum chemistry

The animals were bled to death from the orbital plexus under light ether anesthesia. Samples of approximately 0.1 mL (13 – 15 drops) were individually collected into plastic vials coated with EDTA (Microtainer Brand Tube 0.5 mL, Beckton Dickinson Cat. No. 365973) and processed within 2-3 hours by means of an automated cell analyzer (ARGOS Hematologic Analyzer, AVL Instruments) in the Clinical Institute for Laboratory Diagnosis, Clinical Hospital Zagreb. The following hematologic parameters were determined:

- erythrocyte (RBC), leukocyte (WBC) and platelet (Plt) counts per L,
- hemoglobin concentration, g/L
- percentages of lymphocytes, monocytes and granulocytes.

The hematologic analyzer also yielded the average erythrocyte size and hemoglobin concentration (MCV, MCH, MCHC), platelet volume (MPV) and the lymphocyte count.

After collection of the individual samples, additional 2-3 drops of blood from each mouse were collected into 1.5 or 2.0 mL safe-lock microcentrifuge tubes (Eppendorf) and pooled, as to obtain enough material for the serum analyses. As noted, experimental groups consisted of 6 mice divided into subgroups of 3, hence, the pools (0.5 – 0.8 mL) were collected from subgroups of 3 mice. Pooled blood was allowed to clot at 37 ^{o}C; the serum was harvested and frozen until analysis. Serum chemistry was determined in the Institute of Clinical Chemistry, Clinical Hospital Merkur, Zagreb, Croatia.

The concentrations of Na, K, Cl, inorganic P, urea, creatinine and total bilirubin were determined by means of the Olympus AU-600 autoanalyzer equipped with ion-selective electrodes using the following methods: indirect potentiometry (Worth, 1985) (for Na, K, Cl), molybdenum blue (Munoz *et al.* 1983) (for P), urease – UV (Taylor and Vadgama, 1992) (for urea), Jaffe kinetic method (Gennaro *et al.* 1995) (for creatinine) and DPD tetrafluoroborate sample blank method (Tietz, 1976) (for bilirubin). The concentrations of total Ca (Gambino, 1973), Mg (Kűlpmann *et al.* 1989), and Zn (Weinstock, 1980) were determined by means of the Pye Unicam SP-9 atomic absorption spectrometer. Quality controls were performed using lyophilised universal control sera of human origin: Olympus control serum level 1 ODC 0003 and Olympus control serum level 2, ODC 0004 for the Olympus AU-600 analyzer and Precinorm U Cat. No. 171735; and Precipath U Cat. No. 171760 Boehringer Mannheim GmbH, Germany, for the Pye Unicam SP-9 atomic absorption spectrometer.

Bone marrow cellularity and the progenitor cell content

The femora of the bled mice were aseptically excised and scraped free of muscle. The bone marrow of each femoral pair was flushed out with 3 mL of Iscove’s modified Dulbecco’s medium (IMDM, Sigma Life Technologies), the resulting cell suspension was carefully dispersed by repeated pipetting and the cell count was determined in a hemocytometer. Total and viable cell counts per femur were determined after dilution and staining with Tűrk and trypan blue solutions respectively. The cell suspensions were adjusted to $2x10^{5}$ viable (Trypan blue excluding) cells per mL of Iscove' s medium and used for the granulocyte-macrophage colony-forming unit (GM-CFU) assay.

The GM-CFU assay

The cells were cultured in Iscove' s medium containing 20% pretested fetal calf serum, 10% conditioned medium derived from a cell line constitutively producing interleukin-3 (Karasayuma and Melchers, 1988) and 0.33% Bacto-agar (Difco, USA). One mL samples of the final cultivation mixture containing $2x10^4$ cells/mL were cultured in 35-mm plastic Petri dishes at 37 ^oC, 5% CO_2 and 100% humidity. Granulocyte-macrophage (GM) colonies (above 50 cells) were scored under an inverted microscope after 7 days. Each sample was cultured in triplicate and the mean colony count was calculated. That information served for calculation of the content of GM-CFU per 10^5 viable cells and per femur (Heyworth *et al.* 1993).

Statistics

The data were analyzed by means of a computerized statistical program using nonparametric tests – the Kruskal-Wallis analysis of variance and the Mann-Whitney comparison of two independent samples. Nonparametric statistics was used because the groups were small (6 samples) and because the testing for normal distribution of the data is not needed. The level of significance was set at $p < 0.05$.

RESULTS

Body weight and food tolerance

Body mass was measured at the end of the experiment. No statistical difference was found between the control, and the NGCp or MTCp treated animals (Table 2). All animals accepted and tolerated the clinoptilolite-supplemented food without problems. No constipation was noted.

Peripheral blood counts in normal mice

The erythrocyte counts and hemoglobin concentrations were comparable in control mice and in mice supplied with clinoptilolite for 40 days. Erythrocyte counts (x 10^{12}/L) ranged from 9.1 to 10.2 in the controls and the hemoglobin (g/L) from 150 to 159; the respective values for the clinoptilolite-treated mice were 9.1 – 10.0 and 150 – 160. The platelet counts (x 10^{9}/L) were 410 – 540 in the controls and 380 – 475 in the clinoptilolite mice, i.e. 10 – 20% lower, but without statistical significance.

Leukocyte counts, however, were significantly higher in mice supplied with ground clinoptilolite (NGCp) for 20 or 30 days than in the controls and in mice supplied with the mechanically treated preparation (MTCp) (Table 3). The increases were due to the lymphocytes. It should be noted that lymphocytes and lymphocyte-like mononuclear cells constitute about 90% of the white cell population in mouse peripheral blood (Matsumoto *et al.* 1995).

Serum chemistry of normal mice

Concentrations of serum electrolytes, trace metals, urea, creatinine, and bilirubin in serum of mice receiving zeolite-containing rations are shown in Table 4. The analysis was done on pooled serum and the values were compared to the reference values from the literature (Foster *et al.* 1983). In this limited experiment, the mice receiving either form of the zeolite (MTCp or NGCp) for 20 and 30 days had serum potassium levels as much as 20% greater than those of the control mice eating non-supplemented food. Sodium and chloride concentrations were within the reference values of 132 – 162 mmol/L and 92 – 106 mmol/L, respectively (Foster *et al.* 1983), and did not differ between the clinoptilolite-supplied and control groups (Table 4).

The concentrations of total calcium and inorganic phosphorus, although slightly above the reference values for mice (Foster *et al*, 1983), as well as the concentrations of magnesium and zinc, were not different among the groups. The same was true for creatinine and total bilirubin, indicators of kidney and liver function, respectively. Urea was elevated in all animals including the controls, probably due to food contamination by urine (see Discussion).

Hematopoiesis in normal mice

The bone marrow cellularities (cells per femur x 10^6) of the clinoptilolite-supplied mice varied over a broader range (8.6 – 19.5) than in the controls (10.7 – 15.6), but without a clear pattern. The progenitor cell (GM-CFU) counts per 10^5 plated cells and the GM-CFU contents per femur were below the control level in all mice supplied with clinoptilolite for 30 and 40 days, with significant Kruskal-Wallis and Mann-Whitney tests. Calculated GM-CFU contents per femur followed a similar pattern, but significant drops below the controls were registered only on day 40 for the 12.5% and 25% MTCp mice (Table 5).

Summary of the findings in normal mice

A succint summary of the hematological and serum chemistry changes is given in Table 6.

Mice with transplanted mammary carcinoma

In this limited experiment, the mice were in poor condition at the time of sacrifice (30 days after tumor inoculation) with massive tumors (0.7 – 1.2 cm^3) and evident emaciation. A half of them died in terminal stage, during the weekend just before the planned sacrifice (3 of 6 in the control group without clinoptilolite, 7/12 in the 25% and 50% NGCp groups and 8/12 in the 25% and 50% MTCp groups). The animals were anemic – the erythrocyte counts (x 10^{12}/L) were approximately 50% of the normal level (4.4 as compared to 9.0) and the hemoglobin concentrations (g/L) approximately 60% (90 as compared to 150). The platelet counts were likewise about 60% of the normal level (260 compared to 425). On the other hand, the white blood cell counts (x 10^9/L) were approximately six-fold higher in the tumor-bearing animals than in the controls free of tumor (38,7 compared to 6.0). The increase involved both the lymphocytes and the granulocytes, but the granulocyte increment was relatively greater, so that the proportion of granulocytes in peripheral blood reached 20%, as compared to the normal level which is below 10% (data not presented). The bone marrow cellularity (cells per femur x 10^6) was reduced to 50% of the normal level (5.4 compared to 10.9), but the GM-CFU content per femur (x 10^6) was essentially normal (22.9 compared to 24.2). That was due to an increased colony-forming ability (seeding efficiency) of the bone marrow cells, i.e. 430 CFU per 10^5 plated cells as compared to 225 in normal bone marrow. The supply of clinoptilolite in food ameliorated to some extent the hematological changes of the tumor bearing mice. Both preparations of zeolites (MTCp and NGCp) reduced anemia and NGCp reduced leukocytosis. These effects did not reach statistical significance (data not presented).

The serum electrolytes in tumor bearing mice were grossly disturbed compared with reference values. The potassium level was 14.3 mmol/L, which is 100% greater than the highest reference value, and the chloride and sodium levels were, respectively, 74 mmol/L and 95 mmol/L, which are 20% and 30% less than the lowest reference values (Table 7). The supply of clinoptilolites ameliorated the declines of chloride and sodium. Chloride levels (89 – 91 mmol/L) were almost in the normal range, and sodium levels (112 – 115 mmol/L) approached the normal minimum by 15% (Table 7).

DISCUSSION

Although zeolites have been widely used in industry, agriculture, animal husbandry and environmental protection, their effects in appropriate animal models and possible medical applications await detailed studies. Experimental models *in vitro* may be confounded by the ion-exchange properties of zeolites, which interfere with finely balanced composition of the nutrient media, and animal experiments require zeolite supplied by gastric intubation (which is cumbersome) or in food (which is open to individual variations and other distortions).

Considerable amounts of clinoptilolite-rich tuff were added to the standard mouse ration. Normal mice were supplied with food containing 12.5% or 25% clinoptilolite, and tumor-bearing hosts with 50%, which amounts to about 35, 70 or 140 g per kg bodyweight. Other authors used lower amounts of clinoptilolite or sodium zeolite A. In studies on pigs, for example, Vrzgula *et al.* (1982) used 5%, Pond and Yen (1983) 3% and Ward *et al.* (1991) 0.5%, and in chickens, Fethiere *et al.* (1994) up to 0.16%, Dwyer *et al.* (1997) 1%, Kececi *et al.* (1998) 3 – 5 g/kg body weight and Olver (1997) 50 g/kg body weight.

The increased bulk of the food did not reduce the caloric intake, since the body masses of the animals remained comparable. Likewise, Olver (1997) found no effect of dietary clinoptilolite on the body weight of laying hens, whereas the egg quality was improved. Other literature indicates favorable effects of dietary zeolites on the growth and body weight of lambs (Pond, 1984), swine (Ward *et al.* 1991), and broiler chicks (Fethiere *et al.* 1994). A four-week supply of zeolite A produced disparate effects in rats and turkeys (Kayongo-Male and Jia, 1999). The growth rates were slowed in both species, in turkeys the heart and liver weights, the hematocrit, and the plasma Mg levels were elevated, but in rats, the organ weights and their mineral contents were not materially changed and the hemoglobin concentration and the plasma Zn levels decreased. When studied – e.g. Vrzgula *et al.* (1982), Kececi *et al.* (1991), Dwyer *et al.* (1997) – no hematological alterations were noted.

Serum chemistry

To accrue information about systemic effects on the body fluids attributable to oral supply of clinoptilolite as an ion exchanger, serum electrolytes and the kidney and liver function indicators were measured. The results were compared with reference values for mice (Foster *et al.* 1983). Inasmuch as the ionic affinity of clinoptilolite is $Cs^+ > Rb^+ > K^+ > NH_4^+ > Ba^{2+} > Sr^{2+} > Na^+ > Ca^{2+} > Fe^{2+/3+} > Al^{3+} > Mg^{2+} > Li^+$, its release of Na^+ or Ca^{2+} and take up of K^+ and NH_4^+ might be expected in the gastrointestinal tract (Mumpton, 1999). Actually, a modest (20%) increase of serum potassium was observed, without alterations of other serum electrolytes. Perhaps the initial potassium load of the clinoptilolite was exchanged for NH_4^+. Ward *et al.* (1993) reported no effect of zeolite A on calcium and phosphorus concentration in chicken plasma, Dwyer *et al.* (1997) found no effect of clinoptilolite or clay on phosphorus concentration in sera of broilers, and Pond and Yen (1983) found no effect of zeolite A or clinoptilolite on plasma potassium, sodium, and magnesium levels in swine. In swine supplied with zeolite A, however, Ward *et al.* (1991) observed a decrease of serum calcium and phosphorus concentrations, without changes in the bone. Relevant to our observations, Severance *et al.* (1988) described hypokalemia and severe myositis in a patient after prolonged clay consumption.

Available evidence indicates that clinoptilolite resists degradation by gastric and intestinal juices and that significant amounts of its major constitutive elements (Al and Si) are not absorbed from the gut into circulation. Overnight incubation of clinoptilolite in acidic or weakly alkaline media at 37 °C resulted in minimal amounts of soluble silicon (50 – 60 mg/L) (Subotić B., Rudjer Bošković Institute, personal communication, June 2000). Traces of silicon have not been detected in serum of Wistar rats (Antić, 1999) or CBA mice (Hršak I., Rudjer Bošković Institute, personal communication, April and June 2000) receiving clinoptilolite in food. Cefali *et al.* (1995), however, found elevated levels of silicon and aluminum in plasma of experimental dogs ingesting synthetic zeolite A as a single dose. Likewise, Roland *et al.* (1993) showed increased excretion of Si and Al in hens receiving zeolite A by intubation. It should be noted that zeolite A is soluble, particularly in acidic media (Nagy *et al.* 1998).

Basic tests for the kidney (creatinine) and liver functions (bilirubin) in the mice ingesting the clinoptilolite-rich tuff were within reference values. Pond and Yen (1983) and Ward *et al.* (1991), likewise, found no effect of sodium zeolite A or clinoptilolite on blood urea nitrogen. Dwyer *et al.* (1997) found no changes in biochemical indicators of kidney and liver functions (albumin, blood urea nitrogen, alkaline phosphatase, γ-glutamyl transferase, alanine aminotransferase, glucose) in broiler chickens supplied with clinoptilolite.

The urea concentration in serum was surprisingly high in our mice, regardless of zeolite supply. It is unlikely that all experimental animals harbored an inapparent kidney dysfunction. As noted, concentration of creatinine was normal. A tentative explanation is that the food was contaminated with urine, because mice consumed food crumbles from the bedding. At any rate, urea in serum was equally high in all four groups of mice, whether supplied with zeolite or not. For that reason, possible effects of zeolite on ammonia (NH_3) uptake and turnover, attributable to ion-exchange phenomena in the gastrointestinal tract, were obscured. In cattle receiving urea in fodder, zeolite-rich ration containing 50% clinoptilolite reduced the ammonium concentration in rumen and in the portal vein (Jacobi *et al.* 1984)

Hematology

In as much clinoptilolite is poorly soluble in water at physiological pH (Subotić B., and Hršak I., Rudjer Bošković Institute, personal communications, 1999) and the particles are not absorbed from the gut into circulation (Antić, 1999; I. Hršak, personal communication, 1999), the effects on hematopoiesis, if any, should be attributed to indirect mechanisms initiated in the gut. The ion exchange properties of the zeolite might alter the pH and buffering capacity of gastrointestinal secretions, interfere with the absorption of iron and other oligoelements, bind alimentary proteins (Klint and Eriksson 1997) and affect intestinal microflora (Varel *et al.* 1987). The particles might irritate intestinal mucosa, as they did in the respiratory tract (Gusev et al. 1997, Adamis *et al.* 2000) or pleural cavity (Varel *et al.* 1987). Such local actions might bring about systemic effects.

The red blood cell counts, hemoglobin concentration, and MCV and MCH values in peripheral blood were within the control range during the clinoptilolite supply for 6 weeks. Mice supplied with zeolite for longer periods of time, however, have had a reduced adaptability of erythropoiesis to increased demands (Kartashev and Baskurin, 1995) and female rats supplied with kaolin (an alumosilicate with ion exchanging properties like zeolites) for several weeks before and during pregnancy developed sideropenic anemia affecting the offspring as well (Paterson and Staszak, 1977). Hence, delayed or long-term effects of clinoptilolite supply on the erythron cannot be excluded. It may be noted that the affinity of clinoptilolite for iron is not high, it is e.g. below that for potassium or NH_4^+ (Mumpton, 1999).

Leukocyte counts were increased in mice supplied with roughly ground clinoptilolite (NGCp) and the increase was due to the lymphocytes. That reaction might be attributed to intestinal irritation and inflammation elicited by rough zeolite particles. Macroscopically, the intestines were hyperemic. Intratracheal instillation of fibrous zeolite mordenite (but not of clinoptilolite) caused inflammation of the respiratory tract (Adamis *et al.* 2000) and intrapleural injection of another fibrous zeolite, erionite, caused local fibrosis and foreign body reaction (Fraire *et al.* 1997). Clinoptilolite, mordenite and erionite particles elicited formation of reactive oxygen radicals and caused cytotoxic effects when ingested by the macrophages (Hogg *et al.* 1996, Suzuki and Hei 1996, Gusev *et al.* 1997, Long *et al.* 1997). Hence, local inflammatory phenomena accompanied by the release of lymphokines in the gut of mice eating NGCp may have stimulated a systemic lympho-hematopoietic response. The finely powderized form (MTCp) was apparently better tolerated as it did not cause leuko/lymphocytosis and visible intestinal irritation. In general, clinoptilolite particles have been found to cause less irritation and tissue damage than the rod- and fiber-shaped particles of other natural zeolites such as erionite or mordenite that resemble the asbestos fibers (Pylev *et al.* 1986, Adamis *et al.* 2000).

The cellularity, seeding efficiency (GM-CFU per 10^5 plated cells) and the calculated GM-CFU contents per femur varied during the experiment, probably due to the age differences of the experimental cohorts. In spite of that variability along the time-scale, comparisons between the groups of a cohort sacrificed on the same day were possible. The seeding efficiencies were lower in mice supplied with either form of clinoptilolite than in the controls. In view of peripheral leukocytosis and intestinal irritation by zeolite particles, that decrease may indicate an inhibition (or exhaustion?) of the bone marrow myelopoietic ability by the inflammatory reaction.

Mice with transplanted mammary carcinoma

Mice having transplanted mammary tumors had decreased sodium and chloride and elevated potassium serum levels (Table 6). Sodium and chloride may have been depleted into the extracellular fluid of the rapidly proliferating tumor mass, and intracellular potassium may have been released from multiple necrotic foci in the tumor. The clinoptilolites (mechanically treated or not) apparently buffered the electrolyte disturbances. In view of the paucity of data, interpretation of this preliminary observation is highly speculative. Note that dietary zeolite did not increase the survival rate of tumor-bearing animals. No substantial differences were noted between the mechanically treated (MTCp) and non-treated zeolite (NGCp) with regard to serum chemistry.

The animals were evidently anemic and thrombocytopenic, most probably due to blood consumption and microhemorrhages in the tumor mass. There was, on the other hand, a pronounced leukocytosis, known to occur in mice with transplanted tumors due to stimulation of hematopoiesis by growth factors produced by the tumor (Johnson *et al.* 1985). The demand depleted the bone marrow (as shown by reduced cellularity) but increased the proliferative activity (as evidenced by increased seeding efficiency of the granulocyte-macrophage precursors). In view of profound changes caused by the tumor, the effects of MTCp or NGCp supply were marginal. Anemia, platelet and leukocyte counts in peripheral blood and concomittant changes of the bone marrow proliferative activity were ameliorated to some extent, but the experimental groups were small, and the changes did not reach statistical significance.

In conclusion, ingestion of powdered clinoptilolite-rich tuff, a natural ion-exchanger, caused a modest (20%) elevation of serum potassium in normal laboratory mice, without changes in other serum electrolytes or in indicators of kidney and liver function. Rougly ground clinoptilolite (NGCp) caused leuko/lymphocytosis, presumably due to intestinal irritation, accompanied by a decline of granulocyte-macrophage progenitors (GM-CFU) in bone marrow. Similar, but less pronounced changes were noted with finely powderized clinoptilolite (MTCp). Mice having transplanted mammary carcinoma had grossly disturbed electrolyte levels (increased K, decreased Na and Cl) and hematopoiesis (anemia, thrombocytopenia, leukocytosis). These changes were partly corrected by the use of dietary zeolite, but the effects were erratic and require further experimental studies.

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REFERENCES

Adamis Z., Tatrai E., Honma K., Six E. and Ungvary G. (2000) In vitro and in vivo tests for determination of the pathogenicity of quartz, diatomaceous earth, mordenite and clinoptilolite. *Annals of Occupational Hygiene* **44**, 67 - 74.

Antić A. (1999) Does tribomechanically activated zeolite (TMAZ) enter into circulation from the gut and its effect in mice with transplanted tumor. Bachelor of Science Thesis, Faculty of Pharmacy and Biochemsitry, University of Zagreb, Croatia, pp 7 – 33.

Bernal M. P. and Lopez-Real J. M. (1993) Natural zeolites and sepiolite as ammonium and ammonia adsorbent materials. *Bioresearch Technology* **43**, 27 - 33.

Cefali E. A., Nolan J. C., McConnell W. R. and Walters D. L. (1995) Pharmacokinteic study of Zeolite A, sodium aluminosilicate, magnesium silicate and aluminum hydroxide in dogs. *Pharmaceutical Research* **12,** 270 - 274.

Dwyer M. R., Kubena L. F., Harvey R. B., Mayura K., Sarr A. B., Buckley S., Bailey R. H. and Phillips T. D. (1997) Effect of inorganic adsorbents and cyclopiazonic acid in broiler chicken. *Poultry Science* **76**, 1141 - 1149.

Eady S . J., Pritchard D. A. and Martin M. D. J. (1980) The effect of sodium bentonite on zeolite on wool growth of sheep fed either mulga (Acacia aneura) or lucerne (Medicago sativa). *Proceedings of the Australian Society of Animal Production* **18**, 188 - 191.

Fethiere R., Miles R D. and Harms R H. (1994) The utilization of sodium zeolite A by broilers. *Poultry Science* **73**, 118 - 121.

Foster H. L., Small J. D. and Fox J. G. (Eds) (1983) *The Mouse in Biomedical Research*, Vol III, pp 313-326. Academic Press, New York, London, Paris, San Diego, San Francisco, Sao Paulo, Sydney, Tokyo, Toronto.

Fraire A. E., Greenberg S. D., Spjut H. J., Dodson R. F., Williams G., Lach-Pasko E. and Roggli V. L. (1997) Effect of erionite on the pleural mesothelium of the Fischer 344 rat. *Chest* **111**, 1375 - 1380.

Gambino S. R. (1973) A reference method for the determination of total calcium in serum. *Clinical Chemistry* **19/10**, 1208 - 1213.

Gennaro M. C., Abrigo C., Marengo E., Baldin C. and Martelletti M. T. (1995) Determination of creatinine in human serum. Statistical intercalibration of methods. *Analyst* **120**, 47 - 51.

Gusev V. A., Lomonosova O. S. and Velichkovskii B. T. (1997) Effect of chrysotile-asbestos and zeolites dust on generation of reactive oxygen species by alveolar macrophages and granulocytes (in Russian) *Voprosy medicinskoj khimii* **43**, 148 - 152.

Heyworth C. M. and Spooncer E. (1993) In vitro clonal assays for murine multipotential and lineage restricted myeloid progenitor cells. In: Progenitor Cells, a Practical Approach. Edited by Testa N. G., Molineux G., pp 37 – 53. IRL Press at Oxford University Press.

Hogg B. D., Dutta P. K. and Long J. F. (1996) In vitro interaction of zeolite fibers with individual cells (macrophages NR8383): measurement of intracellular oxidative burst. *Analytical Chemistry* **68**, 2309 - 2312.

Jacobi U., Vrzgula L., Blazovsky J., Havassy I., Ledecky V. and Bartko P. (1984) The effect of zeolite (clinoptilolite) on the post-feeding dynamics of N metabolism in the portal vein, jugular vein and the rumen fluid of bulls. *Veterinarni Medicina* **29**, 207 - 216 (in Slovak)

Johnson G. R., Whitehead R. and Nicola N. A. (1985) Effects of a murine mammary tumor on *in vivo* and *in vitro* hematopoiesis. *International Journal of Cellular Cloning* **3,** 91 - 105.

Kayongo-Male H.and Jia X. (1999) Silicon bioavailability studies in young rapidly growing rats and turkeys fed semipurified diets: a comparative study. *Biological Trace Elements Research* **67**, 173 - 186.

Kartashev A. G. and Baskurin A. K. (1995) Changes in the blood system of white mice with long-term zeolite administration. *Fiziolohichnyi Zhurnal (Bohomoltsia)* **41**, 14 - 19.

Karasuyama H. and Melchers F. (1988) Establishement of mouse cell lines which constitutively secrete large quantities of interleukin 2, 3, 4 or 5 using modified cDNA expression vectors. *European Journal of Immunology* **18**, 97 – 104.

Kececi T., Oguz H., Kurtoglu V. and Demet O. (1991) Effects of polyvinylpolypyrrolidone, synthetic zeolite and bentonite on serum biochemical and haematological characters. *British Poultry Science* **39,** 452 - 458.

Keshavarz K. and McCormick C. C. (1991) Effect of sodium aluminosilicate, oyster shell, and their combination on acid-base balance and eggshell quality. *Poultry Science* **70**, 313 - 325.

Klint D. and Eriksson H. (1997) Conditions for the adsorption of proteins on ultrastable zeolite Y and its use in protein purification. *Protein Expression and Purification* **10,** 247 - 255.

Korkina L. G., Suslova T. B., Nikolova S. I., Kirov G. N. and Velichovskii B. T. (1984) The mechanism of cytotoxic action of the natural ceolite clinoptilolite. *Farmakologiia i Toksikologiia* **47**, 63 – 67.

Külpmann W. R., Buchholz R., Dyrssen C. and Ruschke D. (1989) A comparison of reference method values for calcium, lithium and magnesium with method-dependent assigned values. *Journal of Clinical Chemistry and Clinical Biochemistry* **27**, 631 - 637.

Long J. F., Dutta P. K. and Hogg B. D. (1997) Fluorescence imaging of reactive oxygen metabolites generated in single macrophage cells (NR8383) upon phagocytosis of natural zeolite (erionite) fibers. *Environmental Health Perspectives* **105**, 706 - 711.

Matsumoto K. and Futamura Y. (1995) Cell counts in peripheral blood and bone marrow of male CBA/N mice. *Journal of Veterinary Medical Science* **57,** 755 - 756.

Miner J. R. (1980) Odor control - the challenge to livestock producers. *Agricultural Engineering* **61**, 22 - 24.

Momčilović B. (1999) Megamin, faith, hope and placebo (Croatian). *Arhiv za higijenu rada i toksikologiju (Zagreb)* **50**, 67 - 78.

Mumpton F. A. (1999) *La roca* magica: Uses of natural zeolites in agriculture and industry. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 3463 - 3470.

Munoz M. A., Balon M. and Fernandez C. (1983) Direct determination of inorganic phosphorus in serum with a single reagent. *Clinical Chemistry* **29**, 372 – 374.

Nagy J. B., Bodart P., Hanus I. and Kiricsi I. (Eds) (1998) Ion exchange, sorption and diffusion in microporous materials. In *Synthesis, characterization and use of zeolitic microporous materials.* pp 93 – 118. DecaGen Ltd., Szeged.

Olver M. D. (1997) Effect of feeding clinoptilolite (zeolite) on the performance of three strains of laying hens. *British Poultry Science* **38**, 220 - 222.

Patterson E. C. and Staszak D. J. (1977) Effects of geophagia (kaolin ingestion) on the maternal blood and embryonic development in the pregnant rat. *Journal of Nutrition*. **107**, 2020 - 2025.

Pavelić K., Hadžija M., Bedrica Lj., Pavelić J., Đikić I., Katić M., Kralj M., Herak Bosnar M., Kapitanović S., Poljak-Blaži M., Križanac Š., Stojković R., Jurin M., Subotić B. and Čolić M. (2000) Natural zeolite clinoptilolite – new adjuvant in anticancer therapy. *Journal of Molecular Medicine,* in press.

Poljak-Blaži M., Boranić M., Maržan B. and Radačić M. A. (1981) Transplantable aplastic mammary carcinoma of CBA mice. *Veterinarski Arhiv* **51**, 99 - 107.

Pond W.G. and Yen J.T. (1983) Protection by clinoptilolite or zeolite NaA against cadmium-induced anemia in growing swine. *Proceedings of the Society for Experimental Biology and Medicine,* **173,** 327 – 337.

Pond W G. (1984) Response of growing lambs to clinoptilolite or zeolite NaA added to corn, corn-fish meal and corn-soybean meal diets. *Journal of Animal Science* **59**, 1320 - 1328.

Pylev L. N., Bostashvili R. G., Kulagina T. F., Vasil'eva L. A. and Chelishchev N. F. (1986) Evaluation of the carcinogenic activity of zeolite and clinoptilolite (in Russian), *Gigiena truda i profesional'nye zabolevaniya* **5,** 29 - 34.

Rodriguez-Fuentes G., Barrios M. A., Iraizoz A., Perdomo I. and Cedre B. (1997) Enterex: Anti-diarrheic drug based on purified natural clinoptilolite. *Zeolites* **19**, 441 - 448.

Roland D. A., Rabon H. W., Rao K. S., Smith R. C., Miller J. W., Barnes D. G. and Laurent S. M. (1993) Evidence for absorption of silicon and aluminium by hens fed sodium zeolite A. *Poultry Science* **72**, 447 – 455.

Severance H. W., Holt J., Patrone N. A. and Chapman L. (1988) Profound muscle weakness and hypokalemia due to clay ingestion. *Southern Medical Journal* **81,** 272 - 274.

Suzuki K. and Hei T. K. (1996) Induction of heme oxygenase in mammalian cells by mineral fibers: distinctive effect of reactive oxygen species. *Carcinogenesis* **17**, 661 - 667.

Taylor A. J.and Vadgama P. (1992) Analytical reviews in clinical biochemistry: the estimation of urea. *Analytical Clinical Biochemistry* **29**, 245 - 264.

Tietz N. W. (Ed.) (1976) *Clinical Guide to Laboratory Tests* p. 1037. W.B. Saunders Co., Philadelphia, PA.

Varel V. H., Robinson I. M. and Pond W. G. (1987) Effect of dietary copper sulfate, Aureo SP250, or clinoptilolite on ureolytic bacteria found in the pig large intestine. *Applied and Experimental Microbiology* **53,** 2009 – 2012.

Vrzgula L., Bartko P., Blažovsky J. and Kozač J. (1982) The effect of feeding clinoptilolite on health status, blood picture and weight gain in pigs. *Veterinarni Medicina* **27,** 267 - 274.

Ward T. L., Watkins K. L., Southern L. L., Hoyt P. G. and French D. D. (1991) Interactive effects of sodium zeolite-A and copper in growing swine: growth, and bone and tissue mineral concentrations. *Journal of Animal Science* **69**, 726 - 733.

Ward T. L , Watkins K. L. and Southern L. L. (1993) Research Note: Interactive effects of sodium zeolite A and Eimeria acervulina infection on growth and tissue minerals in chicks. *Poultry Science* **72,** 2172 - 2175.

Weinstock N. (1980). Routine determination of copper and zinc from untreated and undiluted serum by flame atomic absorption spectrometry. *Journal of Clinical Chemistry Clinical Biochemistry* **18,** 712 - 713.

Worth H. G. J. (1985) A comparison of the measurement of sodium and potassium by flame photometry and ion-selective electrode. *Analytical Clinical Biochemistry* **22**, 343 - 350.

Table 1.

*Chemical composition of the natural clinoptilolite-rich volcanic tuff used in the experiments**

Compound	*wt %*
SiO_2	50 – 55
Al_2O_3	9.3 – 11.4
Fe_2O_3	2.2 – 2.8
Na_2O	0.8 – 1.1
K_2O	2.9 – 4.3
MgO	0.8 – 1.2
CaO	13.7 – 17.2
MnO	0.07 – 0.90
TiO_2	0.14 – 0.22
water (800 ^{o}C)	14 – 16

* From Pavelić *et al. (2000)*

Table 2.

The effect of natural zeolite clinoptilolite on mouse body weight and survival rate

	30-day survival (alive/treated)	*Body weight at sacrifice (g)*
Normal mice		
Controls (normal food)	6/6	25.4 ± 2.07
NGCp (25%)	6/6	25.0 ± 1.92
MTCp (12.5%)	6/6	24.6 ± 1.78
MTCp (25%)	6/6	23.8 ± 2.13
Mice with transplanted mammary tumor		
Controls (normal food)	3/6	nd*
NGCp (25% and 50%)	5/12	nd
MTCp (25% and 50%)	4/12	nd

* nd – not determined

NGCp – normally ground, MTCp – mechanically treated clinoptilolite.

Table 3.

The effect of food supplementation with natural zeolite clinoptilolite on hematological parameters in peripheral blood

		Food supplement				
Parameter	Day of sacrifice*	*none (controls)*	*NGCp (25%)*	***MTCp*** *(12.5%)*	***MTCp*** *(25%)*	*Kruskal-Wallis (p)*
leukocytes ($x10^9$/L)	10	7.9 ± 3.4	7.5 ± 1.5	6.9 ± 1.0	7.5 ± 3.2	0.711
	20	6.1 ± 1.4	9.0 ± 2.0[#]	6.7 ± 1.1	5.5 ± 2.7	0.040
	30	5.9 ± 1.1	7.8 ± 1.5[#]	7.5 ± 1.1[#]	5.7 ± 1.8	0.037
	40	5.9 ± 1.2	7.0 ± 1.1	5.6 ± 2.1	5.8 ± 1.5	0.426
lymphocytes ($x10^9$/L)	10	7.2 ± 3.5	6.5 ± 1.3	6.1 ± 0.9	6.9 ± 3.0	0.869
	20	5.5 ± 1.3	8.0 ± 1.7[#]	6.2 ± 1.1	5.1 ± 2.6	0.047
	30	5.4 ± 1.1	7.1 ± 1.3[#]	6.8 ± 1.1[#]	5.9 ± 1.0	0.072
	40	5.4 ± 1.1	6.4 ± 1.0	5.0 ± 2.0	5.3 ± 1.4	0.321

* After the onset of feeding with clinoptilolite-containing food. Six mice per group

[#] Significant difference from the control group at $p < 0.05$ or better (Mann-Whitney)

The data are means ± standard deviations. NGCp – normally ground clinoptilolite, MTCp – mechanically treated clinoptilolite

Table 4.

The effect of food supplementation with natural zeolite clinoptilolite on serum chemistry.
Six mice per group, measurements on pooled serum from 3 mice. Mean values

Parameter	*Day of sacrifice*	***Food supplement***				*Reference values**
		none (controls)	*NGCp* (25%)	***MTCp*** (12.5%)	***MTCp*** (25%)	
potassium	10	6.9	7.4	6.4	8.0	5.0 – 7.6
(mmol/L)	20	7.6	9.2	9.1	6.7	
	30	7.5	9.2	9.3	8.8	
sodium	10	154	155	153	154	132 – 162
(mmol/L)	20	153	153	153	154	
	30	154	154	154	155	
chloride	10	107	103	104	104	92 – 106
(mmol/L)	20	105	105	105	110	
	30	106	106	104	108	
total calcium	10	2.34	2.17	2.34	2.17	2.0 – 2.5
(mmol/L)	20	2.30	2.25	2.22	2.30	
	30	2.23	2.18	2.27	2.34	
inorganic P	10	4.08	4.93	4.48	4.83	2.35 – 3.58
(mmol/L)	20	4.69	4.27	4.88	4.49	
	30	4.77	4.93	4.95	4.87	
magnesium	10	1.14	1.53	1.31	1.48	0.7 – 1.2[#]
(mmol/L)	20	1.48	1.39	1.41	1.43	
	30	1.39	1.43	1.47	1.52	
zinc	10	18.0	17.9	16.0	18.2	9.9 – 17.9[#]
(µmol/L)	20	21.5	15.5	16.3	20.8	
	30	19.7	19.2	21.3	18.6	
urea[&]	10	12.3	13.0	12.1	13.0	2.6 – 4.2
(mmol/L)	20	9.9	8.6	9.7	9.8	
	30	9.6	11.9	9.2	10.3	
creatinine	10	49	63	47	59	1.0 – 80
(µmol/L)	20	53	55	60	51	
	30	56	60	62	62	
total bilirubin	10	2.0	2.0	1.0	2.0	1.3 – 6.4
	20	2.0	2.0	3.0	2.0	
(µmol/L)	30	2.0	2.0	2.0	2.0	

* from Foster et al., 1983

[#] internal reference values for human serum

[&] erratic data due to food contamination with urine (see text)

Table 5.

The effect of food supplementation with natural zeolite clinoptilolite on bone marrow activity

Parameter	*Day of sacrifice**	***Food supplement***				*Kruskal-Wallis (p)*
		none (controls)	*NGCp (25%)*	***MTCp*** *(12.5%)*	***MTCp*** *(25%)*	
GM-CFU per 10^5 cells	10	105 ± 9	85 ± 7	105 ± 6	95 ± 6	0.192
	20	310 ± 18	310 ± 15	335 ± 13	355 ± 15	0.229
	30	230 ± 9	195 ±13[#]	190 ± 8[#]	200 ± 7[#]	0.006
	40	165 ± 11	115 ± 6[#]	130 ± 8[#]	140 ± 4[#]	0.002
GM-CFU per femur (x 10^3)	10	16.1 ± 6	10.0 ± 3	20.4 ± 3	11.9 ± 3	0.007
	20	32.3 ± 5	28.0 ± 6	28.8 ± 5	33.7 ± 5	0.228
	30	24.2 ± 2	23.4 ± 6	22.9 ± 3	26.1 ± 4	0.424
	40	20.9 ± 3	19.2 ± 3	14.6 ± 4[#]	14.5 ± 2[#]	0.004

* After the onset of feeding with clinoptilolite-containing food. Six mice per group

[#] Significant difference from the control group at $p < 0.05$ or better (Mann-Whitney)

The data are means ± standard deviations. NGCp – normally ground clinoptilolite, MTCp – mechanically treated clinoptilolite

Table 6

Summary effects of clinoptilolite feeding in mice

Parameter studied	*Effect*	*Comment*
Hematology*		
RBC, Hb, MCH, MCHC	no change	
Platelet count and size	decline 10 – 20%	not significant
WBC, lymph's	rise 30 – 45%, lymphocytosis	attributable to intestinal
GM-CFU seeding efficiency and content per femur	decreased 15 – 45%	irritation/inflammation?
Serum electrolytes		
K^+	elevated 20%	exchanged for NH_4?
Na^+, Ca^{++}, Mg^{++}, Zn^{++}, Cl^-, inorganic P	no change	
Indicators of liver and kidney function		
total bilirubin, creatinine	no change	

* RBC – red blood cell count and size, Hb – hemoglobin concentration, MCH – mean cell hemoglobin, MCHC – mean cell hemoglobin concentration WBC – white blood cell count, lymph's - lymphocytes

Table 7.

The effect of food supplementation with natural zeolite clinoptilolite on serum chemistry of mice with transplanted mammary carcinoma

	Food supplement			
Parameter	*None (controls) (3)*	*NGCp (25%, 50%) (5)**	*MGCp (25%, 50%) (4)**	*Reference values[#]*
potassium (mmol/L)	14.3	14.3 10.2-18.4	14.3 11.8-16.9	5.0 – 7.6
sodium (mmol/L)	95	112 75-149	115 111-120	132-162
chloride (mmol/L)	74	89 62-116	91 90-93	92 – 106
total calcium (mmol/L)	2.04	2.11 1.34-2.88	2.28 2.04-2.52	2.0 – 2.5
inorganic P (mmol/L)	4.85	4.54 3.96-5.12	4.82 4.55-5.10	2.35 – 3.58
magnesium (mmol/L)	1.53	1.34 1.05-1.64	1.44 1.21-1.67	0.7 – 1.2[$]
zinc (µmol/L)	14.3	16.0 12.2-19.9	15.35 14.2-16.5	9.9 – 17.9[$]
urea[&] (mmol/L)	6.2	11.2 8.2-14.3	12.05 12.8-11.3	2.6 – 4.2
creatinine (µmol/L)	33	33 26-39	34 31-37	1 – 93
total bilirubin (µmol/L)	3.0	2.5 1.0-4.0	2.5 2.0-3.0	1.3 – 6.4

Measurements on pooled serum of mice sacrificed 30 days after the inoculation of the tumor and the onset of feeding. The number of mice per group is given in parentheses.

* the values in these columns are the means and ranges of two pooled samples
[#] from Foster et al., 1983
[$] internal reference values for human serum
[&] erratic data due to food contamination with urine (see text)
NGCp – normally ground clinoptilolite, MTCp – mechanically treated clinoptilolite