Session 6. INTERACTION OF METAL IONS WITH BIOLOGICAL MOLECULES, SUBSTRATES AND SYSTEMS

CHARACTERIZATION OF LACTOFERRIN FROM DOG NEUTROPHILS

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Background: Lactoferrin is a glycoprotein of mammals localized in milk and other biological fluids and in specifiic granules of neutrophils. Lactoferrin is characterized by the ability to form complexes with some metal ions, mainly with Fe^{3+}. Lactoferrin has many different functions in organism. Among them there are transport of iron from maternal milk, participation in the reactions of innate immunity, regulation of activity of some cells. According to the literature data some species including dog lack lactoferrin in milk.

Aims: The aims of this work were to isolate lactoferrin from dog neutrophils and to investigate its Fe^{3+}-binding properties in comparison with human lactoferrin.

Methods: The procedure of latoferrin isolation included extraction of proteins from neutrophis by cetyltrimethylammonium bromide, ion-exchange chromatography on carboxymethylcellulose and gel-filtration on sephadex G-75. Molecular mass of lactoferrin was estimated by disk-electrophoresis in presence of sodium dodecylsulphate and by gel-filtration. Carbohydrate component in protein was determined by Schiff's reaction. Iron-binding properties of human and dog lactoferrins were investigated by dissociation of lactoferrin-Fe^{3+} complex at different pH values in presence of chelating agents (phosphate, citrate).

Results: Lactoferrin from dog neutrophils was isolated and purified to homogeneity. Molecular mass of dog lactoferrin was determined as 76-80 kDa. Dog lactoferrin was revealed to be a glycoprotein as well as human lactoferrin. It was determined that dog lactoferrin is more effective in iron binding compared with human lactoferrin because it holds Fe^{3+} at lower pH than human lactoferrin does. Spectra of lactoferrins complexed with Fe^{3+} were slight different for human and dog lactoferrins.

Conclusions: Dog lactoferrin is similar to human lactoferrin by some characteristics but it exhibits unusually high affinity to Fe^{3+}.

THE ROLE OF METALS' IONS IN THE CHEMISTRY OF LIFE

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Biosphere is the part of the Earth in which life exists. The famous Russian scientists V.I. Vernadsky, A.P. Vinogradov and others made a great contribution in the development of that science. V.I.Vernadsky said that life was a mutual, persistent flow of chemical elements between organisms and environment.

Nowadays more than 80 elements of the Periodical system by D.I. Mendeleev have been found out to play role in the organisms of people and animals.

Most of these elements are in the structure of ferments, vitamins, hormones and participate in biochemical and physiological processes, regulate colloid state of albumen in cells, osmotic pressure, catalyze processes of oxidation-reduction, etc.

So the study of the role of metals' ions in the chemistry of life, medicine, biology, etc. is very actual.

The work "Bioelementology" (255 p.) is the result of the study of the role of metals' ions in the chemistry of life which was conducted during many years.

Biolementology explains the role of these ions, the role of their simple and complex compounds in the life of organisms, the influence of their abundance and lack in the biosphere on the health. It also studies the concentration of these elements in food-stuffs and how to correct your diet to fulfil their lack in the organism. Biolementology also studies toxic influence on the health by the abundance of organic and inorganic compounds including metals' ions, which can take place as a result of development in technology and industry.

Among the decisions of a number of international Congresses there are some about the improvement of the education of specialists: chemists, biologists, technologists, physicians and so on.

Decisions of above-mentioned congresses said that the task of the protection of the biosphere and health of organisms is not someone's else, but our common task.

COPPER-INDUCIBLE GENE EXPRESSION REGULATED BY METAL AND OXIDATIVE STRESS RESPONSIVE PATHWAYS

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Due to its persistent nature and toxicity, copper poses a concern as an environmental and human health threat. Although copper is an essential metal, it is capable of catalyzing the formation of reactive oxygen species (ROS) to produce intracellular oxidative damage. We propose that both metal-responsive and ROS-mediated signal transduction pathways modulate the molecular and cellular responses associated with copper exposure. Transient transfection assays using COS-7 cells were performed to determine the ability of copper to induce the transcription of reporter genes containing upstream regulatory elements from metal- and oxidative stress-responsive genes: mouse metallothionein-I (MT-I) and rat NAD(P)H:quinone oxidoreductase 1 (NQO1). Exposure to copper decreased intracellular glutathione levels and increased levels of oxidized glutathione. In addition, cytoxicity studies demonstrated that depletion of glutathione increased copper cytotoxicity, while pretreatment with aspirin or vitamin E provided partial protection. Copper activated transcription via both metal- and ROS-responsive pathways. Depletion of glutathione increased copper-inducible expression in both MT-1- and NQO1-based reporters. The addition of aspirin or vitamin E prior to copper exposure, reduced the expression of reporter genes driven by metal response elements (MREs) or antioxidant response elements.

To identify the signal transduction pathways that are involved in controlling copper-activated transcription, cells were pre-treated with inhibitors that affect signal transduction cascades. The results indicated that protein kinase C, mitogen-activated protein kinase signaling pathways (ERK-1/2, JNK/SAPK, and p38), and metal-responsive transcription factor-1 (MTF-1) are involved in MT-I transcriptional regulation. Experiments with a MTF-1 double knockout cell line demonstrated copper can induce the transcription of reporter genes under the control of ARE sequences in the absence of MTF-1, but not MRE sequences or the full promoter of MT-I. These results suggest metal- and oxidative stress-responsive signal transduction pathways mediate molecular and cellular responses associated with copper exposure.

THE METAL INTERFERENCE INTO HUMAN COMPLEMENT CASCADE

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Background: During medical interventions some metals can contact human blood. As the process of complement activation causes formation of metal-dependent proteases (activated C1 and convertases) we can suppose that metal exposure can influence the function of complement system.

Aims: The goal of the study was to reveal the possible influence of the stainless steel on the human complement.

Methods: The samples of human sera were incubated at 4°C with fragmentised stainless steel wire (0,1 g/ml) for 12 hrs. The indices of complement activity via the classical and alternative pathways were determined in control and exposed sera. Washed rabbit erythrocytes were used for complement initiation and as complement cascade targets. The lag-period and the velocity of complement-dependent hemolysis were measured at 37°C monitoring the optical density change at 800 nm. For determination of alternative cascade parameters the incubation mixture was supplied with EGTA (10 mM) which blocks the classical pathway.

Results: Sera incubation with stainless steel samples didn't influence significantly the activity if the classical complement cascade, but caused 2-fold inhibition of the alternative pathway. The same effect was observed when bivalent iron (its sulphur salt) was added to control serum in concentrations 0.1–1.2 mM.

Conclusions: The change of complement system parameters after serum exposure to steel, shows that steel components can be effectively ionised and interfere an alternative pathway of complement activation. The experiments with $FeSO_4$ evidence that iron, being the main component of steel, must be responsible for the observed phenomenon. The substitution of alternative convertase magnesium by iron ion seems to be the reason of complement inhibition.

METAL COMPLEX COMPOSITION IN PHARMACOLOGICAL MODIFICATION OF POSTRADIATION EFFECTS IN HEMOPOIESIS SYSTEM

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The unique role of metals in formation of hemopoiesis system is well known. But the significance of metal substances in positive tolerance promotion of oncology radiotherapy remains scantily explored. Nevertheless, we can expect the special success of the metal complexes as prospective drugs for pharmacological modification of post-radiation negative effects. It is connected with indispensable microelements functions in formation and protection of the body enzyme's pool and also with the estimated positive pharmacodynamics and low toxicity of metal complexes as compared with ion metal substances. The polymetal complex compositions are capable of optimal influence on the important homeostasis links. In the first instance such composition can reduce the risk of postradiation complications of hemopoiesis system.

The propose of present investigation is the corroboration of this concept at the analysis of hemopoietic competence of the original microelement composition (MC). MC contains d-biometals in a state of coordination compounds with amine carbonic acid (HL) and other essential metals – such as salts of oxygen acids. In the MC composition the microelement ratio is (mg/1 g): Fe : Zn : Mn : Cu : Co : Cr : Mo : Se : V : HL = 12.8 : 15.2 : 3.6 : 3.3 : 0.32 : 0.30 : 0.55 : 0.22 : 0.04 : 366.0.

We proposed the route of CM creation by the principle new method of "summary synthesis". This method assumes the next reaction scheme:

Rapid contact

Phase 1: $\{(Fe^{3+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Cr^{3+})(\text{anti-ion})_n\} + nHL =$

$= \{(Fe^{3+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Cr^{3+})L_n + nH(\text{anti-ion})$

↑ → **MC**

Phase 2: Mo, V, Se (oxygen acid salts)

CATALITIC OXIDATION OF OXYMYOGLOBIN BY COPPER IONS

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Transformation of muscle oxy-Mb into oxidized met-form, which is catalyzed by Cu^{2+}ions, is of special interest as this reaction is believed to be the main saurce of active O_2 forms produced at ischemia and reperfusion of myocard. The reaction proceeds through formation of the specific reagent-protein complex, which is followed by electron transfer in the complex. Reduced copper is then reoxidized by oxygen to Cu^{2+} with participation protons of the medium, providing the closure of the catalytic cycle. Catalysis is not observed under anaerobic conditions.

The detailed physical mechanism of oxy-Mb oxidation by copper compounds is not clear now. One of the reasons is that sperm whale Mb containing maximal number of histidines was the sole object of its examination. Up to six Cu^{2+} ions can bind to sperm whale met-Mb with different affinities. From the equilibrium dialysis and NMR data, three binding sites have apparently the highest affinity for Cu^{2+}, at His113, His116 and His48 (binding constants correspond to $10^5 – 10^6$ M^{-1}).

In the present work, the influence of Cu^{2+} concentration, pH and ionic strength of the solution, as well as redox-inactive Zn^{2+} ions on the rate of oxidation of oxymyoglobins from sperm whale, horse and pig by copper ions has been studied. These myoglobins have equal redox potentials and homologous spatial structures, but differ in a number of histidines located on the protein surface. It was shown that oxy-Mb can be oxidized in the presence of Cu^{2+} through two distinct pathways, depending on which histidine binds the reagent and how stable is the complex. A slow pH-dependent catalytic process is observed for sperm whale and horse oxymyoglobins in the presence of equimolar Cu^{2+} concentration. The process is shown to be caused by the strong binding of Cu^{2+} to His113 and His116 that are absent in pig Mb. On the contrary, rapid oxidation of 10-15% of pig oxy-Mb (the fast phase) is observed under the same conditions, which is not accompanied by the catalysis because reoxidation of the reduced copper evidently did not occur. Complexation of Cu^{2+} with His97 situated near the heme is most probably responsible for the fast phase of the reaction. The affinity of His97 for Cu^{2+} must be essentially lower than of the "catalytic" His 113 and His 116 residues since the fast phase does not contribute markedly to the rate of sperm whale and horse oxy-Mb oxidation. The pH-dependence curve in both cases is sigmoidal with pK_{eff} corresponding to the His ionization. Since the pK_{eff} values for two proteins differ by 0.3–0.4 pH unit, it is possible to conclude that the rate of oxy-Mb oxidation is influenced by ionization of His 116, which is protonated with pK 6.5 in sperm whale Mb and pK 6.7 in Mb from horse It has been found that increase in copper concentration does not produce proportional growth in the oxidation rate of both oxy-Mbs. It is discussed, which Cu^{2+} binding sites of Mb make main contributions to His reaction rate at different Cu^{2+}: Mb ratios, from 0.25 to 10.

EFFECT OF METAL CATION SUBSTITUTION WITH ORGANIC CATIONS ON THE PARAMETERS OF UV-MELTING CURVES OF DNA

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It has been well documented now that DNA-lipid interactions play an important role in many cellular processes. Therefore, the elucidation of physicochemical regularities of this interaction is an actual, but difficult task due to extremely low solubility of natural lipids. We have studied the influence of cationic amphiphiles, as easily soluble structural analogs of lipids, on the structure and stability of DNA double helix. High molecular weight surgeon sperm DNA was used in this study. The UV-melting curves of DNA with dodecyl-, tetradecil-, hexadecyltrimethylammonium bromide in 10^{-2} M NaCl were measured on the spectrophotometer VSU-2P. The shift of the melting curves of DNA with increase of concentration of cationic amphiphiles to the region of higher temperatures was demonstrated. As in the case of Na^+ cations this shift points to the stabilization of DNA double helix as a result of screening of the repulsion between negatively charged phosphate groups, but the stabilizing effect is about 3 order higher. In contrast to Na^+, the cationic amphiphiles result to the increase of transition width (DDT) and to the decrease of hyperchromicity. Such behavior of melting curves is the consequence of hydrophobic fragment in amphiphile molecules providing for their specific binding to DNA in form of clusters. It provides for specific, sequence-dependent, binding of cationic amphiphiles in form of clusters. Such non-uniform distribution of amphiphile molecules along the DNA double helix results to strong heterogeneity stability manifested itself in the increase of DDT. Besides, the formation DNA-cationic amphiphile complex results to the light scattering of solution, which is responsible for the observed decrease of hyperchromicity at UV-melting of DNA.

POSSIBLE ROLE OF MN-BICARBONATE COMPLEXES IN THE PHOTOSYNTHETIC OXIDATION OF WATER

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Photosynthetic oxidation of water is a fundamental biological process and the main source of both electrons used for CO_2-fixation during plant photosynthesis and molecular oxygen in the atmosphere. This process occurs in a multi-component pigment-protein complex called photosystem II (PSII) that immersed in the inner membrane of chloroplasts (thylakoids). The inorganic core of the water-oxidizing complex (WOC) of PSII consists of three ions (Mn (II), Ca (II) and Cl^-) with stoichiometry of $Mn_4Ca_1Cl_x$. Strong evidence for a bicarbonate (BC) requirement within the water-oxidizing complex (WOC) (both O_2-evolving and assembling from apo-WOC and Mn(II)) of PSII has been presented recently (reviewed in: Klimov and Baranov, BBA 2001, 1503, 187). The following explanations for the involvement of BC in the events within the WOC are considered: 1) BC serves es an electron donor (alternative to water or as a way of involvement of water molecules in the oxidative reactions) to the Mn-containing WOC; 2) BC facilitates re-assembly of the WOC from apo-WOC and Mn(II); 3) BC is an integral component of the WOC essential for its function and stability; it may be considered as a direct ligand to the Mn-cluster. Comparative studies of electrochemical, spectroscopic (EPR) and functional properties of complexes of Mn(II) and Mn(III) with BC and other carboxylates (formate and acetate) show that the unique capability of BC to initiate the assembly of the tetramanganese cluster of the WOC from Mn(II) and apo-WOC-PSII can be attributed to formation of electroneutral, easily oxidizable, oligomeric Mn/BC-complexes that serve as building blocks for the WOC (Kozlov et al., submitted). It is suggested that due to this property BC might have been critical to the evolutionary origin of the first O_2-evolving cyanobacteria from a non-oxygenic bacterial precursor in the Archean period (>2.2 BYA) (Dismukes et al., PNAS, 2001, 98, 2170).

Acknowledgments: Supported by the FIRCA, grant 1R03TW00553-01.

THE FORMATION OF METALS COMPLEXES AND INTERACTION WITH MODEL CELLULAR MEMBRANES

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The determination of correlation between the physical and chemical properties of coordination substances of metals and their physiological activity remains the most important task for purposeful search of new medical products on the basis of metals complexes. Therefore the investigation of the interaction of metals complexes with model and cellular membranes with simultaneous determining the existence forms is important for interrelation determination between physiological activity and molecular structure of substances.

The influence of metals complexes structure on electrochemical properties of model membranes (bilayer lipid membranes BLM and monolayers) was investigated. The composition and share of each complex form had been calculated with the help of step complexation constants, which were determined by the pH-metry, potentiometry and spectrometry of the concrete physiological conditions or used values from literary. For BLM — the influence of complexes on the electroconductivity, the membrane potential, the elastic properties were determined and for monolayers — the change of surface tension at complexes adding.

It was revealed, that in most cases the intensive interaction with model membranes is observed in the predominance field of the coordinately saturated complexes with organic ligands, including bioligands:

1. The maximal change of BLM conductivity was observed in predominance conditions of saturated ethylenediamine, dipyridyl complexes of Cu, Ni and Zn $[Me(bpy)_3]^{2+}$ and $[Me(En)_3]^{2+}$. In the saturated complex ion the metal is completely surrounded by ligands and a share of these ions in a solution determines the conductivity change. So in case of dipyridyl complexes with Cu and Zn, the interaction with BLM does not depend on the nature of central atom. The difference in the interaction of ethylenediamine and dipyridyl complexes is connected with significant difference in the ligands lipophily.
2. The maximal influence on elastic properties of BLM was observed in conditions of predominance saturated cysteine complex of Cr^{3+}.
3. The maximal interaction with a monolayer from phospholipids was observed in conditions of predominance saturated quinolinolato complex of gallium.

The presence of organic ligands, completely surrounding the central atom, prevents the hydrolysis and changes the hydrophilic-hydrophobic balance that results in the increasing of complex influence on membranes of cells.

However in some cases the inorganic ligands can influence the interaction with model membranes:

1. In case of formation the tetrameric complex containing double bounded hydroxide groups ($[Zr_4(OH)_{12}(H_2O)_{12}]^{4+}$ and similar) the electro-conductivity changes of membranes is observed at presence of a pH difference in solutions washing BLM.
2. The change of BLM electro-conductivity in solutions containing Hg^{2+} depends on concentration of ions $HgHal^+$ (where $Hal^- = Cl^-, Br^-, I^-$). The permeability coefficients for the given particles have allowed making the assumption about the dependence of $HgCl^+$ - HgI^+ permeability on the change of bond polarity.

VASORELAXANT EFFECT OF MANGANESE IN ISOLATED AORTA FROM NORMOTENSIVE AND DOCA-SALT HYPERTENSIVE RATS

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Experimental studies have shown that manganese (Mn) modifies vascular tone and reactivity by decreasing intracellular calcium level in smooth muscle cells and by increasing endothelium-dependent vascular relaxation. Little is known about the effects of Mn in blood vessels of hypertensive animals. The aim of this study was to know whether hypertension alters vasorelaxation induced by Mn in isolated arteries of rat. One group of Sprague Dawley rats was made hypertensive by subcutaneously implantating DOCA pellets and adding 0.9% NaCl in drinking water (DOCA HT). The normotensive rats (NT) received no DOCA and no salt. After 12 weeks of DOCA-salt hypertension, thoracic aortae with and without endothelium were suspended in an organ chamber containing physiological solution. Increased Mn concentration (10^{-8}-10^{-4} M) in the bath, induced relaxation of noradrenaline-precontracted aortae without significant difference between NT and DOCA HT rats. In absence of endothelium, the vasorelaxation induced by Mn was markedly attenuated in both groups of rats. In DOCA HT rats, the relaxation was markedly inhibited by endothelium removal in comparison to NT rats. Indomethacin (inhibitor of cyclooxygenase) did not modify Mn-induced relaxation in both groups of rats. L-NAME (inhibitor of NO synthase) attenuated Mn-induced relaxation in DOCA HT rats but not in NT rats. These finding suggest that relaxation induced by Mn is partly mediated by the release of endothelial factors. In NT rats, neither NO nor prostaglandins seems to be involved in the Mn-induced relaxation. In DOCA HT rats, Mn-induced relaxation seems to be partly mediated by endothelial NO. Other endothelial factors may be involved in the Mn-induced relaxation. Although the precise effect of Mn on endothelium has yet to be defined, the differential effect of Mn between NT and DOCA HT rat may involve some mechanisms related to the pathophysiology of hypertension.

INFLUENCE OF CHROMIUM ON GLUCOSE UPTAKE IN MOUSE MYOTUBES

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Background: Chromium is known as a trace element necessary for optimal metabolism of lipids and carbohydrates. Oligopeptide LMWCr (low molecular weight chromium binding substance) containing four Cr^{3+} ions binds to insulin-activated insulin receptor stimulating its tyrosine kinase activity. Cr^{3+} ion acts like insulin-mimetic compound, decreasing blood glucose concentration and it seems to be a part of insulin signal amplification mechanism. Malfunction in glucose homeostasis cause many disorders comparable to those associated with adult-onset diabetes and cardiovascular diseases.

Aims: The aim of this experiment was to compare influence of inorganic chromium compound and new biomimetic complex $[Cr_3O(O_2CCH_2CH_3)_6]H_2O^+$ on glucose utilization.

Methods: Experiment was performed *in vitro* on C2C12 cell culture. Confluent mouse myoblasts were differentiated for 3 days to myotubes, treated with chromium solution at concentrations: 0.01, 0.1, 1, 10 and 100 μg/L (as $CrCl_3\ H_2O$) for 9 hours and insulin 100nM. 2-deoxy-d-[2,3-^{3}H] glucose (1μCi/ml) was added for 10 minutes, cells was incubated in room temperature, lysed and digested with 0.05 N sodium hydroxide. Aliquots of the solubilised cells were counted by liquid scintillation in a scintillation counter.

Results: Chromium produced changes in insulin-independent glucose uptake, scientifically increased this level over control. It reach maximum at chromium concentration 10μg/L (+20%). Positive effect of inorganic chromium is reduced at levels higher than 10мg/L. Insulin-dependent glucose uptake was reduced by chromium at all concentration, because in the presence of insulin the cells are more sensitive and the necessary amount of chromium is lover than without insulin.

CHROMIUM SALTS – DNA INTERACTIONS

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Polyvalent metal ions such as molybdenum, nickel, copper, chromium, cobalt, zinc are known as mutagenic and carcinogenic agents. Chromium compounds are the least studied in this respect despite of their wide application in a number of industrial processes.

We have studied the effect of three chromium salt influence on the structure of DNA double helix using IR-spectroscopy to detect DNA structural changes. $CrCl_3$, $Cr_2(SO_4)_3$, $K_2Cr_2O_7$ were used where chromium was tri- or hexavalent ion. IR-spectra of DNA films containing the above salts in an amount of 0.005 to 0.24 Cr ion per nucleotide were obtained for the samples placed in the atmosphere with relative humidity (r.h.) 0–93%. Chromium cations were found to interact with phosphate groups of DNA. Small amount of the salts in DNA films at Cr/P<0.01 prevents B-to-A transition of DNA induced by r.h. decreasing. The efficiency of the Cr salts to prevent the transition decreases in the row: $Cr_2(SO_4)_3$ > $K_2Cr_2O_7$ ~ $CrCl_3$. When $Cr_2(SO_4)_3$ or $K_2Cr_2O_7$ content in DNA exceeds 0.1 Cr/P, sugar-phosphate backbone of the double helix looses its regularity both with nucleic bases stacking, i.e. DNA denaturation occurs.

THE ESSENTIAL ROLE OF NICKEL AFFECTS PHYSIOLOGICAL FUNCTIONS REGULATED BY THE CYCLIC-GMP SIGNAL TRANSDUCTION SYSTEM

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Unequivocal acceptance of nickel as an essential nutrient awaits the definition of a specific biochemical function in higher animals. This acceptance may be forthcoming because findings have been accumulating suggesting that nickel has a role that involves cyclic nucleotide-gated (CNG) cation channels. Nickel potentiates cyclic guanosine monophosphate (cGMP)-gated cation channels in the rod outer segment of retinae, and desensitizes CNG channels of olfactory receptor cells (1). A number of organs including some in the central nervous, urogentital, and reproductive systems contain CNG channels. If nickel is involved or needed for CNG channel function and /or for various guanylate cyclases upstream to CNG channels, nickel deprivation should affect blood pressure control, sperm physiology, and sodium metabolism. Thus, experiments were conducted

with the objective of demonstrating that nickel deprivation affects spermatozoa and kidney in such a way to support the hypothesis that nickel has an essential role involving the cGMP signal transduction system. In an effort to enhance the effect or need for nickel, two stressors of systems in which CNG channels are important were used as treatment variables; these were NG-nitro-L-arginine methyl ester (L-NAME), a nitric oxide (NO) synthase inhibitor that hinders blood pressure control, and dietary sodium chloride (NaCl) in an amount that acted as a stressor of sodium metabolism. In experiment1, rats (15–16/group) were fed a nickel-deficient basal diet (27 ng Ni/g) supplemented with 0 and 1 ug Ni/g. After 5 weeks of feeding the experimental diets, L-NAME was added to the drinking water (0.5 g/L) provided to half of the rats in each group. Blood pressure was measured one week later. Epididymal sperm motility and density were measured three weeks later. In experiment 2, the treatments (8 rats/treatment) were supplements to the basal diet (27 ng Ni and 1 mg NaCl/g) of 0 and 1 ug Ni/g and 0 and 80 mg NaCl/g. After being exposed to the treatments for 9 weeks, blood pressures were measured. At 10 weeks, urine was collected to assess kidney function. At 16 weeks, sperm motility and production were determined. Nickel deprivation increased systolic blood pressure; decreased spermatozoa motility and density in the epididymides, epididymal transit time of spermatozoa, and testes sperm production rate; and induced kidney damage that resulted in hematuria and microalbuminuria. High NaCl exacerbated changes induced by nickel deprivation. The changes in blood pressure and sperm density caused by nickel deprivation were similar to those induced by L-NAME. Because L-NAME is a stressor of NO metabolism and excessive NaCl is a stressor of atrial natriuretic peptide (ANP) need, the changes suggest that nickel deficiency has detrimental consequences to the ANP-cGMP and NO-cGMP signal transduction systems, and support the hypothesis that nickel has an essential function involving CNG channels.

This research was partially supported by a grant from the Nickel Producers Environmental Research Association (NiPERA), Durham, NC.

PROTEOLYSIS OF CERULOPLASMIN AND COPPER TRANSFER TO LACTOFERRIN

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Acute phase reactants ceruloplasmin (CP), a copper protein, and lactoferrin (LF), an iron protein, form a complex (Kd 1,8 x 10^{-6} M) both *in vitro* and *in vivo* [Zakharova et al., 2000]. Reciprocal affinity of the two proteins increases when CP is partly proteolysed [Pulina et al, 2002]. Limited proteolysis facilitates release of copper ions from CP, which may have its deleterious effect in foci of inflammation. We were interested to study whether LF associated with CP affects its proteolysis and loss of copper. Affinity chromatography with subsequent electrophoresis and spectral studies of metalloproteins were used in this study. Neutrophil proteases elastase and cathepsin G efficiently cleave CP. Along with CP these two proteases are found in increased concentrations in the foci of inflammation where the share of proteolyzed CP may also increase. Anti-oxidant properties of proteolyzed CP are more pronounced and so is its affinity towards apo-LF that is released from neutrophils in the inflammation foci. Incubation of apo-LF/CP complex with trypsin resulted in limited proteolysis of CP, while LF remained intact. Copper ions released from CP were incorporated in increasing amounts by apo-LF, which was evidenced by concomitant decrease of CP absorption at 610 nm and increase of the band at 435 nm corresponding to Cu-LF. Apo-LF did not incorporate copper ions if CP was intact. The observed mechanism might be of importance for protection against pro-oxidative transition metals in the foci of inflammation.

MECHANISMS OF BIOLOGICAL EFFECTS OF METALS MEDIATED BY INTERACTIONS WITH NUCLEOTIDE COFACTORS OF PROTEINS

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To elucidate the mechanisms of biological effects of metals mediated by their interaction with nucleotide cofactors, quantum chemistry analysis of model systems, involving magnesium ion, was carried out. We undertook quantum chemistry calculations of six-coordinated Mg(2+) complexes with water, glutamic acid and ATP/GTP. For us, effects of magnesium ions are of special interest since they initiate biochemical processes leading to the process of physiologically meaningful self-aggregation of tubulin in microtubules. Acting on tubulin within the complex with GTP, Mg2+ coordinates two terminal phosphate groups and initiates transfer of a proton to a singly bound water molecule of the second and subsequent coordination shells. Realization of singlet (S) and triplet (T) states of the considered model complexes was investigated. It was shown that in the triplet state the magnesium complex

concentrates its spin density on a coordinated water molecule (inner or outer coordination shell). Within the molecule a redox reaction occurs giving rise to hydrogen that is pushed away from the complex at a speed of ~ 125 m/s. In water solution, energy of the triplet state is higher than that of the singlet state. In the mixed environment composed of water, amino acids and ATP/GTP, energy of the magnesium complex in the triplet state is lower than that in the singlet state by 1.5–2.0 kcal/mol. A little difference in T and S states allows the Mg(2+)-ATP/GTP complex to switch easily between two reaction mechanisms. Thus, magnesium complex may act via different mechanisms in the singlet and triplet states. The singlet mechanism suggests the proton transfer on the outer-shell water molecule and then on amino acid residues. This relatively slow transfer process obeys the "push-pull" mechanism and leads to ATP/GTP hydrolysis. Ejection of a hydrogen atom from the magnesium complex according to the triplet mechanism (under-barrier tunneling) is a fast process, which lifts most limitations on the velocity and distance range of the transferred energy in biological macromolecules and supramolecular structures.

INTERACTION OF RHENIUM CLUSTER COMPOUNDS WITH HUMAN BLOOD PROTEINS

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Molecular mechanism of interaction of organometallic compounds with biological objects with different structural complexity is still attracting attention of biochemistrers due to promising data about the activity of such compounds. Recently we have shown antitumour, antianemic, cytostabilizing and other activity of rhenium cluster compounds with organic ligands among which the most interesting were: I – dichlorotetra-μ-(i-butirato)dirhenium(III) and II – tetrachlorodi-μ-(γ-aminobutirato)dirhenium(III) chloride. Reaction between antigene (Ag – standart human blood serum) and antibody (Ab – antiserum against IgA, IgM, IgG) was studied in the presence I and II in the range of concentration from 10^{-3} to 10^{-12} M . The process was studied in several modifications and effectiveness of immuno precipitating test was appreciated. In all experiments with IgA and IgM no essential changes were discovered. The most shifts in characteristics of immunoprecepitation lines were noticed in experiments with IgG. I and II interacted with Ag and Ab at several ratios of reagents. We think that changes of antigenic properties can be connected with conformational shifts of proteins, which doesn't bring perturbation of complementarity of the antigen-antibody reactive sites. We consider that Re atoms are coordinated to protein molecules via the imidazole ring of His.

THE EFFECTS OF DIVALENT CATIONS ON THE FIBRIN CLOT FORMATION AND ITS LYSIS

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Background: As hemocoagulation is a Ca^{2+}-dependent process, we suppose that other divalent cations (DC) can also influence this system.

Aims: The effects of some DC - Ni^{2+}, Fe^{2+}, Cd^{2+}, Zn^{2+}, and Ni^{2+} - on thrombin-induced plasma clotting and clot lysis caused by streptokinase have been studied.

Methods: The pool of citrated human plasma was 20-fold diluted with isotonic Na^{2+}-free buffer and incubated (37°C, 3 min) with DC sulfates (0.01–1.0 mM). Than streptokinase was added and the coagulation was initiated with thrombin. The optical density changes were monitored at 340 nm.

Results: All DC studied, except Fe^{2+}, maintained the formation of fibrin clot followed by its lysis. The rank of the ion effectiveness was as followed: Zn^{2+}>Cd^{2+}>Ni^{2+}= Ni^{2+}. At DC presence the coagulation part of the turbidimetry curve was changing dose-dependently in: (1) the decrease of the induction period which seems to be due to thrombin activation or to accelerated fibrin self-association; (2) the elevation of the maximal optical density of the fibrin clot and the velocity of its achievement. The fibrinolytic part of the curve was characterized by the optical density downfall acceleration at low Zn^{2+}, Cd^{2+} and Ni^{2+} concentrations, but marked delay of the process at DC higher concentrations.

Conclusions: Ions of Zn^{2+}, Cd^{2+} ,$Co^{2+}$and Ni^{2+}, but not Fe^{2+}, significantly influence the dynamics of thrombin-induced clot formation and optical clot characteristics. The DC action in fibrinolysis can be inhibiting or activating at different ion concentrations.

LIPID OXIDATION AND BEHAVIOR ARE CORRELATED IN DEPLETED URANIUM EXPOSED MICE

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Depleted uranium (DU) is a heavy metal seeing increased levels of use and corresponding greater entry into the environment. The physiological effects of DU are debated but are generally expected to follow those associated with other heavy metals such as lead and mercury. Many metals are know to enhance oxidation of lipids in living systems, it is reasonable to expect that DU may oxidize lipids with those in the central nervous system being of particular interest. Oxidation of CNS lipids may also affect the behavior of exposed animals.

Swiss-Webster mice, housed under standard laboratory conditions, were exposed to DU in drinking water at 0 (control), 19, 37, or 75 mg/L for two weeks. Following exposure to DU the animals were assessed using a standard animal neurologic screening tool and tested in the open-field maze. Afterward, the brains were removed and the amount of lipid oxidation determined using the thiobarbituric acid assay.

DU exposed animals displayed greater reactivity and fewer rears in the open-field than control animals. Lipid oxidation was increased in a dose-dependent fashion in the brains of DU exposed mice. Preliminary analysis indicates that lipid oxidation is correlated with both reactivity and rearing activity in the open-field.

These data suggest that DU does cross the blood-brain-barrier and increase lipid oxidation. It also appears that lipid oxidation has an effect on animal behavior and may be one mechanism by which DU and other metals effect behavior. Additional analysis of brain DU content of these animals is planned.

CONSTRUCTION AND CLONING OF PSEUDOPHYTOCHELATIN (PPC) GENE FOR BINDING OF HEAVY METALS BY PPC PEPTIDE

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Enzymatically synthesized from gluthathion peptides cadystins or phytochelatins are known to appear in yeast and plants in response to the presence of heavy metals. The given peptides have a high cysteine content and the most typical among them is phytochelatin with the following amino acid sequence — (gamma-GluCys)$_n$ Gly, where n is from 2 to 11. The existence of gamma-bond between glutamine acid and cysteine may be considered as their peculiarity, because alpha-bonds are formed during the process of protein synthesis on ribosomes. The considerable similarity of the peptide complexes with cadmium, mercury and lead was revealed for both natural gamma-phytochelatins and artificial alpha-phytochelatins [Bae, Mehra, 1997, J. Inorg. Biochem., *68*, 201; Pickering et al., 1999, Biochim. Biophys. Acta, *1429*, 351]. It allows to hope for the possibilty of using artificial alpha-phytochelatins for binding of heavy metals, especially when the increase of resistance to cadmium of recombinant strain cells of *Escherichia coli* carrying plasmids with synthetic phytochelatin genes has been lately demonstrated [Bae et al., 2000, Biotechnol. Bioeng., *70*, 518]. For constructing and cloning of pseudophytochelatin (PPC) genes with Met(GluCys)$_n$Gly amino acid sequences and TAG termination codon the corresponding oligonucleotides were synthesized which were selected in such a way that during their annealing and formation of double-stranded molecules cohesive ends suitable for directed cloning in phagemid vector pBluesript II KS(–) appeared. After stages of ligation and transformation of *E. coli* competent cells, search of recombinant colonies among transformants, production of single-stranded matrices, DNA sequencing was carried out by Sanger method, and it showed the presence of chemically synthesized PPC genes in a number of recombinant clones. On the basis of created series of these PPC genes under the control of 35S promoter the constructions in binary Ti vectors with the aim of creation of tobacco transgenic plants will be prepared.

SURFACE-ENHANCED FTIR SPECTRA OF PROTEIN A CONJUGATED WITH COLLOIDAL GOLD

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In infrared (IR) spectroscopy, the effect of roughened surface of a noble metal (mostly Au or Ag) can result in surface-induced enhancement of intensities of bands for functional groups adjacent to the surface. The enhancement factors are of the orders of several units up to 100, i.e. essentially lower than for surface-enhanced Raman scattering (SERS). Nevertheless, the effect may include specific shifts of relevant bands and thus be sensitive to direct metal–molecule contact interactions. We studied Fourier transform infrared (FTIR) spectra of films prepared from sols of colloidal gold (CG) conjugates with Staphylococcal protein A (containing 6 mmg of protein per ml) which are widely used in immunoassay. Conjugation of CG with a biospecific probe occurs

due to the net effect of relatively weak non-covalent interactions (electrostatic and hydrophobic), which allows the nativity of biomacromolecules to be conserved. The results have shown that certain characteristic bands of the protein (e.g., amide I, amide II and some other vibration modes) are essentially affected by the CG surface. The spectroscopic data obtained confirm that the biomolecules are attached directly to the CG surface, which is of primary importance for the synthesis of haptens with CG for subsequent immunization of animals, so that the method may be used for controlling the quality of such bioconjugates.

***Acknowledgements*:** This work was supported in parts by INTAS (EC), NATO (Grant LST.CLG.977664), Russian Foundation for Basic Research (Grant 01-04-48736) and the Russian Academy of Sciences' Commission (Grant 20; the 6th Competition-Expertise of projects).

DNA AS AN ENZYME AND EFFECT OF METAL IONS IN ACTIVATION AND INHIBITION, A ROUTE IN MECHANISM OF CARCINOGENESIS

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Background: There has been reports concerning the electrical properties of DNA and its possible importance in many cellular mechanisms as well as in mechanisms of pathogenesis of cancer (1-2). This property is solely related to the double helical structure and is absent in a single strand. We had previously reported the enzyme like properties of DNA using a dipeptide carnosine (beta alanyl –histidine) as a cofactor. In this report, we have studied this effect with different imidazole containing compounds and metal ions. It was found that in an electron transfer reaction in which an electron is transferred to carnosine the rate is enhanced in presence of DNA, In present study several metal ions are used to investigate the effect of metal ions in such reactions.

Aims*:* To Investigate the nature of electron transfer via an electron scavenger carnosine and the possible role of DNA in such a process. The role of metal ions in similar reactions in enzymes are well known. The acid base catalysis that is mediated by metal ions could be observed in such reactions.

Methods: A fluorometric method was used to study the rate of an oxidation process in which dichloroflourocein by losing an electron in the presence of peroxides and hematin is transformed to fluorescent dichlorofluorecine. This reaction was enhanced several time in presence of DNA purified from calf thymus. The kinetics of catalysis was investigated further using several metal ions such as Pb(II), Ni(II), Fe(II), Zn (II) and Mg(II).

Results: The results obtained indicates that the rate of the electron transfer reaction was enhanced several times in presence of DNA and carnosine. Metal ions such as Mg(II) and Zn(II) could further increase the rate of the reaction. In contrast to the catalyzing properties of the above metals, Ni(II), Pb(II) and Fe(II) inhibited the reaction.

Conclusions: On the basis of the results obtained a mechanism was proposed in which the metal ion Mg(II) and Zn(II) are acting as cofactors mediating the transfer of electrons through DNA chain. Metals such as Ni(II) and Pb(II) could act as electron pool to cause inhibition in such electron transfer reaction. This phenomenon could be related to the carcinogenic effect of these metals.

INTERACTION OF METAL IONS AND NANOPARTICLES OF SILVER WITH DIFFERENT BIOLOGICAL ACTIVE FLAVONOIDS

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It is currently topical to gain insight into the influence of metal ions on mechanism of major biological processes with participation of natural antioxidants – flavonoids (Fl).

In our work we used Fl, such as quercetin (Qu), rutin (Ru) and morin (Mo). It is well-known antioxidant activity of these substances is dependent on the presence of metal ions. So, it's to be interested to investigate of the structural effect of studied flavonoids on the stabilization of different complexes Fl and metal ions in solutions.

It's very important to note, that biological systems environment, consisting of membranes, cells etc. have very high level of organisation, in which metal ions could be present as polymetallic centers or clasters. That is why the reverse micellar solutions were choosen by us as a closed model for investigation of mechanisms of intramolecular interactions.

Our task is to investigate complexes of flavonoids with silver ions in molecular solutions and in micellar ones. Earlier we found the formation of silver nanoparticles in micellar system of Qu/ AOT/ iso-octane after

adding of water solution of silver ions (Ag^+). These nanoparticles are very stable and they have intensive absorption in visible range. We obtained the similar nanoparticles in reverse micellas containing Ag^+ by radiation chemical method in which solvated electrons are as reducing agent.

In order to investigate the interaction of ions and nanoparticles of silver with flavonoids in dependence of their structures we used the methods of UV-VIS spectroscopy, RP-HPLC and TLC.

In all cases we observed the formation of complexes of silver ions with flavonoids in water-alcoholic solutions ($Ag^+ + Fl \rightarrow [Ag^+ \ldots Fl]$) and the formation of silver clasters ($Ag^+ Fl \rightarrow Ag_n^{m+}$) in reverse micellar solutions. Also we registered of complexes of silver clasters with flavonoids ($Ag_n^{m+} + Fl \leftrightarrow [Ag_n^{m+} \ldots Fl]$). The latter fact may be confirmed by the decreasing of intensity of initial optical adsorption band of Ag_n^{m+} ($\lambda \sim 420$ nm) and the appearance of new band with $\lambda \sim 295$ nm.

In the near future our investigations will be concerned with a study of the antioxidant activity of complexes of flavonoids with silver ions and nanoparticles.

Acknowledgments: This work was partially supported by RFFR No. 01-03-32783.

COMPUTATIONAL MODELLING OF SEQUENCE-DEPENDENT METAL ION BINDING TO DNA DUPLEXES

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Three-dimensional geometry of hydrated divalent metal ion and electrostatic potential (EP) around 12 bp DNA duplexes were applied together to analyze the specificity of the metal ion binding to DNA sequences. Variations of electrostatic potential along the double helix have been compared with experimentally observed sequence-selective binding of metal ion to DNA oligonucleotides. Calculations of EP have been performed for three atomic models of the oligonucleotide duplex [d(CGCGAATTCGCG)2] using several variants of EP calculations, including a solution of non-linear Poisson-Boltzmann equation (NPBE). N7 atom of guanine adjacent to adenine base was identified as a region with the most negative electrostatic potential in the major groove. The EP value for the Me ion binding site surpasses the value for N7 of other guanines by 10–26% depending on particular duplex conformation. Qualitatively, the sequence dependent variations of EP near guanine N7 atoms are in agreement with the sequence-selective behavior of Mn(II) and Zn(II) ions as revealed by NMR experiments. But the difference in EP between the two most negative regions near guanine N7 atoms does not exceed 1.25 kT/e. Simple geometrical model suggests that metal ions are capable to form ion-hydrate complexes with **G-Pu** steps of DNA duplex. These complexes are formed via one **Me**···**G** and five **Me**···**water** coordination bonds with water molecules hydrogen bonded to two adjacent purine bases in the same chain. We suppose that such a stereospecific structural possibility is the main factor which control the sequence-selectivity in the metal ion binding. A combination of both mechanisms allows to explain sequence specific Mn(II) and Zn(II) binding to the set of 12 different oligonucleotides.

EFFECT OF CADMIUM AND CADMIUM ANTAGONISTS ON CORTICOMEDULLARY ENZYMES OF RAT

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Background: Cadmium is known to produce environmental stress. Hypertensive nature of this biometal has worked out by several groups. Hypertension is known to modulate the renin-angiotensin and renal structures. Cadmium is nephrotoxic. However, effect of cadmium on corticomedullary regions of kidney in relation to angiotensin converting enzyme activity and oxidant enzymes has not been worked out.

Aim: Therefore we investigated effect of cadmium and its antagonist 24 on these enzyme systems in Sprague- Dawley rats.

Methods: SD rats were treated with cadmium (1mg/kg, i.v.) and antagonist 24 (10 mg/kg, i.v). Rats were anaesthetized by pentobarbitone and blood pressure and heart rate were recorded on Grass polygraph VII. Blood was collected and both kidneys were dissected after one hour of observation period. Cortical and medullary portions were dissected and serum, tissue ACE was estimated using tripeptide HHL. Serum and tissue TBARS were also estimated.

Results: Results of this investigation indicate that cadmium produced hypertension; serum ACE levels were inhibited while TBARS levels were increased. Antagonist 24 upregulated serum ACE while serum MDA did not show significant changes. Cadmium decreased cortical and medullary ACE levels 8.6 ± 3.6, 7.8 ± 1.2 U/mg respectively as compared to 10.3 ± 3, 12 ± 3 U/mg in control rats. Antagonist inhibited medullary

ACE to 3.4 ± 1.1 U/mg and effect was statistically significant. Cortical and medullary TBARS levels were increased and effect was statistically significant ($p<0.01$). Blood pressure was increased on cadmium administration and heart rate was decreased. Antagonist 24 produced lowering of blood pressure 6.4 ± 3.4 mmHg. Heart rate did not change significantly. Serum proteins were decreased on cadmium treatment. Cortical and medullary DNA and RNA levels were altered. Cadmium induced increased serum, cortical and medullary concentration of cadmium ($p<0.01$), while antagonist did not produce significant changes as compared to controls.

Conclusion: These results indicate that cadmium ions produce hypertension and upregulation of TBARS while antagonist 24 lacks such pharmacological properties.

Acknowledgments: UPCST support is highly appreciated.

LEAD- INDUCED CHANGES OF PHYSICAL STATE OF HUMAN ERYTHROCYTE MEMBRANE LIPIDS *IN VITRO*

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Lead (Pb^{2+}) is one of the commonest elements which contaminates the environments. After incorporation of this metal by organism it induces broad range of physiological and biochemical dysfunctions, including changes structural-functional state of cellular membrane. It is established that Pb^{2+} induces changes in the composition erythrocyte membrane phospholipids, fatty acids and proteins, has influence on some membrane enzymes and interfere in processes, which are regulated by calcium. However, the molecular-membrane mechanisms of lead effects are not clear.

We investigated effect of lead acetate *in vitro* on physical state of human erythrocyte membrane lipids by lipophilic fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH) and spin labels 16-doxyl-stearat (16DS), 15-iminoxyl-palmitinic acid (C15).

It was shown values of intensity and the degree of polarization of DPH fluorescence had no considerable differences from control for membranes isolated from erythrocytes treated 1–4 μM of toxin for 1 hour at 37° C, whereas for isolated membranes treated 2–10 μM of lead acetate these parameters increased by 20 % and 10 % respectively. It is evidence that membrane lipid microviscosity of erythrocytes reduced at the effect of lead acetate *in vitro*.

The EPR-spectrum of 16DS demonstrated heterogeneity of microenvironment of label radical. Changes of EPR-spectrum were estimated on portion of more mobile label in total spectrum. It was increased by 10 %. The motion of a EPR-probe C15 in ghosts was close to isotropic rotation, therefore its mobility was estimated on time of correlation τ [$\tau = 2.7 \cdot 10^{-10} \cdot H_0 \cdot (h_1 / h_{-1} - 1)^{1/2}$, H_0 – width of central component in gausses, h_0, h_{-1} – amplitudes of central and highfield lines]. It had a tend to be increased with rising of toxic concentration.

The findings in the present study testify about change of physical state of membrane lipids at effect of lead acetate in vitro. The increase of DPH fluorescence and 16DS EPR-parameters corresponds to decrease of microviscosity in a deep hydrophobic layer of membrane lipids, which may influence on functional characteristics of erythrocytes.