

## **Session 7. METAL IONS IN INFLAMMATION AND IMMUNOLOGY**

### **ZINC INTERVENTION IN THE GENE EXPRESSION OF IL-1 AND TNF ALPHA OF MACROPHAGES FROM MICE IN PERINATAL STAGES**

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The role played by zinc in the immune system and the mechanisms through which it modulates diverse functions have been subjects of study for decades. Zinc status directly affects the monocyte-macrophage cells, that secrete interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF alpha), among others cytokines. There is evidence that lipopolysaccharide (LPS) stimulated peripheral blood monocytes from preterm infants have a decreased secretion of IL-1 and TNF alpha.

We have previously shown that both phagocytosis and phagocytic metabolism increase significantly in animals supplemented with zinc.

IL-1 has multiple biological activities and is a regulator of the host response to infection and injury. Mononuclear

phagocytes are the major source of IL-1, but all other nucleated cells are capable of producing it. IL-1 is a pleiotropic mediator of the host response to infections and injurious insults and it coordinates the activities of other cells and cytokines. TNF alpha with IL-1 set in motion an array of parallel signaling pathways, so exposure of cells and tissues to TNF results in a multitude of biological actions. Thus zinc intervention at the macrophage level could have a direct action of the mentioned cytokines production.

To further study zinc effects on the macrophages functions, we studied the interleukin-1 and TNF alpha gene expression and protein production in peritoneal macrophages from BALB/c mice supplemented with zinc from gestation to weaning.

### **IMMUNOLOGICAL-RELATED PERTURBATIONS INDUCED BY A SUBLETHAL CONCENTRATION OF GALLIUM IN CARP (*CYPRINUS CARPIO* L.)**

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As the manufacture of semiconductor technology using gallium can produce negative consequences for the environment especially for aquatic ecosystems, it is useful to assess and predict the potential risk of gallium for aquatic animal health (Chepesiuk, 1999). Fish is one of the most important indicators of environmental contamination of water and its immunophysiological system is particularly sensitive to water quality. In term of ecotoxicological risk assessment, we reported here the sublethal impact of gallium on immune parameters of carp. Fish were exposed to a sublethal concentration of gallium (chosen as 5% of 96 h LC<sub>50</sub> in carp) for 96 hours. Selected parameters [e.g., phagocyte killing activity, total plasma immunoglobulin, plasma lysozyme activity, plasma ceruloplasmin content and total plasma proteins] were evaluated at 24, 48 and 96 h of exposure. The phagocyte killing and plasma lysozyme activities were

not altered by exposure to gallium. Total plasma immunoglobulin was decreased after 48 h exposure. A same result was observed in level of total proteins in plasma of fish exposed to gallium from 24 h of exposure while the ceruloplasmin content was significantly increased at the same time. Measurements of blood gallium content indicated an elevation of this concentration across metal ion treatment. In case of an acute environmental contamination gallium could be implicated as an immunopathological metal ion in fish. Such aquatic gallium contamination of carp may induced an inflammatory process where the non specific humoral immunity should be the first immune function of fish implicated. Changes in immune parameters observed reflected a decrease in fish health (e.g. inflammatory response) associated with stressful conditions and could increase the sensibility of fish to pathogens.

### **DIFFERENT SENSITIVITY OF BOVINE AND HUMAN CU,ZN –SUPEROXIDE DISMUTASE TO INACTIVATION BY HYPOCHLORITE**

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The superoxide dismutase (SOD, EC 1.15.1.1) belongs to oxidation-reduction enzymes and is abundant in

almost all pro- and eucariotic cells. Superoxide dismutases are a family of metalloproteins. Three classes of

SOD have been described, each characterized by catalytic metal at the active site, namely Cu,Zn – SOD, Mn-SOD and Fe- SOD. SOD is a key enzyme of antioxidant system of cells which defends cells from toxic effects of active oxygen species. Disbalance between prooxidants and antioxidants may be a reason of oxidative damage of cells, and utilization of exogenous antioxidants may be useful for normalization of cell's metabolism. There is a serious dasis to consider exogenous SOD as a powerful pharmacological agent and some types of SOD are intensively investigated as an antiinflammatory agent. However, contradictive results about antiinflammatory effectivity of various types SOD exists, and causes of their different action are not known.

We studied the comparative inactivation and structural desorganization of Cu,Zn-SOD's from human and bovine erythrocytes, and recombinant human Cu,Zn-SOD, produced from *Saccharomyces cerevisiae* by hypochlorite ( $\text{ClO}^-$ ), which is the main prooxidant at the

locus of inflammation.

Small concentrations of  $\text{ClO}^-$  (up to 100  $\mu\text{M}$ ) induced a noticeable inactivation of bSOD but hSOD practically wasn't inactivated. The increase of  $\text{ClO}^-$  resulted in decrease of difference between residual activity of human and bovine enzymes. Various sensitivity of enzymes to action of  $\text{ClO}^-$  also confirmed by electroforetic analysis. The difference of oxidative stability of enzymes may be connected with various amount of free cysteines which are primary target for  $\text{ClO}^-$  in proteins. The greater resistance of hSOD to oxidative action of hypochlorite may be related with the presence of the Cys 111, because modification of human enzyme by iodoacetamide increases the oxidative lability. It is suggested that Cys 111 in human enzyme actively react with hypochlorite and thus the effective concentration of oxidant reacting with catalytically significant residues is decreased. So Cys 111 plays a role of "false" target for hypochlorite in human SOD molecule.

## ROLE OF MERCURY IN LIPID PEROXIDATION

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The effects of mercury, copper and iron on the non-enzymatic oxidation of LDL and the enzymatic oxidation of dihydroxyfumaric acid were investigated. Mercury did not cause non-enzymatic oxidation of LDL but copper appeared to activate peroxidase by 93%. We conclude that mercury and methylmercury do not directly cause or promote the non-enzymatic radical peroxi-

dation of lipids but, that a simultaneous exposure to copper and mercury, the former via acceleration of both enzymatic and non-enzymatic peroxidation, and the latter via the inhibition of glutathione peroxidase, promote lipid peroxidation in biological systems, and thus may promote development of atherosclerosis.

## SELENIUM (SE) STATUS AND INFLAMMATION MARKERS IN DISEASES INFLUENCED BY AIR POLLUTION

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**Background:** the incidence of allergic and respiratory diseases are increasing not only in Europe, but all over the world. The prevalence of asthma bronchiale remarkably increased in Hungary similarly to European, overseas as well as to developing countries. Hungarian soil is low in Se and Se intake is lower than the Recommended Daily allowance.

**Our aim** was to investigate the possible associations between blood Se status, clinical symptoms and inflammation markers in diseases strongly influenced by air pollution.

**Patients:** asthmatic children (n=64), and adults (n=23), and children with allergic rhinitis and or atopic dermatitis (n=40) were investigated. Se and immune parameters were compared to healthy, age and sex

mached controls (n1=81, n2=46, n3=27 respectively).

**Method:** Se concentrations in erythrocytes, whole blood and plasma were determined by atomic absorption spectrophotometry (AAS). Granulocyte macrophage colonic stimulating factor (GM-CSF), intracellular adhesion molecules (ICAM 1) by ELISA.

**Results:** patients with chronic rhinitis and atopic dermatitis had plasma and whole blood Se levels similar to the healthy children, but Se in their erythrocytes decreased. Asthmatic children had less erythrocyte Se ( $p<0.001$ ) and in adult asthmatics Se content significantly decreased in all blood compartments ( $p<0.001$ ). Total IgE increased in asthmatics irrespective of the severity of symptoms. 20 fold increase of IgE has been observed in status asthmaticus. GM-CSF levels increased in chronic

rhinitis and asthma in active phases of the diseases. ICAM-1 concentration was higher in rhinitis and asthma patients but those with severe viral infections had a 300% increase compared to values of healthy children.

In **conclusion** we could say, that in atopic diseases

the blood Se parameters associated with the severity of clinical symptoms and with an altered synthesis of immune proteins, inflammation markers.

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## ZINC EFFECTS OVER IL-12 GENE EXPRESSION AND IL-12 PROTEIN SECRETION IN MICE MACROPHAGES

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The cell that appears to be most directly susceptible to zinc modulation is the macrophage, that produces IL-12, among other monokines. Because IL-12 appears to play a central role in a variety of immunological processes, it is critically important to understand the regulation of its synthesis and its modulators.

With this in mind, we studied zinc supplementation effects (500 mg/L) over IL-12 secretion by RT-PCR and ELISA assays in LPS activated peritoneal macrophages from BALB/c mice, from gestation throughout postweaning. The IL-12 serum concentrations (ELISA assay

evaluates the dual expression of the p70 form and the p40 subunit) was significantly increased in Zn treated mice, group II (6 wk old, 9 wk Zn treatment). The IL-12 gene expression evaluated by RT-PCR (mRNA), showed an increase in mice group I (3 wk old, 6 wk Zn treatment) and an 1.5 fold IL-12 mRNA elevation in mice group II, relative to group I animals.

Results suggest that zinc administration, carefully monitored, could potentiate the host ability to self regulate monokines bioactivity, in this case by an IL-12 positive modulation.

## STRONTIUM EFFECTS ON LIPID PEROXIDATION DUE TO $FeCl_3$ AND ASCORBIC ACID IN RAT SYNAPTOSOMES

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The objective of this work was to test the effects of several strontium (Sr) concentrations in the lipid peroxidation initiated by  $FeCl_3$  and ascorbic acid in rat synaptosomal membranes. In addition, it was tested the combination of Sr with pinoline, an antioxidant that reduces efficiently iron mediated lipid peroxidation in biological membranes. Aliquots of membranes were incubated at 37°C in the presence or absence of either  $FeCl_3$ ,

ascorbic acid,  $SrCl_2$  and pinoline. Lipid peroxidation was estimated by measuring malondialdehyde (MDA) and 4-hydroxyalkenals (4-HDA) concentrations. Under these experimental conditions, Sr did not have significant influence on lipid peroxidation initiated by  $FeCl_3$  and ascorbic acid. Moreover, Sr did not improve the antioxidant ability of pinoline against iron-induced lipid peroxidation.

## IMMUNE PLASTICITY AND SUCCESSFUL AGEING: ROLE OF ZN-METALLOTHIONEINS (I+II) AS BIOLOGICAL AND GENETIC MARKER OF IMMUNOSENESCENCE

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**Background:** Healthy centenarians differ from aged individuals for their optimal metabolic compensation and immune response and for the ability to efficiently counter the alteration of the oxidative status typical of ageing. The molecular basis of such exceptionality is not clear yet. However, a special asset of zinc-bound metallothioneins (Zn-MTs) (I+II) exists playing a central role

both in zinc-related cell homeostasis during oxidative stress, inflammation and in immune response.

**Aim:** The aim is to demonstrate Zn-MTs (I+II) involvement in immunosenescence.

**Methods:** Partial hepatectomy/liver regeneration (pHx) is good model to study constant oxidative stress and inflammation because high Zn-MTs (I+II) are

present in young rats during liver regeneration. Therefore, we have applied this model to young, old and very old mice (mimicking these latter human centenarians). Liver MTmRNA (RT-PCR) and liver NK cells activity ( $^{51}\text{Cr}$  incorporation using YAC cells as target) were tested.

**Results:** At 48 h from pHx, young animals suffer conditions similar to ageing, as MTmRNA and NK cells activity are concerned. Metabolic crude zinc balance is negative at time 0 h. in old mice indicating zinc loss. At time 0 h., no significant differences are detectable in liver MTmRNA between old and very old mice. Dramatic differences emerge at 48 h after pHx. Very old mice display MTmRNA and immune pattern during liver regeneration similar to that observed in young-adult animals. In contrast, old mice display the same values observed at time 0. Negative crude zinc balance is always associated with high MTmRNA and depressed liver NK

cells activity after pHx. This phenomenon is correlated with enhanced corticosterone and IL-6 after pHx.

**Conclusions:** Constant stress, via corticosterone and IL-6, provokes zinc loss from the body and drives MTs to steal cellular zinc removing free zinc ions for liver NK cells activity. Unexpected data in very old mice suggest thymus necessary for immunity during early development, but not in ageing, as the extrathymic T-cell pathway is determinant in old mice. Following that, Zn-MTs (I+II) homeostasis grants immune plasticity and longevity, rather than the thymus. On the other hand, old mice grafted with neonatal thymus display no prolonged survival. Therefore, a well conserved interplay between Zn-MTs (I+II) and extrathymic T-cell pathway, rather than thymus functionality, is involved in successful ageing. This entitles Zn-MTs (I+II) novel biological and genetic marker of immunosenescence.

## RHENIUM CLUSTER COMPOUNDS IN THE MAINTENANCE OF THE RBC REDOX STATE

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According to our recent investigations some binuclear rhenium compounds with a quadruple metal-metal bond like  $\text{Re}_2(\text{i-C}_3\text{H}_7\text{COO})_4\text{Cl}_2$  (I) or  $\text{Re}_2(\text{GABA})_2\text{Cl}_6$  (II) revealed ability to take part in biochemical red-oxidative processes. Therefore lipid peroxidation (POL) intensity and two defence enzyme systems: superoxydismutase (SOD)–catalase (CAT) and glutathione-peroxidase (GP) – glutathione-reductase (GR) were examined during red blood cells (RBC) treatment with compounds I and II in vitro (1-h incubation with  $10^{-4}$ – $10^{-12}$  M) and in vivo on rats. I has demonstrated strong antioxidant effect with POL inhibition on 15–35% (maximal effect was found for

$10^{-8}$  M concentration) while complex II inhibited POL only in the range of low concentrations ( $10^{-10}$ – $10^{-12}$  M). These results prompted our investigations to elucidate the mechanism of action of compounds I and II on enzyme antioxidant systems. Our data showed that compound I decreased activity of SOD, CAT, GP and GR whereas compound II didn't influence on SOD, but diminished GP activity and considerably stimulated CAT and GR activity. Thus, it seems that compound I can be used as an effective scavenger of free radicals with essential influence on enzymes activity on substrate level while compound II has showed certain enzymatic modulation properties.

## NON-SPECIFIC AND SPECIFIC IMMUNE RESPONSE ALTERATIONS IN MAGNESIUM DEFICIENCY: EXPERIMENTAL DATA

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Magnesium (Mg) plays an essential role in fundamental cellular reactions and it was recognized that Mg deficiency exerts deleterious consequences on the immune system. The aim of our studies was to determine alterations of the immune response due to Mg deficiency and to precise mechanisms at the origin of these alterations. Experimental Mg deficiency in rats induces after few days characteristic inflammatory syndrome including increase in plasma levels of IL-6 and several acute phase proteins. The phagocytic cells from deficient rats present characteristics of activated cells (increase in respiratory burst and phagocytic activity) and their response to im-

mune stress is exacerbated. On the opposite, increasing concentrations of extracellular Mg reduce the response of rat and human phagocytic cells to the immune stress. This point out on the potential beneficial anti-inflammatory role of pharmacological concentrations of Mg. The proposed mechanism for the activation of phagocytic cells involves an abnormal Ca handling induced by extracellular Mg depression. Moreover, results in Mg-deficient rats fed a normal or low-Ca diet suggest that the proinflammatory effect of Mg deficiency is the consequence of a reduced extracellular  $\text{Mg}^{2+}/\text{Ca}^{2+}$  antagonism resulting in an increased intracellular free  $\text{Ca}^{2+}$  concentration. The

low concentrations of Mg, which lead to the inflammation, could be related to several pathological consequences observed during this deficiency i.e. exacerbated response to the immune stress, hyperlipemia and cardiovascular damages. Several alterations of specific immunity were also recorded in Mg-deficient animals i.e. accelerated thymus involution, reduced specific IgA produc-

tion by intestinal mucosa. In summary, it appears from these experimental data that Mg deficiency in early stages induces an acute phase response with activation of non-specific immune defense system. If prolonged, this deficiency could contribute to exhausting phagocytic cell activity. It also appears that Mg deficiency rapidly affects immune cells involved in the specific immunity.

## SELENIUM, ZINC AND MAGNESIUM — ESSENTIAL COMPONENTS OF THE ANTIOXIDANT SYSTEM

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The Czech Republic ranks among the countries with highest prevalence of dyslipoproteinaemia and cardiovascular diseases (CVD). As a valid tool in the primary prevention of the CVD the authors consider the monitoring of the selected components (including metal ions and vitamins) of antioxidant system in the organism.

The study was carried out focused on monitoring of the health state in personnel of the Czech Republic Rescue Fire Brigade. In the project there were involved 933 healthy volunteers. Selected anthropometrical and biochemical parameters (including Se, Zn and Mg serum concentrations) were observed.

The results contribute to information about normal values of serum magnesium, zinc and selenium concentrations in Czech population. Mean serum concentration of Mg was  $0.816 \pm 0.057$  mmol/l, that of Zn was  $18.25 \pm 2.54$   $\mu$ mol/l and mean Se serum concentration was  $0.802 \pm 0.14$   $\mu$ mol/l. The distribution of Mg, Zn and Se serum levels in examined population group approached normal distribution.

In the monitored group, the average serum concentrations of Mg and Se showed a tendency towards lower

values of so-called physiological range, however, they corresponded with other findings within the Czech population. The concentrations of Zn corresponded with commonly used normal values. The concept of the study made it possible to reveal relations between the serum Mg, Zn, Se levels, and the age or biochemical and anthropometrical parameters which are generally used as risk indices of cardiovascular disease. No statistically significant relations among the age of examined subjects and their serum Mg, Zn and Se concentrations were proved. Statistically significant negative correlations were revealed for relations among Zn serum concentration and cholesterolemia ( $p \leq 0.05$ ) and triacylglycerolemia ( $p \leq 0.01$ ). High incidence of the obesity and overweight in examined population group prompted to evaluation of Mg, Zn and Se saturation in subjects classified to 4 BMI (body mass index) categories. Mg concentrations showed the tendency to decrease with higher BMI, while by Zn and Se serum concentrations the tendency to increase in higher BMI categories was found. No statistical significance of these changes was proved.

## ISOLATION CHARACTERIZATION AND VALORATION OF METHALLOTIONEIN IN RENAL TRANSPLANTE

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Metallothionein is a protein of low molecular weight. Between 6000 to 9000 daltons, which has many sulphidril groups, due the high concentration in cysteine, it is induced by metals like Mercury, Copper, Silver Zinc and Cadmiun, because of them has a detosifiant function due the propriety to link toxic metals and also at the same time inhive the free radicals catalyced by metals development a roll antitoxic.

Metallothionein is expressed in epithelial renal glomeruls cells and is codified for many gens which expresion live ten isomomrfs metalotioneines, Met-1, Met-2, Met-10.

The kidney is the principal organ expous the action of metals specially by Cadmiun which concentration up 80 micrograms produce renal damage inthe, proximal renal tubules where is the place the Cadmiun act because there Cad-Met is concentrated.

Metallothionein can play and important roll in trasplant, exist evidence that during this process the patients presents oxidative estres by the attack of free radicals and, the same hapen to the patients exposed hemodialysis and peritonealdialisys. Metallothioneine can be a reply agains to free radicals and is considered like a scavenger of these radicals, also certains enzymes, especially glutation peroxidase which is sintetized in the kidney and their action mechanism consist in descompose the peroxide radicall has been considered for Scavio to be an index of renal function.

In this work it has been study the metallothionein values in plasma of patients with renal trasplante, founding low concentration of this protein which would be mean to play renal marker.

## ALUMINIUM IONS IN THE PROPHYLAXIS OF PARODONTIUM PATHOLOGIES

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Generalized inflammation of the parodontium of various severity is a common pathology that accompanies postmenopausal osteoporosis. Parodontium includes the alveolar bone, the gingiva and the periodontium. Parodontitis is subjectively characterized by bleeding, painful sensations, pruritis, gingival tension, increased sensitivity of the dental necks and dental looseness of various degrees. Individual hygiene of the oral cavity plays a very important part in the prophylaxis and treatment of parodontium pathologies.

We have conducted a study on 565 females in the postmenopausal period who were from 45 to 55 years of age. We have included into our study those females who did not have any recordings of lichen planus in their case histories because the saliva of patients with lichen planus contains great amount of aluminium (Al). The control group consisted of 65 females who used prophylactic means of oral hygiene no less than times a day. The first group consisting of 240 females used therapeutic and prophylactic hygienic means with the similar periodicity. The second group included 260 females who

used hygienic prophylactic preparations of anti-inflammatory action containing aluminium lactate. Soluble Al salts are used in medicine as astringent, cauterizing and bactericidal means. Water-soluble Al combinations produce an astringent effect on the oral mucosa. Hygienic and prophylactic preparations contain salts in an ionic state that is why they produce a favourable effect on the gingiva mucosa, facilitate better blood circulation, enhance metabolic processes to some degree, increase outflow of the tissue liquid from the inflamed gingiva and have some analgetic effect.

Hygienic and prophylactic preparations for the oral cavity that contain Al lactate have been found to produce a positive effect on the parodontium state in the examined females gingiva bleeding, pruritis and inflammation of the gingiva border have been determined to be decreased or completely disappear. Positive dynamics is observed in the data of objective investigation and is confirmed by Schiller-Pisarev test and by the change of the parodontium index that is characteristic of the improved state of parodontium tissues.

## METALLIC IONS IN THE ALTERNATIVE PROPHYLAXIS OF GENERALIZED PARADONTIUM INFLAMMATION

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Generalized parodontitis may be the cause of the intensification of electrochemical processes in the oral cavity due to pH change toward more acid side. The products of electrochemical reactions occurring in the oral cavity are metallic ions (microelements) that pass into the saliva from the corroding metallic structures. The dynamics of microelement changes in the saliva composition depends on the electrochemical processes that take place in the oral cavity.

Parodontium inflammation is aggravated if the metallic prostheses and restorations are present in the oral cavity because they are affected by the aggressive media. These aggressive media are some medical preparations that are used for general and local treatment. The use of fluorine-containing preparations in the gel form that have various percentages of fluorine ions and orthophosphoric acid affects the metallic structures of the oral cavity and aggravates corrosion processes. Metallic prostheses in the oral cavity are subject to general, local and fissure corrosion. The aggressive medium also causes chemical corrosion of the outer amalgam layers. Corrosion of amalgam fillings is caused by the absorption of saliva proteins and enzymes by amalgam microstructures.

Corrosive products of all metallic inclusions (mi-

croadmixture of iron, magnesium, nickel and others) pass into the saliva and then into the organism. Apart from haptens of nickel, chromium, cobalt and manganese that are present in the saliva some other admixtures such as zinc, lead, tin, gold, silver appear in the saliva in the result of electrochemical processes. The content of copper and its various combinations that produce toxic effect on the regional tissues and organs and systems of the organism is several times increased.

Generalized parodontium is frequently accompanied by such non-carious lesions as wedge-shaped defect, enamel erosion, hyperesthesia. These nosologies require special treatment and quite often they require treatment by fluorine-containing preparations. Taking this into account we use therapeutic and prophylactic preparations, containing aluminium hydroxide, zirconium silicate, silicon dioxide, titanium dioxide and tannin as an alternative treatment. The results of the study that we conducted on 85 patients have indicated that local inflammation of the oral mucosa that occurs after a long-term use of fluorine-containing gels is observed 5.6 times less when we use therapeutic and prophylactic preparations that contain aluminium hydroxide, zirconium silicate, silicon dioxide, titanium dioxide and tannin.

## EFFECT OF METAL IONS ON ADRENALINE AUTOXIDATION COUPLED WITH THE FORMATION OF SUPEROXIDE RADICALS

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The reaction of adrenaline autoxidation in alkaline medium [1] serving as a model system presenting quinoid pathway coupled with superoxide radical formation via adrenochrome semiquinone [2], was found to be regulated by ions of bivalent metals. Absorption spectra and kinetics of adrenaline autoxidation to adrenochrome were measured on a Uvikon-923 spectrophotometer (Italy) at 347 nm in 0.1 M carbonate buffer, pH 10.55, thermostated cuvette at 22°C during 5 min. Concentration of adrenaline was 260 mM. One of us found [2, 3] that adrenaline autoxidation in alkaline medium can be observed absorption within the range of 320–380 nm or in the middle at 347 nm. This assay is more sensitive and more consistent than that measured at 480 nm. Formation of adrenochrome measured at 347 nm is superoxide dismutase sensitive [2, 3].

Na<sup>2+</sup>, Sr<sup>2+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup> accelerated the initial rate of adrenaline autoxidation showing prooxidant effect. The latent period of the reaction was considerably shortened or even absent in the presence of Mg<sup>2+</sup> and Mn<sup>2+</sup>. The highest prooxidant effect was found for 2.5–5.0 μM Mn<sup>2+</sup>. 25–500 μM Ca<sup>2+</sup> exhibits lower prooxidant dose-dependent effect. Similar effect was induced by low (50–100 μM) concentrations of Sr<sup>2+</sup>, which is chemically analogous to calcium. However higher concentrations of Sr<sup>2+</sup> (250 μM) induces inhibition instead of activation. Effect of Mg<sup>2+</sup> ions is close to the effect of high Sr<sup>2+</sup> concentrations: activation of the initial rate with the following inhibition. The described regulation by physiological cations can modulate adrenaline effect in organism changing the rate of oxygen radical formation.

## LACTOFERRIN AND ALBUMIN PROTECT CERULOPLASMIN AGAINST CU-MEDIATED DEGRADATION INDUCED BY H<sub>2</sub>O<sub>2</sub>

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**Abbreviations:** CP — ceruloplasmin (EC 1.16.3.1); LF — lactoferrin; LYZ — lysozyme from chicken egg white (EC 3.2.1.17); HSA — human serum albumin; DMSO — dimethyl sulfoxide; PAG — polyacrylamide gel.

It is known that EDTA inhibits pro-oxidative effect of copper ions of ceruloplasmin (CP) exerted as the latter is peroxidized. We previously demonstrated that CP is able to form a specific complex with lactoferrin (LF). LF is known as a natural chelator, so we studied its ability to prevent CP peroxidation by scavenging the copper ions released from CP. Degradation of peroxidized CP molecule was followed by electrophoresis in PAG and by changes of the oxidase activity of the

enzyme. Apo-LF more efficiently protects CP against peroxidative destruction as compared to EDTA. Like LF, serum albumin no less efficiently protects CP from peroxidation, which may be explained by its ability to scavenge copper ions. In contrast, lysozyme has no protective effect, as it is unable to bind copper ions. When pH dropped to 5.5, both LF and albumin lost their protective effect. We suggest that LF that is normally secreted in its apo-form can serve as a natural chelator of highly pro-oxidant copper ions, thus preventing the deleterious reactions involving oxygen radical species. Protective function of LF and albumin in the foci of inflammation where pH is low must be questioned in view of the results obtained.