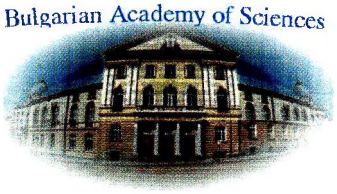
W O R K S H O P
ON
BIOLOGICAL ACTIVITY
OF
METALS AND METAL COMPOUNDS

PROGRAMME AND ABSTRACTS

NOVEMBER 2-3, 2006

SOFIA, BULGARIA

THE WORKSHOP IS ORGANIZED BY
THE INSTITUTE OF EXPERIMENTAL PATOLOGY AND PARASITOLOGY
UNDER THE AUSPICES OF
THE BULGARIAN ACADEMY OF SCIENCES
AND
THE ROMANIAN ACADEMY



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Supported by National Science Fund, Bulgarian Ministry of Education and Science, Sofia, Bulgaria

THE PROGRAM OF THE WORKSHOP

Thursday, November 2

9.45-10.00 *OPENING REMARKS*

Session A: METAL COMPOUNDS IN CANCER TREATMENT

Chairpersons: Prof. Elena Nikolova
Assist. Prof. Radostina Alexandrova

10.00-10.30 Metal compounds in cancer treatment
R. Alexandrova

10.30-11.00 New dimensions in Comet assay applications
G. Milloshev, M. Georgieva

11.00-11.30 Coffee Break

11.30-12.00 Cytotoxic and antiproliferative activities of Cu(II), Co(II) and Fe(II,III) mixed ligand complexes on tumor cell lines
R. Alexandrova, M. Kirilova, G. Rashkova, G. Miloshev, A. Vacheva, E. Nikolova, Y. Martinova, R. Kalfin, E.M. Mosooarca, R. Tudose, O. Costisor, K. Nemet

12.00-12.30 Investigations on cytotoxic and antiproliferative activities of Zn(II), Cu(II) and Co(II) complexes with lithocholic and dehydrocholic acids on tumour cell lines
R. Alexandrova, **I. Todorova**, M. Kirilova, G. Rashkova, Y. Martinova, E. Nikolova, G. Miloshev, R. Kalfin, D. Culita, L. Patron

12.30-13.30 Poster Sessions A, B and Lunch

Session B: METAL COMPOUNDS AS ANTIMICROBIAL AGENTS

Chairpersons: Assoc. Prof. Margarita Gabrashanska
Assoc. Prof. Teodora Popova

13.30-14.00 Metal compounds in the treatment of infectious and other diseases
R. Alexandrova

14.00-14.30 Investigations on antimicrobial activity *in vitro* of eighteen cooper, cobalt, iron and nickel complexes with Mannich type ligands

T. Popova, R. Alexandrova, R. Tudose, E.-M. Mosoarca, O. Costisor

14.30-15.00 Investigations on antimicrobial activity *in vitro* of newly synthesized metal complexes with cholic acids

T. Popova, R. Alexandrova, D. Culita, L. Patron

15.00-15.30 Platinum(II) and palladium(II) complexes of pyridine-2-carbaldehyde thiosemicarbazone with promising anti-HSV effect

P. Genova, T. Varadinova, D. Kovala-Demertzi, P. Souza, M. Demertzis

Friday, November 3

Session C: PARASITES AND METALS

Chairpersons: Assoc. Prof. Yana Mizinska-Boevska
Assoc. Prof. Igiika Nedeva

10.00-10.20 Antioxidant status in helminthoses after treatment with new metal compounds

M. Gabrashanska, M. Anisimova, S. Tepavitcharova, J. Manga Gonzalez, Y. Mizinska, S. Ermidou-Pollet, S. Pollet

10.20-10.40 Coffee Break

Session D: METAL TOXICITY AND CANCEROGENESIS

Chairpersons: Assoc. Prof. Reni Kalfin
Assoc. Prof. Anna Damianova

10.40-11.10 Metal toxicity and cancerogenesis – a brief overview of literature data

R. Alexandrova

11.10-11.30 Fish parasites as bioindicators of heavy metals

M. Gabrashanska

11.30-13.00 Poster Sessions C, D, E and Lunch

Session E: NEWLY SYNTHESIZED COMPOUNDS

Chairpersons: Prof. Luminita Patron
Assoc. Prof. Stefka Tepavitcharova

13.00-13.30 Design of coordination compounds with potential biological activity

L. Patron

13.30-14.00 Physico-chemical characterization and theoretical studies on the magnetite nanoparticles coated with aminoacids, bile salts and various types of dextran

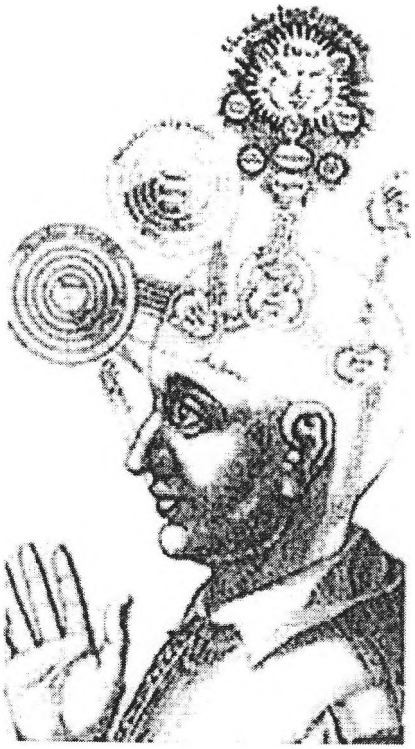
D.C. Culita

14.00-14.30 Poly(alkylcyanoacrylate) nanoparticles - a feasible drug carriers of anticancer drugs

M. Simeonova

14.30 *CLOSING REMARKS*

ABSTRACTS



Main Topics:

METAL COMPOUNDS IN CANCER TREATMENT

METAL COMPOUNDS AS ANTIMICROBIAL AGENTS

PARASITES AND METALS

METAL TOXICITY AND CANCEROGENESIS

NEWLY SYNTHESIZED COMPOUNDS

Session A.

METAL COMPOUNDS IN CANCER TREATMENT

Chairpersons:

Prof. Elena Nikolova (Institute of Experimental Morphology and Anthropology with Museum, BAS)

Assist. Prof. Radostina Alexandrova (Institute of Experimental Pathology and Parasitology, BAS)

AO1

METAL COMPOUNDS IN CANCER TREATMENT

R. Alexandrova

[1]*Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 25, Sofia 1113, Bulgaria*

The need to find a safe and highly selective cure for neoplastic diseases remains a major challenge for modern science. The discovery of the antitumor efficacy of cisplatin and some related platinum complexes has stimulated the search for other metals with antineoplastic properties. Different metal complexes, including such of gold, ruthenium, gallium, vanadium are found to be promising compounds for the design of new anticancer agents and some of them are in different stages of preclinical and clinical investigations.

Acknowledgement: Supported by Grant CC-1402/04 from National Science Fund, Bulgarian Ministry of Education and Science.

AO2

NEW DIMENSIONS IN COMET ASSAY APPLICATIONS

G. Miloshev, M. Georgieva

Laboratory of Molecular Genetics, Institute of Molecular Biology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, Sofia 1113, Bulgaria; e-mail:miloshev@bio21.bas.bg

The methods based on the single-cell observations are more representative and more sensitive than the cell population-based biochemical approaches and high throughput analyses.

The method Single Cell Gel Electrophoresis (SCGE), also known as Comet Assay, has been used for two decades in genotoxic studies. Nowadays, by broadening its applications the Comet Assay is gaining more popularity on the basis of its versatility and broadening of its possible applications.

We have been used the method Comet assay for several years in the field of genetic toxicology. Simultaneously we were able to design numerous modifications of the assay and to develop its novel applications. This allowed us to utilize it in yeast genetics, chromatin structure studies and apoptosis.

The results with Comet assay in epigenetic research, ecology and forensic science will be discussed.

AO3

CYTOTOXIC AND ANTIPROLIFERATIVE ACTIVITIES OF Cu(II), Co(II) AND Fe(II, III) MIXED LIGAND COMPLEXES ON TUMOR CELL LINES

R. Alexandrova[1], M. Kirilova[2], G. Rashkova[1], G. Miloshev[2], A. Vacheva[1], E. Nikolova[3], Y. Martinova[3], R. Kalfin[4], E.M. Mosooarca[5], R. Tudose[5], O. Costisor[5], K. Nemet[5]

[1]Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 25, Sofia 1113, Bulgaria;
[2]Institute of Molecular Biology, BAS, Acad. Georgi Bonchev Str., Block 21, Sofia 1113, Bulgaria;
[3]Institute of Experimental Morphology and Anthropology with Museum, BAS, Acad. Georgi Bonchev Str., Block 25, Sofia 1113, Bulgaria
[4]Institute of Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 23, Sofia 1113, Bulgaria
[5]Institute of Chemistry Timisoara of the Romanian Academy, 24 Mihai Viteazu Blvd, RO-300223, Timisoara, Romania;
[6]Institute of Hematology and Immunology, National Medical Center, Dioszegi Str 64, 1113 Budapest, Hungary

The aim of the present study was to evaluate the cytotoxic and antiproliferative activities of newly synthesized Cu(II), Co(II) and Fe(II, III) mixed ligand complexes. The following cell lines were used in the experiments: K562 (human erythroleukaemia), 8-MG-BA (human glioblastoma), A431 (human squamous cell carcinoma), P3U1 (mouse myeloma), LSR-SF-SR (transplantable sarcoma in rat, induced by Rous sarcoma virus strain Schmidt-Ruppin), LSCC-SF-Mc29. (transplantable chicken hepatoma induced by the myelocytomatosis virus Mc29). The influence on cell viability and proliferation was examined by neutral red uptake cytotoxicity test, trypan blue dye exclusion technique, FACS analysis, colony-forming assay and autoradiography. Concentrations that reduce cell viability by 50% (CC_{50}) were determined using concentration-effect curves. The results obtained revealed that among the compounds investigated the copper (II) complex Cu_2(BAMP)dipyCl_4 [BAMP = N,N'-bis(4-antipyrylmethyl)-piperazine; dipy = 2,2-dipyridyl] expressed the most pronounced cytotoxic and antiproliferative properties. Thus, CC_{50} determined on 24 h were found to vary between 3 μg/ml (LSCC-SF-Mc29) and 34 μg/ml (8 MG BA) and on 48 h between 1 μg/ml (K562) and 23 μg/ml (8-MG-BA). Applied at concentration of 1 to 10 μg/ml this compound completely inhibited the growth of tumor cells in semisolid medium and reduced significantly the incorporation of ^{3}H-thymidine. Shrinkage of the cell and its nucleus, plasma membrane blebbing, chromatin condensation and segmentation of the nuclei as well as formation of apoptotic bodies were observed after acridine orange staining in the cells treated with ≥ 5 μg/ml Cu_2(BAMP)dipyCl_4. DNA damages were found by single cell gel electrophoresis in 60% of the tumor cells cultured for 48 h in the presence of this compound (10 μg/ml). The mixed ligand copper (II) complex expressed more pronounced cytotoxic and cytostatic activities against LSCC-SF-Mc29 hepatoma and P3U1 mouse myeloma cells than on primary chicken embryo cells and BALB/c 3T3 mouse fibroblasts.

The cobalt (II) complex Co_2BAMPpy$_2Cl_4$ (py = pyridine) also decreased the viability and proliferation of tumor cells but to a lower extent than Cu_2(BAMP)dipyCl_4 whereas Fe(II, III) complexes Fe_2BAMPpy$_2Cl_4$, Fe_2BAMPpy$_2Cl_6$, Fe_2TAMENdipyCl_4 and Fe_2TAMENdipyCl_6 (TAMEN = N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane) were without or with very weak effect.

Acknowledgement: Supported by Grant CC-1402/2004 from National Science Fund, Bulgarian Ministry of Education and Science and a Bilateral Project between Bulgarian Academy of Science (Institute of Experimental Pathology and Parasitology) and Romanian Academy (Institute of Chemistry, Timisoara).

AO4

INVESTIGATIONS ON CYTOTOXIC AND ANTIPROLIFERATIVE ACTIVITIES OF Zn(II), Cu(II) AND Co(II) COMPLEXES WITH LITHOCHOLIC AND DEHYDROCHOLIC ACIDS ON TUMOUR CELL LINES

R. Alexandrova[1], I. Todorova[1], M. Kirilova[2], G. Rashkova[1], Y. Martinova[3], E. Nikolova[3], G. Miloshev[2], R. Kalfin[4], D. Culita[5], L. Patron[5]

[1]Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 25, Sofia 1113, Bulgaria
[2]Institute of Molecular Biology, BAS, Acad. Georgi Bonchev Str., Block 21, Sofia 1113, Bulgaria;
[3]Institute of Experimental Morphology and Anthropology with Museum, BAS, Acad. Georgi Bonchev Str., Block 25, Sofia 1113, Bulgaria
[4]Institute of Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 23, Sofia 1113, Bulgaria
[5]Institute of Physical Chemistry "I. G. Murgulescu", Splaiul Independentei 202, sect.6, 060021 Bucharest, Romania

The potential antineoplastic activities of different metals and metal compounds have been under special interest during the recent years (1, 2). The two main reasons for such interest are: 1) It has been found that the disturbed balance in the essential metal metabolism of mammals results in increased susceptibility to infections and malignancies; 2) Being involved in the regulation of some definite processes of the animal organisms, several metals are biological response modifiers. On the other hand the multiple physiological functions of bile acids are well known. Furthermore, it has been suggested that bile acid conjugates could be helpful in the development of new approaches for target anticancer therapy (3). It was found in our previous investigations (4) that Zn(II), Cu(II) and Co(II) complexes with cholic acid expressed cytotoxic and antiproliferative effects on animal and human cancer cells. The aim of the present study was to evaluate the potential antitumour activities *in vitro* of five newly synthesized Zn(II), Cu(II) and Co(II) complexes with lithocholic or dehydrocholic acid. The following cell lines were used in the experiments: MCF-7 (human breast adenocarcinoma), 8-MG-BA (human glioblastoma), K562 (human erythroleukaemia), LSCC-SF-Mc29 (transplantable chicken hepatoma induced by the myelocytomatosis virus Mc29). The influence on cell viability and proliferation was examined by neutral red uptake cytotoxicity test, trypan blue dye exclusion technique and colony-forming assay. Using dose-response curves the concentrations that reduce cell viability by 50% (CC_{50}) and 90% (CC_{90}) were determined. The results obtained showed that applied at concentrations of 1, 10, 50 and 100 μg/ml for 24 h and 48 h the metal complexes investigated decreased in a time- and dose-dependent manner the total number of tumour cells and their viability, and reduced the incorporation of ^3H thymidin in DNA molecules. Single cell gel electrophoresis revealed DNA damages in cells cultured for 24 h and 48 h in the presence of the compounds tested. Cytopathological changes such as appearance of cells with giant, sickle-shaped nuclei or nuclear fragmentation were observed after acridin orange staining. In conclusion, Cu(II) dehydrocholate complexes were found to be the most active among the samples investigated, especially against virus-transformed LSCC-SF(Mc29) cells.

Acknowledgement: Supported by Grants CC-1402/04 and L-1305/03 from Bulgarian National Science Fund.

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4. Alexandrova, R. et al., Annals. Oncol., 15, Suppl. 3, 2005, P112.

AP1

MANNICH TYPE LIGAND COMPLEXES AND THEIR CYTOTOXIC AND ANTIPROLIFERATIVE PROPERTIES

R. Alexandrova[1], G. Rashkova[1], M. Kirilova[2], G. Miloshev[2], E. Nikolova[3], Y. Martinova[3], R. Kalfin[4], E.M. Mosoarca[5], R. Tudose[5], O. Costisor[5]

[1]*Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., Block 25, Sofia 1113, Bulgaria*
[2]*Institute of Molecular Biology, BAS, Acad. Georgi Bonchev Str., Block 21, Sofia 1113, Bulgaria*
[3]*Institute of Experimental Morphology and Anthropology with Museum, BAS, Acad. Georgi Bonchev Str., Block 25, Sofia 1113, Bulgaria*
[4]*Institute of Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 23, Sofia 1113, Bulgaria*
[5]*Institute of Chemistry Timisoara of the Romanian Academy, 24 Mihai Viteazu Blvd, RO-300223, Timisoara, Romania*

In 1912 Mannich and Krösche discovered the property of formaldehyde to bind an amine with a carbon acid *via* a methylene bridge [1]. The method was utilised to obtain pharmaceutical products by implication of acids components which were recognised like substances with therapeutic action. Our main point of interest refers to study the Mannich base complexes of some first row metal ions, in order to explain their biological activity as well as to find new compounds with biological effects. In this frame, the Mannich bases N,N'-bis-(antipyryl-4-methyl- piperazine (BAMP) (Fig. 1) and N,N'-tetra-(antipyrylmethyl)-1,2-ethanediamine (TAMEN) (Fig. 2) as well as their metal complexes have been synthesised and structurally characterised. The aim of the present study was to evaluate the influence of twenty copper (II), cobalt (II), iron (II, III) and nickel (II) complexes with BAMP or TAMEN (Table 1) on viability and proliferation of tumor and nontumor cells by neutral red uptake cytotoxicity test, autoradiography, colony-forming assay and single cell gel elevtrophoresis. The following cell lines served as model systems: MCF-7 (human breast adenocarcinoma), 8-MG-BA (human glioblastoma multiforme), HepG2 (human hepatocellular carcinoma), LSR-SF-SR (transplantable rat sarcoma, induced by Rous sarcoma virus strain Schmidt-Ruppin) and LSCC-SF-Mc29. (transplantable chicken hepatoma induced by the myelocytomatosis virus Mc29). The results obtained indicated that: 1) Among the complexes examined $Cu_2(BAMP)(NCS)_4$ showed the highest ability to inhibit tumor cell surveillance and division. This compounds induces DNA damages in 100% of the treated tumor cells (100 μg/ml, 48h) and is much more toxic for chicken hepaoma LSCC-SF-Mc29 cells than to primary chicken embryo cells. Cu(I, II), Co(II) and Ni(II) complexes with BAMP exhibited more pronounced cytotoxic and cytostatic activities than TAMEN containing compounds of the same metals. On the contrary, Fe(II, III) complexes with TAMEN are found to be more active cytototoxic and antiproliferative agenns as compared to iron complexes of BAMP; 3) Among the cell lines used in the experiments chicken hepatoma cells LSCC-SF(Mc29) are the most sensitive to the cytotoxic effect of the compounds; 4) Applied at concentrations examined both ligands - BAMP and TAMEN do not significantly reduce the viability of tumor cell lines tested; 5) Tested independently, DMSO (the initial solvent of the complexes) did not affect significantly cell viability and proliferation.

The metal complexes examined differ from each other in ligand (BAMP or TAMEN) and anion (NCS^-, I^-, NO_3^-). Each of these components (ligands and anions) as well as metal ions influences in different way physical and chemical properties of the complexes obtained which could explain the differences in their biological effects.

Acknowledgement: This study was supported by Grant CC-1402/04 from National Science Fund, Bulgarian Ministry of Education and Science, and a Bilateral Project between Bulgarian Academy of Sciences (Institute of Experimental Pathology and Parasitology) and Romanian Academy (Institute of Chemistry, Timisoara).

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Mannich C., B. Kather. Ueber Kondensationsprodukte aus Aminsaltzen, Formaldehyd und Antipyrin. - Arch. Pharm., **257**, 1919, 18-33.

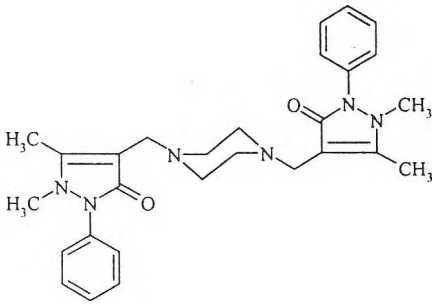

Fig. 1. N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP)

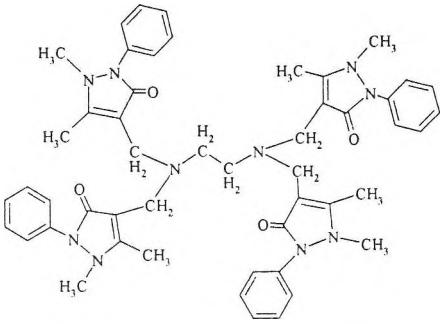

Fig. 2. N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN)

Table 1. Metal complexes with Mannich type ligands

Code	Chemical formula	Molecular weight (g/mol)
TS-1	$Cu_2(BAMP)(NCS)_4$	846.00
TS-2	$Cu_2(BAMP)I_3$	1007.00
TS-3	$Co_2(BAMP)Cl_4$	746.00
TS-4	$Co(BAMP)(NCS)_2$	661.00
TS-5	$Fe_2(BAMP)Cl_4$	740.00
TS-6	$Fe_2(BAMP)Cl_6$	811.00
TS-7	$Cu(TAMEN)(NO_3)_2$	1046.00
TS-8	$Co_2(TAMEN)Cl_4$	1121.00
TS-9	$Co_2(TAMEN)(NCS)_4$	1211.00
TS-10	$Fe_2(TAMEN)Cl_6$	1186.00
TS-11	$Ni(TAMEN)(ClO_4)_2$	1119.69
TS-12	$Ni(TAMEN)(NCS)_2$	1036.69
TS-13	$Ni_2(BAMP)(Ac)_4$	839.38
TS-14	$Ni_2(BAMP)(Cl)_4$	745.38
TS-15	$Fe(TAMEN)(NO_3)_3$	1103.85
TS-16	$Fe(BAMP)(NO_3)_3$	727.85
TS-17	$Cu(TAMEN)(ClO_4)_2$	1124.55
TS-18	$Cu(TAMEN)(Ac)_2$	1043.55
TS-19	$Cu_2(BAMP)(ClO_4)_4$	1011.10
TS-20	$Co(TAMEN)(ClO_4)_2$	119.93

AP2

BASIC SALTS OF ZINC AND COPPER AND THEIR INFLUENCE ON VIABILITY AND PROLIFERATION OF HUMAN AND ANIMAL TUMOR CELL LINES

R. Alexandrova[1], M. Gabrashanska[1], Y. Martinova[2], T. Popova[3], S. Tepavitcharova[4], A. Pastorakova[5], K. Hlubinova[5]

[1]*Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 25, Sofia 1113, Bulgaria*
[2]*Institute of Experimental Morphology and Anthropology with a Museum, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 25, Sofia 1113, Bulgaria*
[3]*Faculty of Veterinary Medicine, Forestry Technical University, Kliment Ohridsky 10 blvd, Sofia 1756, Bulgaria*
[4]*Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences*
[5]*Cancer Research Institute, Slovak Academy of Sciences, Bratislava, Slovakia*

The aim of the study presented here was to study cytotoxic and antiproliferative activities of two basic salts of zinc and copper - $Zn_{5-x}Cu(OH)_8Cl_2.H_2O$ and $CuCO_3Cu(OH)_2.nH_2O$. The following cell lines were used as model systems: i) Human - SK-BR-3 (breast adenocarcinoma), 8-MG-BA (glioblatoma multiforme), Caco2 (colon adenocarcinoma); ii) Rat - BP6 (benzo[a]pyrene-induced fibrosarcoma), LSR-SF-SR - transplantable sarcoma induced by Rous sarcoma virus strain Schmidt-Ruppin) and iii) Chicken - LSCC-SF-Mc29 (transplantable hepatoma induced by the myelocytomatosis virus Mc29). Primary chicken embryo cell (CEC) cultures were also included in the experiments. The effects of the compounds on cell viability and proliferation were investigated by neutral red uptake cytotoxicity test, colony-forming assay and autoradiography. The results obtained revealed that $CuCO_3Cu(OH)_2.nH_2O$ possessed more pronounced cytotoxic and antiproliferative potential than $Zn_{5-x}Cu(OH)_8Cl_2.H_2O$. This salt inhibited the colony-forming ability of tumor cell lines at concentrations > 0.1 μg/ml (BP6, LSR-SF-SR), > 1 μg/ml (Caco-2) and > 10 μg/ml (SK-BR-3; 8-MG-BA). When applied at a concentration of 0.1 μg/ml $CuCO_3Cu(OH)_2.nH_2O$decreased the number of LSR-SF-SR and LSCC-SF-Mc29 ^{3}H-Thymidine labeled cells by 35% ($P < 0.05$) and 43% ($P < 0.01$), respectively. CEC were found to be less sensitive to the influence of both compounds as compared to chicken tumor cells.
Acknowledgement: BP6 tumor cells were a generous gift from Dr. I. Chalupa, Cancer Research Institute, Slovak Academy of Sciences, Bratislava, Slovakia.

AP3

CYTOTOXIC EFFECTS AND CHARACTERIZATION OF APOPTOTIC HEPATOCYTES CELLS AFTER TREATMENT WITH DICLOFENAC AND ITS ORGANOMETALIC SULFUR-CONTAINING COMPLEXES

P.Genova[1], J. Prabhakar Kailash[2], D. Dundarova[1]

[1]*National Center of Infections and Parasitic Diseases, Department of Virology, Laboratory of Cell cultures. E-mail: petia.d.genova@abv.bg.*
[2]*University of Delhi, CPWD Colony, 16-Q, Vasant Vihar, New Delhi – 110 057, India.*

Diclofenac is a non-steroidal anti-inflammatory drug bearing a carboxylic acid functional group. It usually reacts as ligand with metal cations. Moreover, organometalic sulfur-containing compounds are well known as biologically active substances. Nevertheless, Diclofenac is a drug for which a certain number of severe adverse hepatic reactions have been reported. In the present study

we investigated possible linkages between structure of the Diclofenac and its tested organometalic sulfur-containing compounds, drug metabolism, cytotoxicity and putative targets of action to hepatocytes. The cytotoxic effects of tested complexes were evaluated using by the tripan blue exclusion test on Hep G2 cells, at 24h and 72h. In order to confirm or reject the suggestion for an induced destruction of DNA we performed an agarose gel electrophoresis of DNA from sample containing non-treated and treated Hep G2 cells with the corresponding inhibitors. According to the data of cytotoxic effects of the investigated complexes it was found: (i) there was a direct correlation between cytotoxicity and the extent of Diclofenac metabolization by hepatocytes; (ii) the most cytotoxic were $(PhS_4OL)_2$ **3** and the ligand Diclofenac **4**; (iii) the most tolerant was $(PhS_4OL)_2$ **3**; (iv) incubation of the Hep G2 cells with the MNC of $Ph_2S_4L_2$ **2** and $(PhS_4OL)_2$ **3** resulted in a "DNA smear" (non-specific fragmentation of genomic DNA), however, a "DNA ladder" (fragmentation of genomic DNA innucleosome units) indicative of apoptosis was obtained in Hep G2 cells treated with the MNC of the ligand Diclofenac **4**; (v) in Hep G2 cells the higher cytotoxic activity of $(PhS_4OL)_2$ **3** and Diclofenac **4** may be due to a specific induction of apoptosis.

AP4

EFFECT OF Co(III) COMPLEXES OF ARGININE ON VIABILITY OF TUMOR AND NONTUMOR CELLS

P. Genova[1], R. Alexandrova[2], S. Trifunovic[3], P. Radivojsa[4] and T. Varadinova[5]

[1]National Center of Infections and Parasitic Diseases, Department of Virology, Laboratory of Cell cultures. E-mail: petia.d.genova@abv.bg.

[2]Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. Geogi Binchev Str., Bl. 25, Sofia 1113, Bulgaria

[3]Department of Chemistry, Faculty of Science, University of Kragujevac, Yugoslavia

[4]Faculty of Chemistry, University of Belgrade, Belgrade, Yugoslavia

[5]Faculty of Biology, Sofia University "St. Kliment Ohridski"

Being essential metal cobalt influences different physiological and enzymatic functions. Participating in the corrin ring of vitamin B12, cobalt plays a crucial role in a number of biological functions [1]. Furthermore, cobalt speeds up ATP turnover [2], activates arginase and inhibits δ-aminolevulinic acid synthetase [3], effects liver mixed-function oxidase [4], enhances acylamino acid hydrolase [5] and yeast enolase [6]. The antitumor activity of cobalt compoundshas also been reported [7].

The aim of the study presented here was to evaluate the effect of Co(III) complexes of arginine (Table 1) on viability of tumor - LACC-SF(Mc29), LSR-SF(SR) and nontumor - MDBK, cells.

Based on the cytotoxic concentration required to inhibit cell surveilance by 50% (CC_{50}) it was found that: i) the cytotoxicity of the complexes tested increases when the concentration recreased; ii) the cell surveilance depends on both complex and cell specificities. The complex specificity was illustrated by the order $1 > 4 > 2 \geq 3$. The cell specific response was demonstrated by the fact that LSCC-SF(Mc29) cells were up to 60 times more sensitive to 1 while LSR-SF(SR) cells were up to 1000 times more sensitive to 2 as compared to MDBK cells. Furthermore, with the prolongation of action on nontumor cells the cytotoxicity of 4 decreased up to 300 times while for both tumor cells it was independent on the duration of time.

Nr	Complex	M.w.
1	L-/+/$_D$-mer[Co(S-argH)$_3$](NO$_3$)$_3$2H$_2$O	587.34
2	D-/+/$_D$-fac-[Co(S-argH)$_3$](NO$_3$)$_3$3H$_2$O	632.55
3	D-(-)$_D$-cis(NO$_2$)-trans(N)-[Co(S-argH)$_2$(NO$_2$)$_2$]ClO.5H$_2$O	532.83
4	(-)$_D$-anti(N)-D-cis(N), cis(O)-L-cis(N), cis(O)-[Co$_2$(s-argH)$_4$(OH)$_2$]Cl$_4$.4H$_2$O	1058.48

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AP5

Zn(II), Cu(II)AND Co(II) COMPLEXES WITH L-PICOLINIC AND DL-ASPARTIC ACIDS: CYTOTOXIC AND ANTIPROLIFERATIVE PROPERTIES

R. Alexandrova[1], P. Genova[2], E. Nikolova[3], P. Bonchev[4]

[1]*Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. Geogi Binchev Str., Bl. 25, Sofia 1113, Bulgaria*

[2]*National Center of Infections and Parasitic Diseases, Department of Virology, Laboratory of Cell cultures, Sofia, Bulgaria.*

[3]*Institute of Experimental Morphology and Anthropology with a Museum, Bulgarian Academy of Sciences*

[4]*Faculty of Chemistry, Sofia University "St. K. Ohridski", Sofia, Bulgaria*

The antiviral activity of Zn(II), Cu(II) and Co(II) complexes with L-picolinic and DL-aspartic acids was studied in earlier experiments. It was found that $Zn(pic)_2$ and $Zn(asp)_2$ inhibited key steps of the replication of Herpes simplex virus type 1 (HSV-1) [1,2] and inactivated free Varicells-zister virions [3]. Cu(asp)2 inhibited virus yield by 73% while Co(asp)2 had no effect on HSV-1 replication [2]. In addition, Zn(pic)2 and Zn(asp)2 were proved not to express mutagenic effect on both prokaryotic (*Salmonella typhimurium*) and eukaryotic (*Saccharomyces cerevisiae*) test systems [4].

In order to study better biological activity of these compounds, we decided to evaluate the ability of Zn(II), Cu(II) and Co(II) complexes with L-picolinic and DL-aspartic acids to affect viability and proliferation of the following cell lines: HeLa (cervix carcinoma), LSR-SF-SR (transplantable rat sarcoma induced by Rous sarcoma virus strain Schmidt-Ruppin) and LSCC-SF-Mc29 (transplantable chicken hepatoma induced by the myelocytomatosis viris Mc29). Relatively the most active among complexes investigated was found to be $Zn(pic)_2$ with CC_{50} = 40 μM, 64 μM and 60 μM for LSR-SF-SR, LSCC-SF-Mc29 and HeLa cells respectively. $Co(asp)_2$ exhibited the lowest cytotoxicity. The compounds inhibited the ability of tumor cells to grow in a semisolid medium and based on their antiproliferative properties they follow the order: $Zn(pic)_2 > Zn(asp)_2 > Cu(pic)_2 > Cu(asp)_2 > Co(pic)_2 > Co(asp)_2$.

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AP6

INFLUENCE OF PLATINUM AND PALLADIUM COMPLEXES WITH L-ASPARTIC ACID ON TUMOR CELL VIABILITY

R. Alexandrova[1], U. Kalinowska[2], J. Ochocki[2]

[1]Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. Geogi Binchev Str., Bl. 25, Sofia 1113, Bulgaria

[2]Pharmaceutical Department, Medical University, St. Muszynskiego 1, 90-151 Lodz, Poland

Platinum (II) and palladium (II) complexes with L-aspartic acid were synthesized and their structure and purity were verified by IR, ^{1}H and ^{195}Pt NMR spectroscopy and elemental analysis. The cytotoxic activity of these compounds was studied after a short-term exposures of 24 h and 48 h by neutral red uptake assay. The following cell lines were used in the experiments: 8-MG-BA and 42-MG-BA (human glioblastoma multiforme), SK-BR-3 and MCF-7 (human breast adenocarcinoma), Caco-2 (human colon adenocarcinoma), BP6 (benzo[a]pyrene-induced rat fibrosarcoma), LSR-SF-SR - transplantable sarcoma in rat induced by Rous sarcoma virus strain Schmidt-Ruppin) and LSCC-SF-Mc29 (transplantable hepatoma induced by the myelocytomatosis virus Mc29). Applied at a concentration of 0.1 mg/ml for 48 h the complex of palladium (II) reduced the viability of LSCC-SF-Mc29 chicken hepatoma cells by 60% ($P < 0.01$) but had no effect on primary chicken embryo cells. At the same time both compounds expressed no or very weak cytotoxic activity against human (42-MG-BA, 8 MG-BA, SK-BR-3, MCF-7, Caco-2) and rat (BP6, LSR-SF-SR) cell lines.

AP7

VANADIUM AS A POTENTIAL ANTITUMOR AGENT: AMMONIUM VANADATE AND ITS ANTINEOPLASTIC ACTIVITY IN DIFFERENT EXPERIMENTAL TUMOR MODELS

R. Alexandrova, I. Alexandrov

Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. Geogi Binchev Str., Bl. 25, Sofia 1113, Bulgaria

Recent studies have suggested that vanadium could be considered a representative of a new class of non-platinum antineoplastic agents. The mechanisms underlying the antitumor properties of this vanadium and its compounds remains unclear. Some of the following actions are probably related to its anticancer properties: 1) Protective effect against the induction of DNA strand breaks and chromosome aberrations by potent hepatocarcinogens; 2) Inhibition of metabolic activation of the procarcinogen, leading to reduced generation and/or binding of the ultimate carcinogen to DNA; 3) Elevated detoxification of the precarcinogen and/or its reactive metabolites through specific induction of activities of some of the xenobiotic biotransforming enzymes; 4) Vanadium (V) has been shown to inhibit as well as to enhance depending on its concentration in the media DNA synthesis in vitro. DNA polymerasaes, nucleotidyl transferases and phosphotransferases were also inhibited by vanadium; 6) Effect on the immune system [1-3]. It was found in our investigations that NH_4VO_3 dissolved in the drinking water at a concentration of 0.5 ppm and given *ad libitum* expressed antineoplastic activity against different experimental tumor models: Transplantable sarcoma in rat induced by Rous sarcoma virus strain Schmidt-Ruppin, solid form of tumour of Ehrlich and tumor of Guerin. Stimulation of some immune functions of tumor-bearing bearing was also observed [4, 5].

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AP8

STUDY ON CARBOHYDRATE-BINDING PROTEIN EXPRESSION IN BREAST ADENOCARCINOMA CELLS CULTURED IN THE PRESENCE OF Cu(II), Co(II) AND La(III) COMPLEXES OF CHOLIC ACID

J. Stoyloff[1], R. Alexandrova[1], S. Ivanov[1], D.C. Culita[2], L. Patron[2]

[1]*Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. Geogi Binchev Str., Bl. 25, Sofia 1113, Bulgaria*

[2]*Institute of Physical Chemistry "Ilie Murgulescu", Splaiul Independentei 202, 060021 Bucharest, Romania*

Differential expression of many molecules, as a consequence of the neoplastic transformation, reflects the characteristic phenotype of cancer cells, which are specific for each type of tumor. Looking for differentially expressed proteins expressed proteins, in hope that their expression would be tumor-specific, is a goal for an attempt to create a panel with tumor markers for diagnostic purposes. Our study was focussed on the expression and secretion of carbohydrate-binding proteins (CBPs) in culture media from nontreated and treated Cu(II), Co(II) and La(III) complexes with cholic acid. We found lower levels of CBPs with specificity towards N-Ac-β-D-mannosamine in culture media from MCF-7 human breast adenocarcinoma cells treated with metal complexes of cholic acid, where as mannose-binding proteins and galactose-binding proteins were elevated. Treatment of the MCF-7 tumor cell led to increased expression of galactosamine-binding proteins in the following order: $La(Chol)_3 \cdot 2H_2O > Cu(Chol)_2.4H_2O > Co(Chol)_2.5H_2O$. On the other hand, levels of CBPs with specificity towards D -glucosamine were found to be lower in culture in media from treated tumor cells. Fucose-binding proteins were elevated in culture media from MCF-7 breast adenocarcinoma cells treated with 10μg/ml $Cu(Chol)_2.4H_2O$, $Co(Chol)_2.5H_2O$ and $La(Chol)_3 \cdot 2H_2O$ complexes, but monnosamine-binding proteins were found to be lower in culture media from MCF-7 cell cultured in presence of 10μg/ml $Cu(Chol)_2.4H_2O$ and $Co(Chol)_2.5H_2O$ complexes.

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Session B.
METAL COMPOUNDS AS ANTIMICROBIAL AGENTS

Chairpersons:

Assoc. Prof. Margarita Gabrashanska (Institute of Experimental Pathology and Parasitology, BAS)

Assoc. Prof. Teodora Popova (Faculty of Veterinary medicine, Forestry Technical University, Kliment Ohridsky 10 blvd, Sofia 1756, Bulgaria)

BO1

METAL COMPOUNDS IN THE TREATMENT OF INFECTIOS AND OTHER DISEASES

R. Alexandrova

Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. Geogi Binchev Str., Bl. 25, Sofia 1113, Bulgaria

Metals have played an important role in medicine for centuries, ever since human have walked the planet. Silver, zinc and copper compounds have been used for years as antimicrobial agents. It has recently been shown that some metals could be useful in the treatment of malaria and Chaga's disease. One of the most virulent strains, Plasmodium falciparum has become resistant to the major antimalarial drugs such as quinolones. In the search for drugs that were highly selective against the quinolone-resistant strains, a number of metal (Ga(III), Al(III), Fe(III), Au(I), Au(III) complexes were found (Goldberg et al., 1997, Harpstrite et al., 2003). Chaga's disease is another parasitic disease currently being treated by a number of organic drugs which require high doses easily leading to toxicity. Complexing the drugs with Ru(II) or Rh(I) were reported to increase the activity of the original drugs as much as 2-5 times (Sanchez del Grado et al., 1996).

Helycobacter pylori is an important human pathogen that colonises the stomach of about half of the world's population. The bacterium has now been accepted as the causitive agent of several gastrointestinal disorders, ranging from chronic active gastritis and peptic ulcer disease to gastric cancer. The anti-Helicobacter pylori of bismuth (III) is well established and it has been prescribed for the treatment of gastrointestinal ailments since 18th century. Colloidal bismuth subcitrate is currently used in combination with antibiotics to reduce enteric H. pylori colonization as a therapy of stomach ulcers. There are data that bismuth citrate and coloidal bismuth subcitrate may also reduce cecal colonization by Campylobacter jejuni in chickens (Farnell et al., 2006; Romano et al., 2006). Cobalt chloride was reported to possess marked activity against H. pylori (minimum inhibitory concentration range was 0.03 - 1 mg/L) without affecting other intestinal bacteria (Bruggraber et al., 2004).

In addition, different metals are used in the treatment of various pathological conditions such as rheumatoid arthitis (Au), anemia (Fe), depressive disorders (Li), wounds (Zn), gastrointestinal disorders (Al, Mg, Bi).Vanadium and chromium complexes have been shown to be insulin-mimetics (Howard-Lock, Lock, 1993; Guo et al., 1999; Srivastava, Mehdi, 2005).

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BO2

INVESTIGATIONS ON ANTIMICROBIAL ACTIVITY *IN VITRO* OF EIGHTEEN COPPER, COBALT, IRON AND NICKEL COMPLEXES WITH MANNICH TYPE LIGANDS

T. Popova[1], R. Alexandrova[2], R. Tudose[3], E.-M. Mosoarca[3], O. Costisor[3]

[1]*Faculty of Veterinary medicine, Forestry Technical University, Kliment Ohridsky 10 blvd, Sofia 1756, Bulgaria*

[2]*Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 25, Sofia 1113*

[3]*Institute of Chemistry Timişoara, Romanian Academy, 24, Mihai Viteazu Blvv., RO-300223, Timişoara, Romania*

In looking for efficacious antibacterial means in this study the *in vitro* antimicrobial activity of five compounds of copper, four cobalt complexes, five compounds of iron and four nickel complexes with ligands containing the antipyrine moiety N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP) or N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN) was evaluated against 43 pathogenic Gram-positive and Gram-negative bacterial strains, as well as control ones. The antifungal activity of the nickel complexes against four *Candida spp.* was established too. Thiamphenicol, the new broad spectrum antibiotic, analog of chloramphenicol, was also included in the experiments as a control. Investigations were performed by the routine agar-diffusion method of Bauer-Kirby and by the method of minimum inhibitory concentrations (MIC).

It was found that the compounds exhibited some antibacterial activity *in vitro* against the tested bacteria, although the established MICs were higher than these of thiamphenicol. The results obtained showed that the examined metal complexes with TAMEN have more pronounced antimicrobial properties than BAMP containing compounds. Highest inhibitory activity showed the respective copper complexes, followed by these of iron, nickel and cobalt. Most expressed sensitivity to the metal compounds manifested the examined Gram-positive microorganisms.

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BO3

INVESTIGATIONS ON ANTIMICROBIAL ACTIVITY *IN VITRO* OF NEWLY SYNTHESIZED METAL COMPLEXES WITH CHOLIC ACIDS

T. Popova[1], R. Alexandrova[2], D. Culita[3], L. Patron[3]

[1]*Faculty of Veterinary Medicine, Forestry Technical University, Kliment Ohridsky 10 blvd, Sofia 1756, Bulgaria*

[2]*Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 25, Sofia 1113, Bulgaria*

[3]*Institute of Physical Chemistry "I. G. Murgulescu", Spaiul Independentei 202, Sect. 6, 060021 Bucharest, Romania*

In the study presented here we report data about the *in vitro* effect of newly synthesized complexes of Zn (II), Cu (II), Co (II) and La (III) with cholic, dehidrocholic and lithocholic acids was examined in Gram-positive and Gram-negative pathogenic bacteria. The antifungal activity of some of the complexes against *Candida spp.* was established too. The experiments were carried out by the routine method of Bauer-Kirby and by determination of minimum inhibitory concentrations (MIC). Weak antimicrobial activity of the compounds was established *in vitro*. More pronounced inhibitory effect manifested the complexes with lithocholic acid and weak – these with cholic acid. The antimicrobial activity of the copper complexes was a little bit better than those of the zinc complexes. Most sensitive to the examined compounds were found to be the Gram-negative bacteria, especially the strains of *P. aeruginosa.*

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BO4

PLATINUM(II) AND PALLADIUM(II) COMPLEXES OF PYRIDINE-2-CARBALDEHYDE THIOSEMICARBAZONE WITH PROMISING ANTI-HERPES SIMPLEX VIRUS EFFECT

P. Genova[1], T. Varadinova[1], D. Kovala-Demertzi[2], P. Souza[3], M. Demertzis[2]

[1]*Laboratory of Virology, Faculty of Biology, Sofia University, 8 Dragan Tzankov Blvd., 1164 Sofia, Bulgaria. E-mail: petia.d.genova@abv.bg;*

[2]*Inorganic and Analytical Chemistry, Department of Chemistry, University of Ioannina, Ioannina, Greece;*

[3]*Departamento de Química Inorgánica, Facultad de Ciencias, C/ Francisco Tomás y Valiente 7, Universidad Autónoma de Madrid, 28049 Madrid, Spain.*

The cytotoxicity and the antiviral activity of Pd(II) and Pt(II) complexes with pyridine-2-carbaldehyde thiosemicarbazone (HFoTsc) against HSV replication was evaluated on four HSV strains – two *wt* strains Victoria (HSV 1) and BJA (HSV 2) and two ACV^R mutants with different *tk* gene mutations R-100 (TK^A, HSV 1) and PU (TK^N, HSV 2). The experiments were performed on continuous MDBK cells and four HSV 1 and HSV 2 strains were used, two sensitive to acyclovir and two resistant mutants. The five complexes of HFoTsc, [Pt(FoTsc)Cl], $[Pt(FoTsc)(H_2FoTsc)]Cl_2$, $[Pt(FoTsc)_2]$, $[Pd(FoTsc)(H_2FoTsc)]Cl_2$ and $[Pd(FoTsc)_2]$, were found to be effective inhibitors of HSV replication. The most promising, active and selective, anti-HSV agent able to overcome the existed to ACV resistance was found to be the complex $[Pt(FoTsc)(H_2FoTsc)]Cl_2$. PCR study of immediate early 300bp ReIV Us1 region reveals that the complex $[Pt(FoTsc)(H_2FoTsc)]Cl_2$ specifically suppressed *wt* HSV-1 genome 2h after the infection,

not inducing apoptosis/necrosis on the 8h after virus infection and the target was found to be the viral but not the host cell DNA.

BP1

STUDY ON ANTIBACTERIAL ACTIVITY IN VITRO OF TWO BASIC SALTS OF ZINC AND COPPER

T. Popova[1], R. Alexandrova[2], M. Gabrashanska[2], S. Tepavitcharova[3]

[1]Faculty of Veterinary Medicine, Forestry Technical University, Kliment Ohridsky 10 blvd, Sofia 1756, Bulgaria;

[2]Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 25, Sofia 1113, Bulgaria

[3]Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 11, Sofia 1113, Bulgaria

Copper and zinc are essential elements possessing broad spectrum of biological activities. A wide variety of zinc and copper compounds have been proved to possess antiviral and/or antibacterial properties. In the study presented here we report the data about the *in vitro* effect of two basic salts of copper and zinc [$CuCO_3Cu(OH)_2.nH_2O$ and $Zn_{5-x}Cu_x(OH)_8Cl_2.H_2O$] on 50 Gram-positive and Gram-negative bacterial strains. The wide spectrum antibiotic gentamicin was also included in the experiments as a control. The investigations were performed by the method of minimum inhibitory concentrations (MICs) and agar diffusion method of Bauer-Kirby. The results obtained revealed that both metal compounds express antibacterial activity *in vitro* although the established MICs were higher than these of gentamicin.

BP2

ANTIHERPES ACTIVITY OF THIOSEMICARBAZONES AND THEIR METAL COMPLEXES

M. Gatzovska[1], N. Vilhelmova[1], S. Shishov[1], T. Varadinova[1] and P. Souza[2]

[1] University of Sofia "St Kliment Ohridski", Sofia 1164, Bulgaria

[2] Universiad Aoutonoma de Madrid, Madrid 28049, Spain

Thiosemicarbazones are large group of organic molecules with potencially beneficial biological activities. They are effective inhibitors of ribonukleotide reductase (RR) and α-(N)-heterocyclic carboxaldehyde are among the most effective RR inhibitors yet identified. In some cases the highest activity of TSC-s is associated with a metal complexes. Metals play essential role in regulation of many processes, they can also increse the biological activity of organic drugs. Metallic complexes of thisemicarbazone derivates have antibacterial, antimalaric, antiviral and antitumor propertis. They are with prove activity against HSV. We tested the effect of two bisthiosemicarbazone - H_2L^1 и H_2L^2 and theirs complexes with Zn(II), Pd(II) and Cd(II) on HSV-1 (Victoria) and HSV-2 (BJ) in cell culture MDBK (Madin Darby bovine kidney). We determined maximal nontoxic concentration (MNC) as well as concentration required to inhibit cell viability by 50% (CC_{50}) of compounds tested. The complexes with Zn(II) and Pd(II) are with the same value of MNC such as their ligands - $1x\ 10^{-8}$. The cadmium complexes in respect to cell viability are the most cytotoxic: Cd_2/H_2L^1 - MNC= $1x10^{-10}$ μM and Cd/H_2L^2 - MNC= $1x10^{-9}$ μM.

The ligands and their zinc complexes express activity against HSV-1 and HSV-2. Toward strain Victoria Zn_2/H_2L^1 is the most active with effective concentration required to inhibit virus yield by 50% (IC_{50})- $0.001x10^{-8}$ μM. The ligands and Zn/H_2L^2 indicate same activity- IC_{50}= $0.01x10^{-8}$ μM. Against strain BJ the strongest effect exhibit H_2L^2 and Zn_2/H_2L^1- IC_{50} = $0.001x10^{-8}$ μM both. IC_{50} of H_2L^1 and Zn/H_2L^2 have value $0.01x10^{-8}$ μM. The most highly selectivity toward both strains have H_2L^1 и Zn_2/H_2L^1.

Session C.
PARASITES AND METALS

Chairpersons:

Assoc. Prof. Yana Mizinska-Boevska (Institute of Experimental Pathology and Parasitology, BAS)

Assoc. Prof. Iglika Nedeva (Institute of Experimental Pathology and Parasitology, BAS)

CO1

ANTIOXIDANT STATUS IN HELMINTHOSES AFTER TREATMENT WITH NEW METAL COMPOUNDS

M. Gabrashanska[1], M. Anisimova[1], S. Tepavitcharova[2], J. Manga Gonzalez[3], Y. Misinska[1], S. Ermidou-Pollet[4], S. Pollet[4]

[1]*Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str. Bl. 25, 1113 Sofia, Bulgaria.*
[2]*Institute of General and Inorganic Chemistry, Bulgarian Academy of Science, Sofia*
[3]*Estacion Agricola Experimental, CSIC,Fca Marzanos, 24 346 Grulleros, Leon, Spain*
[4]*Medical School, University of Athens, Mikras Asias Str. 73, Athens, Greece*

The parasitic diseases are widespread and cause large economic damages in the domestic animals and plants. Mineral substances play an important role in the metabolic disturbances of parasitoses.

The aim of our study was to investigate the effect of newly synthesized compounds of Zn, Mn, Co and Cu on the antioxidant (vitamin C and E, Zn, Co, Mn Cu, SOD and CAT) and physiological (mortality and body weight gain) status in the infected with helminths host. Investigated experimental host-parasite systems were chickens infected with *Ascaridia galli* (Nematoda) and rats infected with *Fasciola hepatica* (Trematoda). Newly synthesized compounds were: $(Zn_xCu_y)_2(OH)_3Cl$; $(Zn_xMn_{1-x})_4(OH)_6SO_4.2H_2O$; $(Zn_xCo_yCu_{1-x-y)4}(OH)_6.SO_42H_2O$ and $(Zn_xCo_yMn_{1-x-y})_4(OH)_6.2H_2O$.The levels of the both vitamins, trace elements and SOD activity were reduced in the livers in the infected hosts. Developed antioxidant imbalance in the host was depend on the parasite and host species, parasite localization and toxicity. The application of $(Zn_xCu_y)_2(OH)_3Cl$ and $(Zn_xCo_yMn_{1-x-y})_4(OH)_6.2H_2O$ influenced positively the antioxidant imbalance and restored body weigh losses and reduced mortality. The rest salts did not show any effect on the infected host. Newly synthesized salts could be used for a control of helminthoses by improvement of the antioxidant defense system of the host.

CP1

ANTIOXIDANT STATUS IN CHICKENS INFECTED WITH PARASITES AND TREATED WITH A NEW ZINC COMPOUND

M .Gabrashanska [1], V. Koinarski [2], S. Ermidou-Pollet [3], S. Pollet [3], N. Georgieva [2]

[1] Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria;
[2] Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria;
[3] Medical School, University of Athens

The effect of $2Gly.ZnCl_2.2H_20$ compound on the antioxidant status in chickens infected with *Eimeria acervulina* (Protozoa) or *Ascaridia galli* (Nematoda). Antioxidant status was measured via determinations of blood plasma malonyl dialdehyde (MDA) reactive products, activities of enzymes superoxide dismutase (SOD) and catalase (CAT), concentrations of carotene, vitamin A, C, E and Zn.

The results showed increased plasma MDA and CAT-activity, decreased SOD-activity, reduced vitamins and Zn concentrations in chickens infected with *E. acervulina* or *A.galli*. Developed antioxidant imbalance was better expressed in chickens with eimeriosis than those with ascaridiosis. $2Gly.Zn.Cl_2.2H_20$ oral administration almost restored vitamin E and Zn losses, as well as reduced CAT-activity but SOD-activity, vitamin C and A, carotene and MDA levels were not statistically changed.

Economical parameters (body weight gain and mortality) in the infected chickens were checked too. They were correlated with the developed oxidative stress due to the infections. $2Gly.ZnCl_2.2H_20$ increased BWG and reduced mortality in the both infections.

CP2

MODULATION OF LIVER ANTIOXIDANT ABILITY UNDER CHRONIC FASCIOLIASIS AND $(Zn_xCu_{1-x})_2(OH)_3Cl$ SUPPLEMENTATION

M. Gabrashanska[1], J. Manga Gonzalez[2], Y. Misinska[1], S. Ermidou-Pollet[3], S. Pollet

[1] Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str. Bl. 25, 1113 Sofia, Bulgaria
[2] Estacion Agricola Experimental, CSIC, Fca Marzanos, 24 346 Grulleros, Leon, Spain.
[3] Medical School, University of Athens, Mikras Asias Str. 73, Athens, Greece

The aim of the study was to assess the antioxidant status (vitamin C, E, A, zinc, copper, catalase, superoxide dismutase, glutathione peroxidase and malondialdehyde) in chronic stage of fascioliasis after treatment with Zn-Cu mixed basic salt.

White Wistar rats were infected per os with 25 metacercariae of *Fasciola hepatica*. Application of $(Zn_xCu_{1-x})_2(OH)_3Cl$ salt started 6 weeks after the infection and was performed during 10 consecutive days.

During the chronic stage of fascioliasis in the liver, a decrease of concentrations of vitamins C, E, A and of the trace elements Zn and Cu were observed together with a reduction of the Cu,Zn-SOD activity and an increase of CAT and GPX activities. MDA concentration was elevated.

Application of $(Zn_xCu_{1-x})_2(OH)_3Cl$ salt increased the concentrations mainly of vitamin E, but also of vitamin C and of the trace element Zn. On the other hand, only the activity of SOD was enhanced after salt treatment. It seems that antioxidant imbalance was developed in the *Fasciola hepatica*

infected liver rats during the chronic fascioliasis and that Zn-Cu salt improved the antioxidant defence abilities.

Acknowledgments: The study was supported by the Spanish-Bulgarian Scientific Cooperation (CSIC-BAS) – 2005BG-Sp 008.

CP3

ANTIOXIDANT STATUS IN *ASCARIDIA GALLI* INFFECTED CHICKS TREATED WITH DOUBLE ZINC-COPPER SALT

M.Gabrashanska[1], M. Galvez-Morros[2], N. Tsocheva-Gaytandzieva[1], S. Pollet[3], S. Ermidou-Pollet[3], M. Mitov[1]

[1]Institute of Experimental Pathology and Parasitology, BAS, Bulgaria
[2]Facultad de Ciencias Quimicas, UCM, Spain
[3]University of Athens, Greece

Antioxidants status (vitamin C and E, copper and zinc) was investigated in the liver of male chicks (un)infected with *Ascaridia galli* and (un)treated with a new symthesized double basic salt. The content of vitamin C was determined spectrophothometrically and that of vitamin E – by fluorimetric method. Levels of Zn and Cu were established by an atomic absorption spectrophothometry. The levels of vitamin C and E, as well as Zn and Cu were reduced in *A.galli* infected chicks. The contents of liver Zn and Cu were significantly increased and that of vitamin E and C were slightly elevated in the infected chicks and controls after the salt supplementation. The salt had the beneficial effect on the body weight gain of the control and infected chicks. The mortality in the treated groups (controls and infected) was decreased Parasite burden was reduced in the infected chicks received the salt. The addition of the double basic salt of Zn and Cu enhanced the antioxidant defence system and the performance in *A.galli* infected chicks.

CP4

INVESTIGATIONS OF THE NH_4VO_3 EFFECT ON GALLING CAUSED BY *MELOIDOGYNE ARENARIA*

D. Salkova[1], O.Baicheva[1], Y. Mizinska-Boevska[1], A. Damianova[2], K. Georgieva[1]

[1]Institute of Experimental Pathology and Parasitology, BAS, Bulgaria
[2]Institute of Nuclear Research and Nuclear Energy, BAS, Bulgaria

The aim of present investigations was to study the effect of NH_4VO_3 on the gall formation caused by root parasitic nematodes belonging to Meloidogyne Göldi, 1887. As a model of our experiments the parasite – host system " Meloidogyne arenaria – Tiny Tyn tomato plants" was chosen. The experiments were carried out under laboratory conditions. Different concentrations of NH_4VO_3 were tested *in vivo* and *in vitro* . On the base of the results the experiment doses for treatment of plants were chosen.

The influence of the NH_4VO_3 was investigated on the life cycle of " Meloidogyne arenaria – hatching of invasive second stage larve (J2), duration of the life cycle, and formation of the developing stages, egg production and gall formation. The effect of the NH_4VO_3 on the structure of the galls was examined on semi tin sections. The changes of the microelement balance of the host as well as the content of vanadium in the plant organs were determined by means of AAS.

In vitro experiments the effect of NH_4VO_3 on the growth and the development of the plants and on the biomasses of the host was studied.

The different methods use made possible a complex estimation of the NH_4VO_3 effect on the parasite and on the host within the framework of experiment.

The result obtained showed that given concentrations of NH_4VO_3 are non toxic for the invaded host and they have pressure effect on the development of the helminthes and limit the pathological transformation of the invaded root tissues which is a cause for gall formation and destructions of the root.

The investigations connected with the physiological role of vanadium are very scanty. As exception there are some data in respect to the insulin – like influence of vanadium, its antitumor activity and effect on some enzymes of vertebrates. That is why we have not possibility to interpret our results in comparative aspect.

The results of the investigations carried out will be a base for further studies. We hope that they will be a contribution to understanding of the specific biological mechanisms and role of vanadium in the system " plant-parasitic nematode-plant host".

Session D.
METAL TOXICITY AND CANCEROGENESIS

Chairpersons:

Assoc. Prof. Reni Kalfin (Institute of Experimental Pathology and Parasitology, BAS)

Assoc. Prof. Anna Damianova (Institute of Nuclear Research and Nuclear Energy, BAS)

DO1

METAL TOXICITY AND CANCEROGENESIS - A BRIEF OVERVIEW OF LITERATURE DATA

R. Alexandrova

Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., Bl. 25, Sofia 1113, Bulgaria, rialexandrova@hotmail.com

Virtually all metals ate toxic it they are ingested in large enough quantities. Human and animal exposure to metals occurs in many diverse circumstances, including environment, therapeutic use, industry, accidents. The following factors affect the exposure of individuals to particular metals: element (atomic radius, valency, mobility, hydration), chemical form (organic, inorganic, oxidation state), compound (sulphide, oxide, salts), physical form (dust, fume, solubility), concentration (high, low, seasonality), duration (acute, chronic, variable), interactions with other metals/compounds (bioavailability), route of entry (oral, inhalation, skin), geography (wind direction, water run-off, distance from source), food chains (plant uptake, agriculture, fishing). Whereas micronutrients (like iron, zinc, copper, cobalt) are toxic if excessive amounts of them are ingested, many other metals (such as cadmium, lead, mercury, etc.) are always toxic if they are ingested. In addition several metals and metal containing compounds have been proved to ve potent mutagens and carcinogens. Although each of them has its own mechanism of action, it is believed that generation of reactive oxygen species (ROS) is of great importance. Metal mediated formation of free radicals causes various modifications to DNA bases, rnhanced lipid peroxidation. and altered calcium and sulfhydryl homeostasis. Lipid peroxides, formed by the attack of radicals on polyubsaturated fatty acid residues of phospholipids can further react with redox metals finally producing mutagenic and carcinogenic products (malondialdehyde, 4-hydroxynonenal, etheno and/or propano DNA addusts). Iron (Fe), copper (Cu), Chromium (Cr), vanadium (V) and cobalt (Co) undergo redox-cycling reaction. For a second group of metals, mercury (Hg), cadmium (Cd) and nickel (Ni), the primary route for their toxicity is depletion of gluthatione and bonding to sulfhydryl groups of proteins. Arsenic (As) activity on cell metabolism is multiple - there are data that cell transformation is induced by long-term exposure to a low level of arsenic. As is thought to bind directly to critical thiols, however, other mechanisms, involving formation of hydrogen peroxide under physiological conditions, have been proposed. Nitric oxide (NO) seems to be involved in arsenic-induced DNA damage.

The body reacts to an intake of metal by several defense mechanisms including sequestration by metallotheonein, formation of nuclear inclusion bodies, biotransformation.

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DO2

FISH PARASITES AS BIOINDICATORS OF HEAVY METALS

M. Gabrashanska

Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. Geogi Binchev Str., Bl. 25, Sofia 1113, Bulgaria

DP1

COPPER-RELATED DISEASES

R. Alexandrova[1], R. Kalfin[2], E. Leventieva-Necheva[2]

[1]Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., Bl. 25, Sofia 1113, Bulgaria, rialexandrova@hotmail.com
[2]Institute of Physiology, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., Bl. 23, Sofia 1113, Bulgaria

Copper is an essential metal with broad spectrum of key biological activities. Most mammals have efficient transport systems to prevent accumulation of excess copper in cells and tissues. Relatively small amount of this metal is stored in the body, compared with other trace elements such as zinc and iron, and the adult body usually contains < 100 mg Cu. Chronic copper toxicity is rare and primarily affects the liver. In long-term copper toxicity, both brain and kidney are also involved. Two genetic disorders of copper metabolism are described – Wilson's disease and Menkes' disease. Resent studies have also implicated copper in the pathogenesis of neuronal injury in Alzheimer's disease and the prion-mediated encephalopathies. There are data that copper excess could be also involved in cancerogenesis.

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DP2

MANAGANESE AND PARKINSON'S DISEASE

R. Kalfin[1], M. Lazarova[1], R. Alexandrova[2]

[1]*Institute of Physiology, Bulgarian Academy of Sciences;*
[2]*Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences*

Manganese (Mn) is an essential metal, widely distributed in the environment - it is the 12th most common element in the Earth's crust and the 4th most widely used metal in the world. Manganese is employed in the manufacture of steel, where it enhances hardness of the metal. Manganese is also used in the manufacture of batteries, in water purification, in bactericidal and fungicide agents, and as an antiknock agent in petrol. Manganism or manganese poisoning is a toxic condition resulting from chronic exposure to manganese. Neurotoxicity of Mn has been observed among miners and industrial workers who had been exposed to high levels of Mn. Manganism occurred also in agricultural workers, due to exposure to Mn-based pesticides such as the fungicides Maneb (Mn ethylene-bis-dithiocarbamate) and Mancozeb (Mn Cu Zn ethylene-bis-dithiocarbamate).

It has been shown that overexposure to Mn plays an important role in causing Parkinsonian disturbances, possibly by enhancing physiological aging of the brain. An increased amount of Mn may cause neurodegeneration in the basal ganglia, generating a pre-Parkinsonian condition. The clinical features of Mn neurotoxicity resemble those of idiopathic Parkinsonism, including the presence of generalized bradykinesia and widespread rigidity. Nevertheless, a careful analysis of Mn poisoning cases have shown clinical and pharmacological differences with Parkinson's disease patients mainly as follows: (i) less frequent resting tremor, (ii) more frequent dystonia, (iii) a particular tendency to fall backwards, (iv) failure to achieve a sustained therapeutic response to levoDOPA, and (v) failure to detect a reduction in fluoroDOPA uptake by positron emission tomography. It is also to be considered that a number of necropsy studies carried out on Mn-induced Parkinsonism have shown degenerative lesions of the globus pallidus and subthalamic nucleus, caudate nucleus, and putamen, with less frequent lesions of the substantia nigra. These findings demonstrate that in contrast to Parkinson's disease, which preferentially damages dopamine neurons in the substantia nigra, Mn is likely to be accumulated within and to damage the pallidum and striatum, while refrains from destroying the nigrostriatal system.

In summary, although Parkinson's disease is one of the most common neurodegenerative disorders in adult individuals, its etiology is still unknown and the hypothesis of an interaction between environmental factors and individual genetic susceptibility, both acting on normal aging, has to be considered. Manganese exposure can definitely play an important role in determining Parkinsonian disturbances. However, there are several differences between manganism and Parkinson's disease. Current evidences indicate that Mn-induced Parkinsonism can be differentiated from Parkinson's disease because of its preference to accumulate in and damage the pallidum and striatum rather than the substantia nigra. It is also important to consider that Mn poisoning is the result of exposure to very high doses of Mn, while prolonged exposure to lower levels of Mn may act differently, and enhance the onset of Parkinsonian disturbances.

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DP3

TOXIC EFFECTS OF IRON IN ORGANISMS

R. Alexandrova[1], R. Kalfin[2], K. Jangyozova[2]

[1]Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 25, Sofia 1113, Bulgaria; rialexandrova@hotmail.com
[2]Institute of Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 23, Sofia 1113, Bulgaria

It is well known that iron is essential for fundamental cell functions, such as DNA synthesis, transport of oxygen and electrons, and cell respiration. The content of body iron is regulated primarily by absorption since humans have no physiological mechanism by which excess iron is excreted. This regulation, however, is not absolute. Many factors such as the content of diets, iron loses, life style, etc. influence iron absorption. There is very little iron excreted under normal conditions. While iron deficiency is a worldwide problem, the risk of inducing iron overload in the general population is low. Genetically determined diseases that may lead to siderosis, such as hereditary haemochromatosis or thalassaemia major, show a limited geographic and ethnic distribution At the same time we have to emphasize that excess body iron can be highly dangerous because the metal is an effective catalyst in the free radical reactions. The resulting oxyradicals have the potential to damage cellular lipids, nucleic acids, proteins, and carbohydrates leading to wide-ranging impairment in cellular function and integrity. High tissue iron concentrations have been associated with the development and progression of several pathological conditions, including certain cancers, liver and heart disease, diabetes, hormonal abnormalities, and immune system dysfunctions. Brains from patients with Alzheimer disease show a disruption in the metabolism of iron, such that there is an accumulation of iron in senile plaques, and an altered distribution of iron transport and storage proteins. It has been suggested that iron loading is among the risk factors for sudden infant death syndrome.

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DP4

NICKEL TOXICITY AND CANCEROGENICITY

R. Alexandrova, D. Teofanova, M. Gabrashanska

Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., Block 25, Sofia 1113, Bulgaria

Nickel is an essential trace element for many species. In animals nickel deficiency has been associated with depressed growth, reduced reproductive rates, alterations in glucose and lipid

metabolism. The element is also important for proper functioning of the immune system. On the other hand, human exposure to highly nickel-polluted environment, such as those associated with nickel refining, electroplating, and welding, has the potential to produce a variety of toxic effects. People working in nickel refineries or nickel-producing plants have experienced chronic bronchitis and reduced lung function. Workers who drank water containing high amounts of nickel had stomachaches and suffered adverse effects to their blood and kidney. Pathological alterations of nickel metabolism are recognized in several human diseases. Almost all cases of acute nickel toxicity result from exposure to nickel carbonyl. Nickel is a common sensitizing agent with a high prevalence of allergic contact dermatitis. The metal is also reported to induce embryotoxic, teratogenic and cancerogenic effects. Almost everyone in the industrially developed countries may be in daily contact with nickel. The metal can be transferred from the mother to an infant in breast milk and can cross the placenta. nickel compounds express antineoplastic activity in vitro and in vivo.

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DP5

HEAVY METALS IN FISH-PARASITE SYSTEMS FROM THE DANUBE RIVER (BULGARIAN AND SERBIAN PARTS)

M.Gabrashanska[1], I. Nedeva[1], P. Cakic[2], M. Galvez-Morros[3], E.Karaivanova[1], G. Atanassov[1], M. Lenhardt[2]

[1]Institute of Experimental Pathology and Parasitology, BAS, Bulgaria
[2]Institute for Biological Research "Sinisa Stankovic", Serbia
[3]Facultad de Ciencias Quimicas , UCM, Spain

Concentrations of heavy metals (Cu, Zn, Cd, Pb and Mn) in fish tissues from *Barbus barbus* and *Siluris glanis* and their parasites – acanthocephalan *Pomphorhynchus laevis* and nematode *Eustrongylides excisus* were analysed by atomic absorption spectrophothometry. The studies were done in two points in the Danube River in comparison – Archar (Bulgaria) and Prahovo (Serbia). The amount of heavy metals was generally higher in Acanthocephalans than in their hosts (fish) from the both points. The metal levels in *E. excisus* were above than in their hosts. Acanthocephalans may serve as very sensitive and useful indicators for available heavy metals, mainly Pb and Cd., in aquatic ecosystems. Nematodes could not serve for this purpose. Based on these data there were no significant differences in heavy metal concentrations from the both sites in the River Danube.

Acknowledgments: The study was supported by Bulgarian-Serbian Scientific Cooperation (Bulgarian Academy of Sciences – Serbian Academy of Sciences).

DP6

COMPARARTVE INVESTIGATION ON MICROELEMENT LEVELS IN SAMPLES FROM SOIL, WATER, FODER AND INTERNAL ORGANS OF WILD ANIMALS

A. Daskalova[1], M. Gabrashanska[2]

[1]National Research Station for Wildlife Management, Biology and Pathology, blvd. "Iskarsko shousse" 5 , 1528 Sofia, Bulgaria
[2]Institute for Experimental Pathology and Parasitology, BAS, 1113 Sofia, Bulgaria

Research on the levels of microelements copper, iron, manganese, zinc, cobalt, molybdenum, lead and cadmium in samples from soil, water and green vegetation, as well as from kidney and liver of cloven-footed wild species such as follow deer, red deer and wild boar were performed. The aforesaid investigation covered four stations set for intensive game breeding in Bulgaria. The microelement levels were established using an atomic absorption spectrophotometry. The selection of studied objects was carried out keeping in mind the requisition for optimal interconnection between them, i.e. vegetation and water samples are from places used by wild animals for pasture and drinking pool, and soil ones – from game's usual residence. The results obtained demonstrated that examined objects are indicative for ecological conditions in game stations and the outcome illustrate lack of toxic contaminants in the environment.

Some distinctions in microelement levels in soil, water and green vegetation between game stations, as well as among different biotops of the same station were established. Correlation was found between levels of mineral substances in all four kinds of objects investigated that diluted in the chain water – soil – vegetation – wild animals

DP7

CHANGES IN CHEMICAL CONTENT OF WATER PLANTS (FONTINALIS ANTIPYRETICA) DUE TO THE VARIATIONS OF CU CONCENTRATION IN THE LOCAL ENVIRONMENT

A. Damianova[1], I. Sivriev[1], N. Lihareva[2]

[1]Institute for Nuclear Research and Nuclear Energy, Bulgarian Academy of Sciences, 72 blvd. Tzarigradsko Shousse, Sofia, Bulgaria
[2]Central Laboratory for Mineralogy and Crystalography, Bulgarian Academy of Sciences, str. G.Bonchev, bl. 107, Sofia, Bulgaria

In the study of global atmospheric changes one of the main tasks is the evaluation of the adverse factors on life. The bryophytes Fontinalis antipyretica is among the most recommended species as biomonitors for their high bioaccumulation rate and large enrichment to different metals. They are particularly sensitive to cooper and cadmium.

The aim of this study was to enhance our knowledge about the processes related to the biological response on the stress due to the relatively high doses of Cu in the water environment. Aquatic bryophytes F.antipyretica collected from Rila mountain rivers were exposed in the laboratory conditions to increasing concentrations of Cu in the water medium.

The bioaccumulation of Cu and the physiological stress response effect on the element content of the bryophytes have been studied.

The results obtained show dynamic in the Cu accumulation in the plants with the time. The accumulation of Cu reach a constant high level in two weeks time independently of the concentrations used. As a results of this accumulation a disbalance with the time in the content of Cu, Mn, Mg, Fe etc. in plants is observed.

Session E.
NEWLY SYNTHESIZED COMPOUNDS

Chairpersons:

Prof. Luminita Patron (Institute of Physical Chemistry, Bucharest, Romania, RA)

Assoc. Prof. Stefka Tepavitcharova (Institute of General and Inorganic Chemistry, BAS)

EO1

DESIGN OF COORDINATION COMPOUNDS WITH POTENTIAL BIOLOGICAL ACTIVITY

L. Patron

Institute of Physical Chemistry "Ilie Murgulescu", Splaiul Independentei 202, 060021 Bucharest, Romania

In the last years, the coordination chemistry has aroused a great interest owing to their applications in biology and medicine. It is well known that the transition metal ions (named – also – essential microelements) are involved in the synthesis and the degradation of biological molecules, in the substitution of functional groups, as oxygen carriers, in the cell redox reactions, etc. To better understand these processes, to find some simple models to explain the behaviour of the biological metal containing systems, an enormous number of chelate complex compounds are synthesized.

The study of the synthetic chelate complex compounds led to the development of the "metal terapy" – now many of these compounds were confirmed as drugs.

We began to work in this fantastic field, thirty years ago. From these years till now, we studied three classes of complex compounds defined after the chelate agent:

- The aminoacid complex compounds;
- The biguanide complec compounds;
- The bile acid complex compounds.

Some aspects about the chemical and physico-chemical properties of these compounds are presented.

EO2

PHYSICO CHEMICAL CHARACTERIZATION AND THEORETICAL STUDIES ON THE MAGNETITE NANOPARTICLES COATED WITH AMINOACIDS, BILE SALTS AND VARIOUS TYPES OF DEXTRAN

D. C. Culiță

Institute of Physical Chemistry "Ilie Murgulescu", Splaiul Independentei 202, 060021 Bucharest, Romania

Magnetite nanoparticles coated with aminoacids, bile salts and various types of dextran were synthesized and characterized by XRD, FTIR, TEM, Mossbauer measurements and elemental analysis. Magnetic properties such as magnetization, ZFC and FC and blocking temperature have been investigated. A theoretical study of the consistant experimental results was performed using

QSPR (Quantitative Structure Property Relationsheep). According with these studies, the synthesized nanoparticles seem to be organized into a core shell system, where the inner core is formed from unit cells of magnetite. A way to control the self assembly and the physical properties of the synthesizsed nanoparticles consists in their correlation with descriptors like chemical structures of the biomolecules. Using quantum chemical as well as the other simplest original descriptors, it was found a relationsheep between the used aminoacids and the magnetization, nanoparticles diameter, magnetic core diameter.

A correlation between the molecular weights of the various types of dextran and the coating degree of the magnetite was performed.

EO3

POLY(ALKYLCYANOACRYLATE) NANOPARTICLES – A FEASIBLE DRUG CARRIERS OF ANTICANCER DRUGS

M. Simeonova

Institute of Polymers, Bulgarian Academy of Sciences , Acad. Georgi Bonchev Str., bl. 103-A, Sofia 1113, Bulgaria

EP1

AB INITIO THEORETICAL STUDY ON CU(II) COMPLEX COMPOUNDS WITH ANIONS OF BILE ACIDS AS LIGANDS

J. Weinberg[1], D. C. Culita[1], L. Patron[1], R. Alexandreva[2]

[1]*Institute of Physical Chemistry "Ilie Murgulescu", Splaiul Independentei 202, 060021 Bucharest, Romania*

[2]*Institute of Experimental Pathology and Parasitology, Acad. Georgi Bonchev Str., bl. 25, Sofia 1113, Bulgaria*

Ab initio theoretical study was performed on the Cu(II) complex compounds with anions of bile acids as ligands: $CuL_2 \cdot nH_2O$, where L = $C_{24}H_{39}O_5^-$ (anion of the cholic acid); $C_{24}H_{33}O_5^-$ (anion of the dehydrocholic acid); $C_{24}H_{39}O_3^-$ (anion of the lithocholic acid).

The calculations were made using G03 program at 3-21g* and 6-31g* levels at Ecole Nationale Superieure de Chimie de Montpellier. Geometry optimization of the complex compounds of Cu(II) with the anions of the three bile acids, at stationary point, lead to a tetraedric configuration of the Cu(II) ion. Among these compounds, the lowest formation energy corresponds to the complex with cholate anion as ligand. It can be observed that HOMO orbital is localized and extended in complex only on the ligand which functions as monodentate. In case of the LUMO orbital, if on the free acids it is localized around of the carboxyl group, in the complexes it is localized around the Cu(II) ion and its bonds with the ligands.

EP2

SYNTHESIS OF MAGNETITE NANOPARTICLES IN THE PRESENCE OF AMINOACIDS AND BILE SALTS

G. Marinescu[1], D. C. Culita[1], L. Patron[1], I. Balint[1], N. Stanica[1], L. Bessais[2], C.B. Cizmas[3]

[1] *"Ilie Murgulescu" Institute of Physical Chemistry, Spl. Independentei 202, Bucharest 060021, Romania;*

[2] *LCMTR, UPR-209 CNRS, 2/8 Rue Henri Dunant, B. P. 28, F-94320 Thiais, France;*

[3] Department of Physics, Transilvania University, B-dul Eroilor 29, Brasov 50036, Romania

A new synthesis route to prepare magnetite nanoparticles by the decomposition of polynuclear coordination compounds in only one step is described. The precipitation of magnetite is performed in the presence of aminoacids and bile salts. The experimental protocol is original. The polynuclear coordination compound precursors containing as ligands: proline, tryptophan, histidine, tyrosine and cholate anion and the nanomagnetites are characterized by XRD, FTIR, TEM and SQUID magnetometry at low temperature. The samples are pure Fe_3O_4 without impurity phases and they have the characteristic of bulk magnetite. Crystallite phase and the broad peaks suggest the nanocrystallite nature of magnetite particles. The average diameter of particles estimated from XRD and TEM is 7-12 nm. The IR spectra recorded on the modified nanomagnetites were shown the presence of carboxylic groups onto the surface of these particles. In order to evidence the superparamagnetic properties of our samples the FC and ZFC thermomagnetic analyze at 100 Oe were performed. The blocking temperature (T_B) demonstrates that for $T > T_B$ is possible to assume superparamagnetic behavior. For example T_B is 166 K or 142 K for the proline and tryptophan samples. The values of T_B can be discussed in terms of particle size distribution and inter-particle dipole-dipole interaction. If the distance between the particles is bigger, then the inter-particle dipole-dipole interaction is less, T_B is shifted to a lower temperature. Our results confirm this theory. The magnetization isotherms at 4 K and 293 K in magnetic field up to 9T were performed. The saturation magnetization at 4K for the proline and tryptophan is 70.6 Am^2/kg and 61.1 Am^2/kg reflect the nanostructural character of the samples. The QSPR analysis confirms the existence of the chemical bonds between the oxygen atoms from the aminoacid molecules in the layer and the iron ions situated at the margin of the magnetite core. Such Fe(magnetite) -O(aminoacid) chemical bonds may induce antiferromagnetic effects and consequently the lowering of the saturation magnetization.

EP3

SYNTHESIS AND STRUCTURAL CHARACTERIZATION OF IRON COMPLEXES CONTAINING MANNICH BASES AS LIGANDS

O. Costisor, R. Tudose, E.-M. Mosoarca, E.-F. Cosma

Institute of Chemistry Timisoara of Romanian Academy, Inorganic Chemistry Laboratory, 24 Mihai Viteazu Blvd, RO-300223, Timisoara, Romania

Mono and/or binycclear complexes of iron (II, III) with N,N'-bis-(antipyril-methyen)-piperazine (BAMP) and N'N-tetra(4-antipyrylmethyl)-1,2-diamonoethane (TAMEN) were synthesized. The complexes obtained were characterized by elemental analysis, electric conductivenes, electronic and IR spectroscopy. The molar conductivities values of the complexes demonstrated their behaviour as electrolytes type. Electronic spectra of the complex indicated the

environment of the central ion whereas the infrared spectra for the comples supported the coordination of the Mannich bases.

EP4

SYNTHESIS AND STRUCTURAL CHARACTERISTICS OF COPPER COMPLEXES WITH MANNICH TYPE LIGANDS

R. Tudose, E. M. Mosoarca, E. F. Cosma, O. Costisor

Institute of Chemistry Timisoara of Romanian Academy, Inorganic Chemistry Laboratory, 24 Mihai Viteazu Blvd, RO-300223, Timisoara, Romania

The synthesis and structural characteristics of mono- and/or binuclear complexes of copper (II) with N,N'-bis-(antipyril-methyen)-piperazine (BAMP) and N'N-tetra(4-antipyrylmethyl)-1,2-diamonoethane (TAMEN) were reported. Depending on the diamine conformation, the BAMP and TAMEN molecules act as a bis-bidentate or bis-tridentate ligands, respectively, leading binuclear complexes, and as tetradentate or hexadentate ligands, respectively, leading mononuclear compounds. The spectroscopic (IR and UV-Vis) and conductivity data of the complex compounds were presented.

EP5

POLY(BUTYLCYANOACRYLATE) NANOPARTICLES AS A DRUG DELIVERY SYSTEM OF 5-FLUOROURACIL DESIGNED FOR SKIN CANCER TREATMENT

M. Simeonova[1], P. Troyanova[2], M. Hadjikirova[2], R. Velichkova[1],Ch. Petkov[3]

[1]Bulgarian Academy of Sciences, Institute of Polymers, Sofia, Bulgaria
[2]National Hospital of Oncology, Sofia, Bulgaria
[3]Central Custom Laboratory, Sofi, Bulgaria

Poly(alkyl cyanoakrylate) nanoparticles have been developed as biodegradable drug carriers for targeting a drug at tissues, cells or subcellular compartments. This approach have been used to improve the efficiency of some cytostatics, which poor specificity causes a toxicological problem what seriously hampers the effective therapy. 5-fluoruracil (5-FU) is one of the most frequently used pyrimidine antimetabolites in the chemotherapy of cancer, including topical treatment of epidermal dysplasia.

We have investigated the capabilities of poly(butylcyanoacrylate) nanoparticles (PBCN) as drug delivery system of 5-FU in the local treatment of basal cell carcinoma. The results suggest that PBCN offer a possibility to improve the therapeutic index and frequency of topical 5-FU.

EP6

PBCN AS DRUG CARRIERS OF DOXORUBYCINE. EFFECT ON SOME MORPHOMETRIC AND HEMATOLOGIC INDICES OF HEALTHY MALE RATS

M. Simeonova[1], R. Velichkova[1], T. Chervenkov[2], D. Gorova[2], B. Galunska[2], D. Ivanova[2]

[1]Bulgarian Academy of Sciences, Institute of Polymers, Sofia, Bulgaria
[2]Medical University, Varna, Bulgaria

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