

SESSION 2
**TRACE ELEMENTS AND MINERALS IN PHYSIOLOGY,
SPORT MEDICINE AND REHABILITATION**

**PLASMA ANTIOXIDANT SYSTEM AND ZINC LEVELS
IN ALLERGIC RHINITIS PATIENTS**

E. Akcil, M. Akiner, N. Yazihan, S. Beton, K. Köse

Ankara University, Ankara, Turkey; eakcil@medicine.ankara.edu.tr

OBJECTIVES: We evaluated the impact of anti-oxidant enzymes and trace element concentrations in the blood and plasma of allergic rhinitis (AR) patients with their symptoms. **PATIENTS AND METHODS:** The study included 45 patients with allergic rhinitis and 20 healthy controls. Blood and serum samples were analyzed spectrophotometrically for catalase and superoxide dismutase activity. Zinc (Zn) and copper (Cu) levels measured by atomic absorbance system. The results were compared with symptoms of AR patients. **RESULTS:** Plasma Zn and Cu levels found significantly low in AR patients ($p < 0.001$) and in parallel to plasma; intra erythro-

cyte Cu ($p < 0.01$) levels were low in AR whereas Zn levels increased ($p < 0.001$) compared to the control group. SOD activity was increased in AR group ($p < 0.001$). We did not find any difference in CAT activity in AR group compared to control. Main symptoms of AR such as sneezing, excess tears in the eyes, cough, headache were found correlated with low plasma Zn levels. **CONCLUSION:** Our results suggest that imbalances in antioxidant system and plasma trace elements especially Zn levels play a role in the etiopathogenesis of allergic rhinitis. Zn treatment might be important for follow-up and treatment of AR patients.

**SE LOW INTAKE AFFECTS OXIDATIVE STRESS PARAMETERS
IN AN AGE DEPENDENT MANNER: AN EXPERIMENTAL STUDY
CONDUCTED ON YOUNG (5 MONTHS) AND OLD RATS (18 MONTHS)**

***R. Benaraba¹, J. Arnaud^{1,2}, V. Ducros², M. Osman¹, A.-M. Roussel¹,
X. Leverve¹, I. Hininger-Favier¹***

¹ INSERM, U884, LBFA, Université Joseph Fourier, Grenoble, France; rachida.benaraba@ujf-grenoble.fr, mireille.osman@ujf-grenoble.fr, a-m.roussel@ujf-grenoble.fr, xavier.leverve@ujf-grenoble.fr, isabelle.hininger@ujf-grenoble.fr

² CHU de Grenoble, Grenoble, France; JArnaud@chu-grenoble.fr, VDucros@chu-grenoble.fr

BACKGROUND: Epidemiological studies indicate that low plasma selenium (Se) concentrations increase the risk of human diseases. High selenium status has been proposed to be an important factor for successful. In Europe the level of Se intake is low regarding the recommendation and decreases with age. Therefore, we investigated the effect of dietary selenium on oxidative stress parameters at different ages, as a possible underlying mechanism. **METHOD:** We conducted a study on four groups ($n = 10$ per group) of young (5 months) and old (18 months) rats submitted for 8 weeks to low (30 ppm) or high (180 ppm) Se diet. The effect of diet was evaluated on plasma, brain, heart and liver oxidative stress parameters. **RESULTS:** Low Se diet decreased significantly Se and GPX activity in old and young rats in plasma and tissues excepted for brain. In plasma and tissues the differences between low and high Se diet were in

average 25% in old rats and 17% in young rats. These results suggest that old rats are more sensitive to low Se diet and confirm that brain is remarkably protected. With regard to oxidative stress parameters in plasma, TBARS were significantly higher in old rats whatever Se regimen. Protein thiol groups were comparable in old and young rats when Se diet was high but they were significantly decreased in old rats in case of Se low diet. Liver thioredoxine reductase was lower in old rats when Se diet was high. When Se diet was low, old rats had significantly higher TBARS, and glutathione reductase. In heart, low Se diet was associated with a significant decrease in thiols groups and glutathione reductase in old rats whereas no effect was observed for young. **CONCLUSION:** Old rats seem more affected by a low selenium diet than younger. These results are in favour of the maintenance of selenium sufficient intake while aging.

SELENIUM STATUS IN WOMEN LIVING IN SÃO PAULO, BRAZIL

M.C. de Bortoli¹, C. Cominetti¹, D.I.T. Fávoro², S.M.F. Cozzolino¹

¹ School of Pharmaceutical Sciences, Department of Food and Experimental Nutrition, University of São Paulo, São Paulo, Brazil; mbortoli@usp.br

² Institute for Energetic and Nuclear Research, University of São Paulo, São Paulo, Brazil

Selenium is an essential nutrient, and has antioxidant properties and other functions, among them, participates in the conversion of thyroid active hormone (triiodothyronine), and protection against intoxication for heavy metals, including mercury. Selenium status is influenced by its content on soil, the presence of heavy metals and the chemical form with which it is ingested. Selenium deficiency affects glutathione peroxidase activity, an enzyme that reacts with a large variety of organic hydroperoxides, and for this property is widely known as the major protector system for lipid peroxidation. Acknowledging selenium importance in human health, the aim of this research was to assess selenium status in women living in São Paulo, Brazil. This project was approved by the Ethics Committee of the University of São Paulo. Seventy women formed the group of study, and they were all in reproductive age, did not suffer from diabetes or thyroid dysfunctions, were no preg-

nant or breast-feeding, did not take multivitamins tablets and lived in São Paulo for more than 20 years in average. A blood sample was collected to assess selenium levels in plasma and erythrocytes by hydride generation-atomic absorption spectrometry, and GPx activity in erythrocytes was measured by commercial kit (RANDOX). Mean selenium concentration in plasma was $81.7 \pm 18.8 \mu\text{g/L}$, and in erythrocytes was $86.3 \pm 34.7 \mu\text{g/L}$. The mean value found for GPx activity in the erythrocyte was $37.1 \pm 12.2 \text{ U/gHb}$. According to these results the values found for selenium and GPx activity were adequate in relationship to the reference values for these parameters, denoting that this population is not at risk of selenium deficiency. However, it is advisable that more studies take place, with larger population, to assess selenium nutritional status.

Financial support: CNPq and FAPESP

THE INFLUENCE OF DIETARY INTAKE AND ELEMENTAL STATUS ON THE STUDENT'S RESULTS

T.I. Burtseva, O.I. Burlutskaya, O.O. Frolova

Orenburg State University, Orenburg, Russia; burtat@yandex.ru

INTRODUCTION: The optimal nutrition is one of the main conditions of healthy and successful life. Correct, balanced nutrition provides growth and evolution of children, assists to improve the school results and workable qualities, help no resist the negative influence of the environment. The lack of essential macro- and trace elements in foodstuffs and drinking water, and in some cases the excess of the toxic substances promotes the disturbance of vital functions, decrease the adaptive possibilities of the organism and deterioration of the individuals and population of the regions. **AIM:** To determine the possible influence of macro- and trace elements provision on the students results. **METHODS:** Totally 15 — 17 y.o. 105 healthy college students were interviewed, tested for daily intake of microelements with food, and their hair were investigated by ICP-MS in laboratory of Centre for Biotic medicine (Moscow). All students were divided in groups: Gr. 1 and Gr. 2 consisted from 34 boys and 31 girls, with good results; Gr. 3 and Gr. 4 included 17 boys and 33 girls with poor results. **RESULTS:** The investigation of dietary

intake of macro- and trace elements, in the students allowed to see that the boys with poor results used significant less ($p < 0.05$) calcium (1.2 times), iron (1.3 times) and zinc (1.4 times), and girls — copper (in 1.2 times) in comparison with Gr. 1, 2. The analyses of macro- and microelements in the student's hair allowed to mark differences ($p < 0.05$) and in their elemental status depending on the student's results. The research have shown that the good results are associated with higher Zn, Se, J, Fe and lower Cu hair content in comparison to Gr. 3 and Gr. 4. It was mentioned that high copper content in boy's hair significantly ($p < 0.05$) correlated ($r = 0.25$) with poor results. **CONCLUSIONS:** This research demonstrated that the lack of dietary intake of essential elements (calcium, iron, zinc, iodine) and copper excess have the negative influence on the student's results.

This investigation was supported by grant of Russian Humanitarian and Scientific Foundation (RGNF) 10-06-81602a/Y and by the government of Orenburg region.

REDUCTION OF THE TRACE ELEMENTS (COPPER AND MANGANESE) FROM LAYER DROPPINGS FOLLOWING THE USE OF DIETARY CHELATES AND AMINO ACIDS

R. Criste, A. Untea, A. Bercaru

National Research and Development Institute for Biology and Animal Nutrition (INCDBNA), Balotesti, Romania; cristerodica@yahoo.com

The poultry production is one of the agricultural operations that may generate, via the droppings, a surplus of trace elements that may pollute the environment. In the attempt to reduce the Cu and Mn levels from the droppings, we conducted a 14 week experiment on 120 Lohmann Brown layers (aged 30 weeks) whose diets included chelates of Cu (TEC/Cu) and Mn (TEC/Mn) with amino acids. The layers were housed in cages (4 birds/cage) and assigned to three groups (C, E1 and E2) fed on the same basal diet of corn and soybean meal. The inorganic premix (IP) added to the control (C) diet contained 6 ppm Cu and 71.9 ppm Mn from inorganic sources. The level of trace elements added to the experimental formulations was calculated using NRC criteria. E1 contained 6.5 ppm Cu (3 from IP, 3.25 from TEC/Cu) and 46.95 ppm Mn (35.95 from IP and 11 from TEC/Mn), E2 contained 4.03 ppm Cu and 28.05 ppm Mn from chelates. Throughout the experiment, we produced batches of feed every three

weeks and starting from the second week of feeding with the new batch, the droppings were collected on a daily basis and average weekly samples/cage were formed. The Cu and Mn were determined by FAAS, after microwave digestion. We observed that the Mn level from the droppings increased over time in all three groups, but it was consistently significantly ($p \leq 0.05$) lower in E1 and E2 as compared to C. The excretion of Cu in the droppings also increased over the time, but during the final 7 weeks it was significantly ($p \leq 0.05$) lower in the experimental groups as compared to group C. This differentiation is accounted for by the lower level of Cu and Mn in groups E1 and E2 compared to C. The feed intakes and the egg production were not statistically different between the three groups; this shows that these lower levels did not affect the bioproduktive parameters of the hens. The Cu and Mn concentration from layer serum, collected at the end of the experiment, was also evaluated.

SELENOPROTEINS AND THEIR ROLES IN REDOX BIOLOGY

V.N. Gladyshev

Center for Redox Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston MA, USA; vgladyshev@rics.bwh.harvard.edu

Selenoproteins contain a rare amino acid, selenocysteine, which occurs in all three domains of life and functions as the catalytic redox group in several classes of oxidoreductases. Full sets of selenoproteins have recently been identified in a variety of organisms, including humans, which have 25 known selenoproteins. These proteins explain the role dietary selenium plays in biology and human health and point to new biological processes that are dependent on this trace element. Selenoproteins are members of diverse protein families. Thioredoxin-like proteins are particularly prone to conversion into selenoprotein forms. Because dietary selenium is required for selenoprotein expression, diets differing in selenium levels provide means

of regulating selenoprotein function and redox homeostasis in mammals. Selenoproteins may also be used as tools to identify proteins that contain catalytic redox-active cysteine residues and determine location of these residues in protein sequences. In addition, studies on selenoproteins provide new information about the genetic code. Being redox catalysts, selenoproteins are involved in the repair of oxidatively damaged proteins, activation and inactivation of thyroid hormone, regulation of the redox state of thioredoxin, removal of hydrogen peroxide, and other functions. In turn, these functions implicate selenoproteins in cancer prevention, regulation of the aging process, and male reproduction.

NO EFFECT OF SUBMAXIMAL EXERCISE INTENSITIES ON SERUM ION LEVELS IN EUHYDRATED ENDURANCE ATHLETES

C. González-Haro¹, M. Soria¹, J.L. López², M.T. Llorente², J. Escanero Marcén¹

¹ School of Medicine, University of Zaragoza, Zaragoza, Spain; E-mail: ghcarlos@gmail.com

² Hospital Central de la Defensa. Madrid, Spain

BACKGROUND: There is controversy about the effect of low to moderate intensity exercise on serum levels of trace elements. The two main causes of these changes are hypothesized to be a decrease of plasma volume and a transient shift from active muscle to extracellular fluid. **AIM:** To assess the effect of relative exercise intensity on different serum ions in euhydrated endurance athletes. **METHODS:** 27 endurance athletes (age: 33.8 ± 6.7 yr, BMI: 23.3 ± 2.2 kg/m²) carried out a warm-up on cycloergometer of 10 min at 2.0 w/kg and increments of 0.5 w/kg until exhaustion. Oxygen uptake (VO₂), blood lactate concentration ([La⁻]_b), and serum ions (Mg, Mn, Co, Zn, and Se) were measured at rest, at the final of each stage and minutes 3, 5 and 7 of post-exercise. Urine density and pH were analyzed before and after the test, and subjects drank water ad libitum. Fat oxidation (FAT_{ox}), carbohydrate oxidation (CHO_{ox}), energy expenditure from fat (EE_{FAT}), energy expenditure from CHO (EE_{CHO}), total EE were estimated by

means stoichiometric equations. Serum ion levels at each exercise intensity (w/kg) were compared by means repeated measures ANOVA. Pearson's correlation was performed to assess the relationship between variables. Significance level was $P < 0.05$. **RESULTS:** Euhydration of subjects was controlled (drank: 842 ± 197 mL of water, no significant differences neither urine density: 1.014 ± 0.004 vs. 1.014 ± 0.004 g/cm³ nor urine pH: 5.90 ± 0.75 vs. 5.98 ± 0.77 pH). No significant differences were observed in any ion respect to exercise intensity. A low significant correlation between Zn ($r = 0.332$, $P < 0.001$) and Se ($r = 0.242$, $P < 0.01$) respect to [La⁻]_b were found; and no relationship with [La⁻]_b, VO₂, FAT_{ox}, CHO_{ox}, EE_{FAT}, EE_{CHO}, EE_T and serum ion level were established. **CONCLUSIONS:** Acute exercise at different submaximal intensities in euhydrated endurance athletes does not have an effect on serum ion levels. This may be due to a low activity of ions in extracellular tissue.

BORON REGULATES TUFTELIN mRNA EXPRESSION OF MC3T3-E1 CELLS

S.S. Hakki, B.S. Bozkurt, E.E. Hakki

Selcuk University, Konya, Turkey; sshakki@yahoo.com, sshakki@selcuk.edu.tr

BACKGROUND AND AIMS: The effects of boron in the formation and maintenance of mineralized structures in molecular level and its precise function is still unclear. Tuftelin has been suggested to play an important role during the development and mineralization of enamel. The purpose of this study was to investigate the effects of boron on the mRNA expressions related to osteogenic differentiation of MC3T3-E1 cells, *in vitro*. **MATERIAL AND METHODS:** In order to evaluate mRNA expression of MC3T3-E1 (clone 4) cells, they were treated with different concentrations of boron (0, 0.1, 1, 10, 100 and 1000 ng/ml) and total RNAs of the groups were isolated on day 3. Cells treated with boron were evaluated for gene expressions profiles using RT2 Profiler PCR micro-array which

includes 84 genes related to osteogenic differentiation. This array contains genes functioning in the development of the skeletal system as well as bone mineral metabolism. **RESULTS:** According to PCR micro-array results, all boron treatments dramatically up-regulated ($1.5E+0$ or greater) tuftelin mRNA expression and these results were confirmed by Quantitative RT-PCR using tuftelin probe. Moreover, boron treatments down-regulated ($0.5E+0$ or lower) integrin- $\alpha 2$ gene transcript. **DISCUSSION:** Results suggested that at the molecular level boron plays important role on not only bone metabolism, but also in amelogenesis process.

This study was supported by National Boron Research Institute of Turkey and The Selcuk University.

HEPCIDIN AND THE IRE/IRP SYSTEM: PHYSIOLOGY AND DISEASES OF CELLULAR AND SYSTEMIC IRON REGULATION

M.W. Hentze

European Molecular Biology Laboratory, Heidelberg, Germany; hentze@embl.de

The regulation and maintenance of systemic iron homeostasis is critical to human health, because both iron deficiency and iron overload cause severe, potentially lethal diseases. In addition to systemic iron balance, iron homeostasis must also be maintained locally within organs and even at the single cell level, because iron is both necessary (e.g. haem synthesis, iron-containing enzymes, Fe-S clusters) and potentially toxic (radical formation, Fenton chemistry). It is now well appreciated that the liver hormone hepcidin and its target protein ferroportin play a critical regulatory role for systemic iron metabolism. Hepcidin deficiency is the common cause of the different forms of hereditary haemochromatosis, including the more severe juvenile form. Conversely, hepcidin overproduction as a consequence of inflammatory and infectious stimuli or due to genetic mutations (e.g. IRIDA syndrome) leads to iron refractory microcytic anaemia. Cellular iron homeostasis is preserved via

the IRE/IRP regulatory network, and recent experimental data also unveiled the importance of this network in systemic iron homeostasis. I will give a systematic overview of the regulation of systemic and cellular iron metabolism and its role in human disease.

Hentze M.W., Muckenthaler M., Andrews N.C. Balancing acts: molecular control of mammalian iron metabolism. *Cell* 117, 285–297, 2004.

Galy B., Ferring-Appel D., Kaden S., Gröne H.-J., Hentze M.W. Iron regulatory proteins are essential for intestinal function and control key iron absorption molecules in the duodenum. *Cell Metab.* 7, 79–85, 2008.

Vijic Spasic M., Kiss J., Herrmann T., Galy B., Martinache S., Stolte J., Gröne H.-J., Stremmel W., Hentze M.W., Muckenthaler M.U. Hfe acts in hepatocytes to prevent hemochromatosis. *Cell Metab.* 7, 173–178, 2008.

Muckenthaler M.U., Galy B., Hentze M.W. Systemic iron homeostasis and the IRE/IRP regulatory network. *Ann. Rev. Nutr.* 28, 197–213, 2008.

A SINGLE INJECTION OF COPPER SULPHATE RESTORES OXIDASE ACTIVITY IN BLOOD SERUM OF MAMMALS FED BY FODDER WITH SILVER IONS

E. Iliecheva¹, A. Skvortsov¹, E. Zatulovsky¹, N. Tsymbalenko², P. Rusconi³, M. Broggin³, L. Puchkova^{1,2}

¹ Saint-Petersburg State Polytechnical University, St.Petersburg, Russia; ikaterina2705@yandex.ru

² Research Institute of Experimental Medicine, St.Petersburg, Russia; puchkovalv@eyandex.ru

³ Research Institute of Pharmacology Mario Negri, Milan, Italy; massimo.broggini@marionegri.it

BACKGROUND: Copper (Cu) is the critical structural and catalytic cofactor of vital enzymes and a member of signalling. At the same time Cu is a potentially toxic agent. So any disturbance of Cu metabolism causes serious diseases. Changes of Cu status (CS), which includes concentration of Cu, the level of ceruloplasmin (Cp) oxidase activity, and Cp concentration, are often a clinical manifestation of such diseases. The link between CS and intracellular Cu metabolism is poorly studied. In work, the rodent model with changing CS is characterized. The decrease of CS was induced by adding Ag(I) to fodder, and its recovery was initiated by CuSO₄ injection. **APPLIED TECHNIQUES:** The work was carried out on Wistar rats and C57Bl mice, which received 50 mg AgCl/kg body weight daily (Ag-animals). Metal concentration was measured by FAAS, oxidase and ferroxidase activity were measured in gel-reactions. Cp-mRNA level was determined by semiquantitative RT-PCR. Cp protein content was measured by Western blot. Cp preparations were isolated by ion-exchange chromatography and analyzed with UV/vis

absorption and circular dichroism (CD) spectroscopy and calorimetric studies. Golgi membranes were isolated by equilibrium centrifugation. **IMPORTANT RESULTS:** After definite time the concentration of Cu in Ag-animals decrease and oxidase and ferroxidase activity could not be detected then. However content of Cp protein does not change. The relative activity of Cp gene and the Cp level in Golgi complexes are similar in control and Ag-animals. Ag(I) was found in preparation of Cp, which was isolated from Ag-animals (Ag-Cp). Tertiary structure of Ag-Cp significantly differs from that of holo-Cp. Single injection of 10 µg CuSO₄·5H₂O per g of body weight causes rapid recovery of oxidase and ferroxidase activity in blood serum, which is easily detected after 1h and persists for 3 days under continuing Ag-diet. Comparison of rates of appearance of [¹⁴C]Cp and «new» oxidase Cp in bloodstream indicates that injected Cu are inserted to Cp molecules in Golgi complex. The possibility of using Ag-animals as an experimental model with switching Cu status is discussed.

INTERRELATIONSHIP BETWEEN METABOLIC SHIFTS AND CONTENT OF SOME CHEMICAL ELEMENTS IN HUMAN BIOSUBSTRATES

M. Karganov¹, I. Alchinova¹, A. Fesenko¹, I. Ivashkiv²

¹ Institute of General Pathology and Pathophysiology RAMS, Moscow, Russia; mkarganov@mail.ru

² Military Medical Department of the Ministry of Internal Affairs of Russian Federation, Moscow, Russia

Laboratory diagnostics allow not only obtaining specific results for each individual, but also combining basically different methods for more precise evaluation of the functional state of various body systems. This approach to the diagnostics implies evaluation of mutual influences and combinations of various parameters, their relationships with clinical symptoms and correlations with other indexes, rather than simple accumulation of laboratory data. We present data obtained during combined use of standard trace element assay protocol in individuals with different content of some chemical elements (Hg, As, Mn, etc.) and laser correlation spectroscopy of blood serum, urine, and oropharyngeal washout fluid (OPWF). This method allowing measuring of particle size in native biological fluids is successfully used in clinical practice. The relative content of particles of different size characterizes the direction of metabolic shifts in the organism. We analyzed biological samples obtained from 18 individuals conceivably exposed to the action of tox-

icants (Hg and As). It was found that the incidence of metabolic shifts correlated with the content of the test elements. In particular:

— the lower was arsenic concentration in urine samples, the higher was the contribution of large particles into light scattering in OPWF samples ($r = -0.75$, $p < 0.05$);

— significant correlations between the shifts in the serum and metal concentration in the urine were found for samples taken during the second examination: Hg ($r = 0.50$, $p < 0.05$) and As ($r = 0.82$, $p < 0.05$).

— analysis of the relationship between metal concentration and the contribution of certain particles into light scattering revealed a correlation between the presence of As in the urine and accumulation of the zone 1 particles (0–10 nm) ($r = 0.53$, $p < 0.05$) in blood serum, while the increase in the content of this element in the urine correlated with an increase in the contribution of zone 2 particles (51–400 nm) ($r = 0.76$, $p < 0.05$).

SELENIUM APPLICATION INHIBITS ATP, ACh, OR K⁺-INDUCED CONTRACTILE RESPONSES IN ISOLATED RAT ILEUM

E. Koç, B. Turan

Ankara University, Ankara, Turkey; ekoc@medicine.ankara.edu.tr; belma.turan@medicine.ankara.edu.tr

Selenium (Se) is a ubiquitous trace element in nature which has proved to be essential for human. Its best known biological role is being a component of an antioxidant enzyme, glutathione peroxidase. Se plays an important role in physiology of many cell types. Previously, it has been shown that extracellular application of sodium selenite/selenate at high concentrations (≥ 1 mM) caused cellular dysfunction in different cell types of mammalian tissues. In the present study, we aimed to investigate the effects of sodium selenite (1 mM) application on the responses to ATP, acetylcholine (ACh), or K⁺-induced contractions of isolated ileal preparations. Healthy adult Wistar rats (200–250 g) were used. Isolated ileum segments were suspended into isolated tissue bath chamber containing 40 ml standard Tyrode solution. The contractile forces were recorded isotonically with a force displacement transducer. The maximum amplitude of the contractile activity was measured from

paper-recording traces. Sodium selenite application alone caused a contractile response in ileal preparations. When it is applied to the samples at 5 min following ATP, ACh or K⁺-induced contraction, it caused about 50% decrease in the contractile responses, as a selenium concentration-dependent manner. Since it has been previously shown that selenium applications, at $\geq 10^{-4}$ M concentrations, depressed contractility, intracellular free Ca²⁺ concentration, and Ca²⁺ current in heart preparations, our present data demonstrate that selenium induces marked inhibition in the contractile responses to muscarinic receptors stimulated as well as depolarization-induced contractile activity, in part due to its toxic effect. Although the biological function of selenium is not known clearly yet, under our previous and present findings, we can conclude that one can take caution about use of selenium compounds, due to its side effects in normal healthy subjects.

BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS IN COMPETITIVE SWIMMERS AFTER VITAMIN AND MINERAL SUPPLEMENTATION

**A.B. Lasierra¹, A.C. Arnal², L. Rello¹, M.L. Calvo¹, J.M. Tricás², O. Lucha²,
C. Vidal², A. García de Jalón¹**

¹ Hospital Universitario Miguel Servet, Zaragoza, Spain; ablasierra@gmail.com

² Universidad de Zaragoza, Zaragoza, Spain

The aim of this study was to assess the effects which vitamin and mineral supplementation can exert on biochemical and haematological parameters in athletes and, thus, achieve a physical performance improvement. 41 healthy competitive swimmers participated in the study. They did not change their dietary habits and training programme during this research and were supplemented for 10 weeks with a vitamin-mineral compound: 90 mg Mg/day, 0.4 mg folate/day, 0.002 mg vitamin B₁₂/day, 70 mg Ca/day, 6 mg Cu/day, 30 mg Zn/day, 28 mg Fe/day, 54 mg P/day, 41.4 mg Si/day, 1.46 mg Mn/day, 0.3 mg I/day, 0.1 mg Cr, Se/day and other ones: Vitamin C, choline, inositol, vitamins B, E, B₆, B₂, B₁, A, K, D, pantotenic acid, biotin and methionin. A comprehensive study of the haematological and biochemical serum parameters was carried out before and after the 10 weeks time of vitamin and mineral supplementation. Significant statistical differences were observed for the following parameters (mean differences between parenthe-

sis): LDH (-13.2 U/L), Mg (0.093 mg/dL), IgA (22.2 mg/dL), IgM (12.7 mg/dL), % Neutrophils (-4.7%), % Lymphocytes (4.4%), MCV (1.5 fL) and MCH (0.54 pg). This study shows a significant increase in the serum levels of Mg, IgA, IgM, % Lymphocytes, MCV and MCH and a significant decrease in the LDH serum levels and % Neutrophils after supplementation. After 10 weeks time of vitamin-mineral supplementation the enzymatic activity of serum LDH was lower what suggests a beneficial effect of the supplementation on muscular metabolism and exercise efficiency, avoiding muscular degradation. The significant increase of MCV and MCH in addition to a slight increase, although not statistical significant, in the red blood cell count ($p = 0.099$), haemoglobin ($p = 0.208$), haematocrit ($p = 0.349$) and CMCH ($p = 0.475$) after the supplementation, indicate a positive influence on the erythropoiesis mechanism. Higher levels of Lymphocyte, IgA and IgM after supplementation can suggest a protective effect on the immune system.

PROFILES OF MACRO- AND TRACE ELEMENT STATUS SHOWED BY MALE RESIDENTS OF MAGADAN REGION'S DIFFERENT CLIMATIC AREAS

A.L. Maximov, Ye.A. Lugovaya

Scientific-Research Center «Arktika» FEB RAS, Magadan, Russia; arktika@online.magadan.su

Hair samples from males aged 16–21 were examined to study 25 macro- and trace elements using the methods of atom emission and mass spectrometry with inductively bonded argon plasma. All subjects were divided into three groups depending on residing areas: group I, Magadan town (seaside area); group II, Ola settlement (seaside area); and group III, Susuman town (continental area). The average values of Al, As, Ca, Cd, Co, Li, I, Mg, Mn, Ni, Pb, Se, and Sn found to be lower through all groups compared to Russia's average values (Skalny, 2000), while Si and Zn values showed to be higher. Fe level was lower in the seaside area residents while in the continental area that parameter was two times as much compared to that of Russia's. Subjects of groups I and II showed their Cu average values lower than those of Russia's. Besides, their P concentrations exceeded the values demonstrated by group III. Ca deficit was observed in 79% of group I, and 44% and 22% of groups II and III, respectively. Co,

Cu, and I deficit was found in most subjects through all examined groups testifying to common lack typical of North residents. Half of group II demonstrated K and Na excess that can be caused by profiles of biogeochemistry and anthropogenic activity. Subjects of group III showed Zn and P excess while subjects of group I showed deficit in those elements. Mn deficit was revealed in groups I and II while group III demonstrated the excess of this element. At the same time most subjects of group I and 50% of group II demonstrated Mg deficit while 35% of group III proved to have its excess. The deficit in Ca, Co, Cu, and I, as well as Si excess can be considered as common North-related profile of the residents' element status. The deficit of Mg, Mn, P, Se, and Zn is typical of the seaside residents. The excess of Zn, P, Fe, Mn, and Cr is typical of the continental area residents. Those findings should be taken into account in correction-and-prevention activities even within a single region.

UNDERSTANDING ZINC HOMEOSTASIS

D. Oberleas¹, B.F. Harland²

¹ Texas Tech University (Emeritus), Lubbock TX, USA; doberlea@aol.com

² Howard University, Washington DC, USA

Understanding zinc homeostasis has been an evolutionary process. Initial interest was associated with absorption or availability to the body metabolism. In 1960, it was shown that phytate, a natural constituent of all plant seeds had some adverse effect on zinc that was perceived to affect absorption. It had been determined in 1943 that zinc was secreted into the GI tract of dogs. This was extended later to be the same physiology in 7 species in 8 laboratories. More recently, evidence has indicated that this represents normal secretion by the pancreas in the form of a metallothionein I complex that contains 7 atoms of zinc per molecule of protein. Of equal importance is that this secretion occurs over two or more weeks after the zinc has been consumed and absorbed. It is secreted into the pancreatic duct along with several protein digestive enzymes. Thus the fragile metallothionein protein is readily hydrolyzed and the zinc is released as inorganic zinc ions that are again vulnerable for complexation by phytate. Since this zinc represents that absorbed at an earlier time, this process takes zinc away from the body, thus the term «homeostasis» becomes more fitting to describe the processes oc-

curing in all monogastric species. A formula has been derived to quantify this process referred to as the phytate:zinc molar ratio. A molar ratio of 10 or less has been established as the critical ratio below which there is adequate zinc homeostasis. Molar ratios above 10 define zinc deficiency with increasing severity. Thus not all of the phytate must be broken down in order to provide adequate zinc homeostasis. Also, recently it has been determined that small amounts of phytate are absorbed. These small amounts are well within the limits of the molar ratio of 10. One important problem that limits the usefulness of the current system is the lack of phytate analyses for an adequate variety of foodstuffs. At some future time, nutrient databases will provide adequate phytate analytical data to improve this process. Another factor that has entered into the process recently is the availability of a broad, effective pH range phytase produced by the fungus *Aspergillus ficuum* that is available for human consumption. The amount of enzyme necessary to hydrolyze most but not all of the phytate has not been adequately studied. The latter appears to be the best and cheapest cure for zinc deficiency.

FUNCTIONAL INDICATORS OF CARDIO-RESPIRATORY SYSTEM, CHARACTERISTIC OF METABOLISM AND DEFENCE SYSTEM STATUS IN GIRLS' ATHLETES

N. Pankova¹, A. Fesenko¹, E. Arkhipova¹, A. Cherepov¹, A. Fesyun², M. Karganov¹

¹ Institute of General Pathology and Pathophysiology RAMS, Moscow, Russia; nbpankova@gmail.com

² Military Medical Department of the Ministry of Internal Affairs of Russian Federation, Moscow, Russia

We examined the state of the body of professional athletes (group S, n = 13), female students (17–25 years), who studied modern dance (group T, n = 8) and a control sample of the same age (group C, n = 8). The objects of study were indicators of the respiratory system and indicators of autonomous regulation (by spectral analysis of HRV and peripheral BPV, and the sensitivity of arterial baroreflex). All of the indicators tested in the sitting position in 2 states: at rest with random breath (1), and in functional test increasing the «dead» breathing space of about half, but allowing evaluate the respiratory parameters, with random breath (2). No differences were revealed between the groups T, C and K in parameters of the respiratory system (respiration rate and respiratory volume). The features of professional athletes were a sports bradycardia, high level of HRV and high sensitivity of the arterial baroreflex. For groups S and T were also experienced higher (in compare with the

group C) the systolic BP and variability of BP during the respiratory cycle. Also it was revealed the highest resistance to the application of functional test in girl' athletes using the evaluation of the reactivity of all the indices by changing the options testing. Laser correlation spectroscopy (LCS) and analysis of cytotoxicity of patient serum and urine for bovine spermatozoa, new methods for laboratory diagnostics and control of the defence systems status and efficiency of prescribed treatment, were first used in sport medicine practice. Method of LCS allows determining the dispersion composition of the studied biological fluid by the relative contribution of particle components into light scattering. In this case it was revealed mainly catabolic shifts, normal values in C3–C4 complement components but differences in biological fluids cytotoxicity. Our results suggest that applied methods are highly informative and safe and should be introduced into sport medicine practice.

HIRUDO MEDICINALIS – MEDICAL PRODUCT WITH TRACE ELEMENTS ACTIVITY?

**T.V. Pleteneva, A.V. Syroeshkin, T.V. Grebennikova, N.A. Hodorovich,
T.V. Maksimova, T.K. Slonskaya, A.A. Mokrousov, A.P. Popel**

Peoples' Friendship University of Russia, Moscow, Russia; pleteneva@med.rudn.ru

Joint influence of trace elements on the human organism is of interest to pharmacists (multicomponent chemotherapy, combined medicines) and toxicologists (joint toxicity). Introduction of medicines with different combinations of essential elements into pharmaceutical practice requires detailed study of biological effects in various combinations in the relevant concentration ranges. The medicinal leech is a natural medical product. It is the source around of 50 metals and amphoteric elements but their combined biological activity during therapeutic using has not yet been investigated. Correlation dependences of accumulation of copper and zinc, iron and nickel and other combinations of elements in the medical leech have been discussed in the report. The general regularities of accumulation (*Hirudo medicinalis*, human hair of healthy and sick persons) and the nature of combined biological activity of different combinations of trace elements on the culture of yeast *Saccharomyces cerevisia* have been investigated. The features of specific ele-

ment profiles for *Spirostomum ambiguae*, *Bacillus subtilis*, *Mycobacteria tuberculosis*, *Rhodococcus ridichrous*, *Allium aflatuense* and *Hirudo medicinalis* have been discussed. The analysis of essential macro- and microelements in medicinal leeches was carried out by (ICP-MS, ELAN DRC II) after devitalisation by freezing, drying to constant mass and microwave mineralization. The correlation of the iron and zinc accumulation in human hair was studied by XRF. The kinetics of the culture growth in individual and combined effects of metal ions investigated for *Saccharomyces cerevisia* by nephelometry method and counting the number of cells/colonies in the Goryaev chamber. The cell distribution on size and shape after the combined effects of ionic forms of essential and impurity elements recorded using the laser light diffraction in a laser diffraction particle sizer «Malvern 3600 Ec». It had been suggested that *Hirudo medicinalis* is able to restore disturbances of essential element homeostasis during the period of contact with patient blood.

DIET CA/P RATIO ALTERS THE SENSITIVITY OF RAT BB CREATINE KINASE ACTIVITY TO VITAMIN D SUPPLY

V. Risnik¹, A. Sokolnikov²

¹ Institute of Nutrition at Russian Academy of Medical Sciences, Moscow, Russia; spirichev@ion.ru

² ANO «Centre of Biotic Medicine», Moscow, Russia; sa221260@yandex.ru

Vitamin D deficiency in rats (25(OH)D plasma concentration 4.0 ± 0.6 ng/ml, Ca — 8.5 ± 0.3 mg/100ml) led to 40 per cent increase of renal creatine kinase activity under 1.2:1 diet Ca/P ratio. When the phosphorus intake was increased (Ca/P ratio = 1:2) renal creatine kinase activity was about 2-fold decreased in animals sufficiently supplied with vitamin D and vitamin D deficit (25(OH)D plasma concentration 1.9 ± 0.2 ng/ml, Ca — 6.0 ± 0.3 mg/100ml) did not effect this enzyme ac-

tivity. Both intestinal and renal phosphorylase activities were the same in sufficiently and insufficiently vitamin D supplied rats. When the phosphorus ration concentration was raised, intestinal phosphorylase activity in cAMP presence was elevated 1.5–2 fold, while renal enzyme activity was 2.5–3 fold reduced. These data demonstrate the possible role of excess dietary phosphorous in the regulation of BB creatine kinase and intestinal and renal phosphorylase activities.

HAEMOPOIETIC PROPERTIES OF THE PLANT *SOLANUM TORVUM SWATZ (SOLANACEAE)*

N. Salama¹, S.B. Tzokov², A. Khan¹, P.H.E. Gardiner¹

¹ Biomedical Research Centre, Sheffield Hallam University, Sheffield, UK; nuri_lb@yahoo.com

² Krebs Institute for Biomolecular Research, The University of Sheffield, Sheffield, UK

Anaemia caused by iron deficiency affects about one in three persons worldwide thus making it the most prevalent mineral deficiency. Therefore the availability of a cheap, safe and effective source of iron for the treatment and prevention of iron deficiency has obvious attractions. *Solanum torvum Swartz (Solanaceae)* a small shrub, and a relative of the eggplant, is found in most tropical and sub-tropical parts of Africa, South East Asia, the Pacific Rim, the West Indies, the Indian sub-continent, Australia, central America and the USA. Although the plant is regarded as an invasive species in some parts of the world, it is used extensively in Asia, Africa and the West Indies for culinary and medicinal purposes. In Africa and India, the fruit is used as a haemopoietic agent and tonic, the fruits and leaves

decoction are used in the treatment of coughs, wounds, skin infections and athlete's foot. Results from the iron determination in the roots, leaves, flowers, and fruits of the plant were found to be 71, 132, 51 and 98 mg/kg, respectively. In addition to iron other trace elements, copper, manganese, and zinc, and minerals, sodium, potassium, calcium, magnesium, and phosphorus were also found to be present. Characterisation of the main iron-containing compound in the leaves, fruits and roots was carried out using mass spectrometry and electron microscopy. In addition, the presence of phenolic acids in the plant extracts was confirmed. The implications of these findings in relation to the haemopoietic properties of *Solanum torvum Swartz* and the bioavailability of iron from the plant are discussed.

TRACE ELEMENTS CONTENT AND HORMONAL PROFILES IN WOMEN WITH ANDROGENETIC ALOPECIA

M.G. Skalnaya^{1,2}, V.P. Tkachev¹

¹ Russian Society of Trace Elements in Medicine, Moscow, Russia

² ANO «Centre for Biotic Medicine», Moscow, Russia; skalnaya@yandex.ru

It is well-known that some trace element imbalances play significant role in pathomechanism of many forms of alopecia. But androgenetic alopecia is specific local sensitivity of hair follicle receptors to androgens. In clinical and laboratory study, 153 women with androgenetic alopecia (AGA) and 32 control women were examined. In AGA patients telogen hair and vellus hair (miniaturization, $D < 30 \mu\text{m}$) significantly differed in frontal and parietal hair comparison with occipital area ($20 \pm 0.9\%$ vs $12 \pm 0.5\%$ and $33 \pm 0.9\%$ vs $12 \pm 0.6\%$ respectively). In AGA group levels of androstenedione and dehydrotestosterone were higher than in control group. Hair elemental content, analyzed by ICP-MS, demonstrated lowered Cu and Zn content in frontal area in comparison to occipital area (13.1 ± 0.6 vs $16.3 \pm 0.4 \mu\text{g/g}$ and 173 ± 3.3 vs $191.7 \pm 3.9 \mu\text{g/g}$, respectively, $P < 0.05$). It is important

to note, that AGA patients with elevated levels of androstenedione and dihydrotestosterone had increased Cu content and decreased Mn, Se, Zn content in occipital area of scalp. The occipital level of Cu positively correlated with concentration of free testosterone in serum. A negative correlation between Zn content in occipital area and dehydroepiandrosterone level in blood was found. Unfortunately, a routine treatment course of AGA patients, including topical inhibitor of 5-alpha-reductase and minoxidil, had no effect on Cu hair content in occipital and frontal areas. However, there were positive changes in morphological structure and other trace elements content. These data let us to hypothesize a key role of Cu metabolism disturbances in the AGA onset, development of AGA, and potential pharmaceutical targets for the treatment of AGA.

SEX-DEPENDENT PECULIARITIES OF MACRO AND TRACE ELEMENTS CONTENT IN VITILIGO PATIENTS

V.V. Skalny¹, S.V. Danilova²

¹ Orenburg State University, Orenburg, Russia; vskalny@mail.ru

² People's Friendship University of Russia, Moscow, Russia

The aim of this study was to investigate the elemental status of patients, suffering from vitiligo (hypopigmentation of skin). In general, 140 vitiligo patients (VP) (49 males and 91 females), aged from 18 till 55 years, were clinically examined. Occipital scalp hair samples were collected and 25 chemical elements were determined in them by ICP-AES/ICP-MS method in laboratory of ANO «Centre for Biotic Medicine» (Moscow, Russia). The significant peculiarities of hair elemental content in male and female VP were observed. Contrary to hypothesis about the main role of Cu deficiency in the pathogenesis of vitiligo, the low Mn hair level in 29.7% of females and 49.0% of males (comparing to 5.9 and 4.8% respectively) was

found. Low Cu, Fe, Co, K, Na content is more typical for female VP, but low Mn, Zn, Mg content is more widespread in male VP. In the group of male VP, the increased incidence of elevated hair Ni, Cd, Pb was observed, while in the female VP group there was increased hair Ti, Al. Thus, the significant sex-dependent differences in macro and trace element hair contents are present in VP. Hair elemental content reflects the involvement of numerous macro and trace element imbalances, especially deficiencies of Mn, Cu, Zn in the pathogenesis of vitiligo. The using of macro and trace elements in the treatment of vitiligo has to be in accordance with the gender and individual metabolic peculiarities.

THE MARVELLOUS ROLES OF BORON MINERALS AND COMPOUNDS ON HUMAN HEALTH

H. Türkez

Atatürk University, Erzurum, Turkey; hasanturkez@gmail.com

Boron has long been known to an essential nutrient for normal plant growth. Except for boron, all elements which are essential for plant growth are also evaluated as essential for herbivorous mammals. Several studies have reported that boron is essential for magnesium and calcium metabolism or homeostasis; however, there have been only a few reports about its other functions. Hence, its biochemical roles have not been clearly defined. On the other hand the limited number of actual reports indicated that boron deficiency and supplementation exerted interesting measurable biological effects in human and animal tissues. The first one of these, the ability to strengthen the tissue antioxidant defences by a yet unidentified mechanism. The second one is non-genotoxic properties of

boron and its compounds. And the third one, anti-mutagenic nature of boron. Many critical disorders like cancer and arteriosclerosis, occurred when the survival mechanisms (antioxidants) were unable to deal adequately with the reactive oxygen and nitrogen (ROS and RNS) species. And it is well known that, ROS and RNS have been appraised to be major mechanisms in DNA damages. In this context, the oxidative and genetic investigations both *in vitro* and *in vivo* model systems are literally pivotal. Thus, the reviewing of these biochemical and genetic studies will serve to introduce the roles of boron and improve its therapeutic gain. So in this review, the association of boron with genetic and biochemical pathways is evaluated from the view of human health.

PROPOLIS PREVENTS THE ALUMINIUM-INDUCED GENETIC AND HEPATIC DAMAGES IN RAT LIVER

H. Türkez¹, M.I. Yousef², F. Geyikoglu¹

¹ Atatürk University, Erzurum, Turkey; hasanturkez@gmail.com

² Alexandria University, Alexandria, Egypt; yousefmokhtar@yahoo.com

Aluminium (Al) is present in several manufactured foods and medicines and is also used in water purification. Therefore, the present experiment was undertaken to determine the effectiveness of propolis in modulating the aluminium chloride (AlCl₃) induced genotoxicity and hepatotoxicity in liver of male Sprague-Dawley rats. Animals were assigned to 1 of 4 groups: control; 34 mg AlCl₃/kg bw; 50 mg propolis per kg bw; AlCl₃ (34 mg/kg bw) plus propolis (50 mg/kg bw), respectively. Rats were orally administered their respective doses daily for 30 days. Rats were anesthetized after Al and propolis treatments and hepatocytes were isolated for counting the number of micronucleated hepatocytes (MNHEPs). In addition, the levels of serum enzymes and histological alterations

in liver were investigated. After exposure with Al, this metal caused a significant increase in MNHEPs incidence. The enzymatic activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) significantly increased. Furthermore, severe pathological damages such as: sinusoidal dilatation, congestion of central vein, lipid accumulation and lymphocyte infiltration were established in liver. On the contrary, the applications of propolis did not cause any adverse effect on above parameters. Moreover, simultaneous treatments with propolis significantly modulated the toxic effects of Al. It can be concluded that propolis has beneficial influences and could be able to antagonize AlCl₃ toxicity.

SELENIUM DEFICIENCY AT THE CONTINGENT OF INTERNATIONAL OPERATIONS

**V.V. Voicehovskis¹, G. Ancane¹, T. Ivascenko¹, J. Micans²,
A. Skesters¹, N. Vaivads²**

¹ Riga Stradins University, Riga, Latvia; www@arctica.lv, pmpk@rsu.lv, anderjs.skesters@rsu.lv

² Medical Support Centre, National Armed Forces, Latvia

BACKGROUND: Selenium (Se) appears to be an essential trace element, it provides human brain protection from Oxidative Stress (OS) and toxic heavy metals. Neuronal membranes phospholipids are especially vulnerable to damage, the injury leads to the receptor-mediated signal transduction and, furthermore, information processing disorders. Se is recognized to be a potentially protective factor in case of brain injury because of scavenging endogenous and environmental oxidant sources. Considering interconnection between OS and brain disorders, including Posttraumatic Stress Disorder (PTSD), the authors suppose to study Se plasma level and Se deficiency (SeD). Until now, processing the data available in literature, had difficulties in rating and interpreting because it is being inhomogeneous on a gender, race, age, and nutrition

factors. **AIM:** to define level of SeD in participants of Contingent of International Operations (CIO). The study met the demands of Helsinki declaration, was approved by Ethics Committee of Riga Stradins University. **METHODS:** Data were received from the retrospective research of Latvian CIO deployed in the same international mission. Totally 29 participants (males, Europeans, of average age 26.19) were examined. Plasma Se was determined using fluorometric method by Alfthan. Data were processed using SPSS 15.0. **RESULTS:** SeD of 13.79% was revealed in participants of Latvian Contingent of International Operations. Se concentration in plasma is less than predicted value of 80 µg/l. The research strengthens the need for the further investigation of the role of Se as well as antioxidants in PTSD therapy.

LITHIUM AND MELATONIN EFFECTS ON THE DAY: NIGHT RHYTHMS OF Na⁺, K⁺, Ca⁺² AND Li⁺ CONTENTS IN BLOOD, BRAIN AND URINE OF RATS

T.A. Zamoshchina, E.V. Ivanova

Siberian Medical University, Tomsk, Russia; beladona@hotmail.ru

The present study was designed to examine in experiment the lithium and melatonin effects on rhythmic organization of daily dynamics of Na⁺, K⁺, Ca⁺² and Li⁺ contents in blood, brain and urine of rats. The studies were carried on 350 adult male rats weighting 220–240 g, which were maintained on constant light illumination (LL24, 120 Lx, 7 days) during z or lithium hydroxybutirate plus melatonin were given at the beginning objective light or dark phase (morning or evening). The control groups were intact or received solvent. One group of rats were maintained on light illumination of winter solstice (LD6:18) and did not received any drug or solvent. After 7 days urinary samples were collected each 4 h for 3 objective light: dark cycles after preliminary animals adaptation to the experimental conditions (metabolic cages). After this observation the brain and blood of rats were used for analyses each 4 h during one light: dark cycle. Concentrations of Na⁺, K⁺, Ca⁺², Li⁺ in tissues were defined by flame pho-

tometry. Statistical analyses of the obtained results were performed by a one-way ANOVA, spectral and «Cosinor» methods. There are internal rhythms' desynchronizations in rats at the constant illumination. Lithium hydroxybutirate only at the beginning of objective light phase prevented the destroying of rats' circadian system and facilitated the forming of entrainment mechanism. At the beginning of objective light phase melatonin alone did not influence on the rats' circadian system but prevented effects of lithium hydroxybutirate. Melatonin alone at the beginning of objective dark phase facilitated of rhythms desynchronization but this drug was given together lithium hydroxybutirate did not influence on the power of rhythms desynchronization. The drugs alone increased the Li⁺ content in brain only in the objective morning and decreased this index in the objective evening. Together lithium hydroxybutirate and melatonin did not influence on concentrations of Na⁺, K⁺, Ca⁺², Li⁺ in brain.

INFLUENCE OF CERULOPLASMIN-ASSOCIATED COPPER DEFICIENCY ON COPPER METABOLISM IN MAMMALS

*E. Zatulovskiy¹, E. Iliecheva¹, L. Puchkova^{1,2}, N. Tsymbalenko², P. Babich¹,
A. Skvortsov¹, E. Caiola³, M. Mazzoletti³, M. Broggin³*

¹ Saint-Petersburg State Polytechnical University, St.Petersburg, Russia; zatulovskye@mail.ru

² Research Institute of Experimental Medicine, St.Petersburg, Russia; iem@iem.spb.ru

³ Research Institute of Pharmacology Mario Negri, Milan, Italy; massimo.broggin@marionegri.it

BACKGROUND: Cu is simultaneously a structural and catalytic co-factor for vital enzymes, a potential toxic agent for all biopolymers and a participant of signalling. Impressive success has been achieved in understanding of the mechanism of safe intracellular distribution of Cu(I). However extracellular Cu turnover remains unclear. We tried to uncover the role of Cu, associated with ceruloplasmin (Cp), the main Cu-transporting serum protein, in intracellular Cu metabolism of various cell lineages. **APPLIED TECHNIQUES:** Wistar rats and C57Bl mice fed by fodder with 50 mg AgCl per kg body weight (Ag-animals) were used. Metal concentration was measured by FAAS. Oxidase, ferroxidase and SOD1 enzymatic activities were tested in gels. Semiquantitative RT-PCR and WB techniques were applied to measure expression level of Cp, GPI-Cp, CTR1, CTR2, ATP7A, ATP7B, SOD1 and COX (Cox4i1). Subcellular fractions were isolated by ultracentrifugation. **RESULTS:** Oxidase and ferroxidase activities

are absent in Ag-animals' serum. Cu concentration decreases 2–9 fold. But Cp protein concentration stays at same level. Ag accumulates in liver, adrenal gland and hypophysis selectively. It's detected in bile. In hepatocytes more then 60% Ag is found in mitochondrial matrix; Fe accumulates in cytosol in large scale. In two weeks after Ag-diet cancellation, Ag concentration drops in all organs. Cytosolic Fe concentration falls as well. Hepatocytes' level and intracellular distribution of Cu and Zn remains unchanged during these experiments. In liver, cuproenzymes and Cu-transporting genes activity, protein concentration, and enzymatic activity doesn't change. In brain, Cu-transporting and Cp genes activity, but not SOD1, decreases. In nude mice, the growth of HCT116 (cell line obtained from human colon cancer) is reduced by Ag-diet when diet is given together with tumour implantation. Possibilities to use Ag-animals model for investigation of the biological roles of Cu associated with Cp are discussed.