

THE SEVENTH WORKSHOP
“BIOLOGICAL ACTIVITY OF METALS, SYNTHETIC COMPOUNDS AND NATURAL PRODUCTS”

IS ORGANIZED BY THE INSTITUTE OF EXPERIMENTAL MORPHOLOGY, PATHOLOGY AND ANTHROPOLOGY WITH MUSEUM (IEMPAM)

UNDER THE AUSPICES OF

THE BULGARIAN ACADEMY OF SCIENCES

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**THE SEVENTH WORKSHOP “BIOLOGICAL ACTIVITY OF METALS,
SYNTHETIC COMPOUNDS AND NATURAL PRODUCTS”**

27 – 29 NOVEMBER, 2012

SOFIA, BULGARIA

Supported by

***European Social Fund and Republic of Bulgaria, Operational Programme
“Development of Human Resources” 2007-2013, Grant № BG051PO001-3.3.06-0048
from 04.10.2012.***

The Program of the Workshop

Tuesday, 27 November 2012

9.30 – 9.50 REGISTRATION
9.50 – 10.00 OPENING CEREMONY
10.00 -10.15 ANNOUNCEMENT FOR THE BEGINNING OF PROJECT
BG051PO001-3.3.06 – 0048 FROM 04.10.2012.

Session A.

Chairpersons:

Assoc. Prof. Radostina Alexandrova, PhD
*Institute of Experimental Morphology, Pathology and Anthropology with Museum,
Bulgarian Academy of Sciences*

Assoc. Prof. Rumiana Tsoneva, PhD
*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of
Sciences*

Secretary: Lora Dyakova
Institute of Neurobiology, Bulgarian Academy of Sciences

10.15 – 10.30

**AO1. THE EFFECT OF ERUFOSINE ON CYTOSKELETON
REORGANIZATION AND CELL DEATH IN MALIGNANT AND NON-
TUMORIGENIC ADHERENT BREAST EPITHELIAL CELL LINES**

Veselina Uzunova, Viktoria Pehlivanova, Iana Tsoneva, Martin Berger, Iva Ugrynova
and Rumiana Tzoneva

10.30-10.45

**AO2. THE INFLUENCE OF ELECTROPORATION ON ADHESIVENESS OF
FIBROBLASTS AND BREAST CANCER CELLS**

V. Pehlivanova, V. Uzunova, I. Tsoneva and R. Tzoneva

10.45 – 11.00

**AO3. GROWTH INHIBITORY ACTIVITY OF SELECTED MICROALGAE AND
CYANOBACTERIA TOWARDS HUMAN CERVICAL CARCINOMA CELLS
(HELA)**

Gergana V. Gacheva, Liliana G. Gigova, Reneta A. Toshkova, Elena G. Gardeva, Natalia
J. Ivanova, Liliya S. Yossifova, Georgi D. Petkov

11.00 – 11.20 COFFEE BREAK

11.20 – 11.35

AO4. ANTIPROLIFERATIVE ACTIVITY OF QUATERNIZED CHITOSAN-COATED NANOFIBROUS MATERIALS CONTAINING GOSSYPOL TOWARD HELA CELLS

Reneta Toshkova, Elena Gardeva, Liliya Yossifova, Milena Ignatova, Nevena Manolova, Iliya Rashkov, Marin Alexandrov

11.35 – 11.50

AO5. AMMONIUM VANADATE AFFECTS VIABILITY AND PROLIFERATION OF CULTURED CELLS

Abdulkadir M. Abudalleh, Tanya D. Zhivkova, Lora V. Dyakova, Kalinka N. Popova, Anna B. Gancheva, Boyka D. Andonova-Lilova, Radostina I. Alexandrova

12.05 – 12.20

AO6. AMMONIUM VANADATE INHIBITS THE IN VITRO AND IN VIVO GROWTH OF VIRUS-TRANSFORMED RAT SARCOMA CELLS

Abdulkadir Mahdi Abudalleh, Tanya Zhivkova, Lora Dyakova, Boyka Andonova-Lilova, Radostina Alexandrova

11.50 – 12.05

AO7. METAL COMPLEXES OF ISOXICAM – EFFECTS ON VIABILITY AND PROLIFERATION OF TUMOR CELLS

Lora Dyakova, Tanya Zhivkova, Anna Gancheva, Reni Kalfin, Daniela-Cristina Culita, Gabriela Marinescu, Luminita Patron, Radostina Alexandrova

12.20 – 12.35

AO8. DIFFERENT INCIDENCE OF PROSTATE CANCER INDUCTION AFTER TESTOSTERONE-N-METHYL-N-NITROSUREA TREATMENT IN TWO MODELS OF METABOLIC SYNDROME

Pavlina Gateva, Mihail Mihailov, Maya Katrafilova, Nadka Boyadjieva

Session B.

Chairpersons:

Prof. Reni Kalfin, PhD

Institute of Neurobiology, Bulgarian Academy of Sciences

Assoc. Prof. Anna Tolekova, MD, PhD

Faculty of Medicine, Trakya University, Stara Zagora

Secretary: Ivelin Vladov

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

13.30 – 13.45

BO1. ANTIOXIDANT ACTIVITY OF NOCICEPTIN AND ITS NEW-SYNTHEZIZED STRUCTURAL ANALOGUES IN RAT LIVER AND PAW TISSUE

Elina Tsvetanova, Stefany Vircheva, Almira Georgieva

13.45 – 14.00

BO2. POTENTIAL ROLE OF VASPIN FOR OBESITY AND APPETITE REGULATION

St. Mihaylova, M. Yakovlieva, R. Sandeva, A. Tolekova

14.00 – 14.15

BO3. ANGIOTENSIN II RECEPTOR BLOCKADE – IMPORTANCE FOR BLOOD PRESSURE CONTROL AND SOME NEW ASPECTS

P. Hadzhibozheva, A. Tolekova, Ts. Georgiev

14.15-14.45

BO4. NEW CHOLINESTERASE REACTIVATORS AND THEIR BIOLOGICAL ACTIVITY

Ahmed Nedzhib, Ivayla N. Pantcheva, Vasil Atanasov, Iskra Petrova , Svetlana Simova

14.45 – 15.05 COFFEE BREAK

15.05-15.35

BO5. PRELIMINARY STUDY ON *IN VIVO* TOXICITY OF MONENSIN, SALINOMYCIN AND THEIR METAL COMPLEXES

Vasil Atanasov, Silviya Stoykova, Yana Goranova, Ahmed Nedzhib, Lyubka Tancheva, Juliana Ivanova, Ivayla N. Pantcheva

15.35-16.05

BO6. ADIPOSE TISSUE - AN IMPORTANT PARTICIPANT IN THE ENVIRONMENTAL MOLECULAR TOXICOLOGY

Stanislav Yanev

Wednesday, 28 November 2012

Session C.

Chairpersons:

Assoc. Prof. Reneta Toshkova, MD, PhD

*Institute of Experimental Morphology, Pathology and Anthropology with Museum,
Bulgarian Academy of Sciences*

Assist. Prof. Dimitar Ivanov, PhD

*Institute of Experimental Morphology, Pathology and Anthropology with Museum,
Bulgarian Academy of Sciences*

Secretary: Tanya Zhivkova

*Institute of Experimental Morphology, Pathology and Anthropology with Museum,
Bulgarian Academy of Sciences*

10.00 – 10.15

**CO1. FRACTIONING OF PROTEIN EXTRACTS OF SIX TRICHINELLA
ISOLATES BY THE METHODS OF ISOELECTROFOCUSING AND SILVER
STAINING**

Valeria Dilcheva, Svetlozara Petkova, Evgeni Gabev

10.15-10.30

**CO2. ALTERNATIVE METHODS FOR CONTROL OF HELMINTH
PARASITES**

D. Salkova, M. Panyotova-Pencheva, S. Movsesyan

10.30 – 10.45

**CO3. MACROPHAGE ACTIVITY IN MICE WITH INFLUENZA INFECTION,
TREATED WITH NATURAL COMPOUNDS**

S. Apostolova, R. Toshkova., L. Yossifova, E. Gardeva, J. Serkedjieva

10.45 – 11.05 COFFEE BREAK

11.05 – 11.20

**CO4. ANTIVIRAL AND VIRUSTATIC ACTIVITIES *IN VITRO* OF MASTIC
GUM EXTRACT, OBTAINED FROM THE TRUNK AND LEAVES OF
*PISTACIA LENTISCUS VAR. CHIA***

P. Genova-Kalou, K. Dimas, S. Dundarov, D. Dundarova, K. Yotovska, J. Ivanova

11.20 – 11.35

**CO5. MICROBIOLOGICAL ASSESSMENT OF NEW COSMETIC PRODUCT
BASED ON NATURAL INGREDIENTS**

Elena Ivanova, Nataliya Gerginova, Kaloyan Bozhanchev, Silvia Geneva, Anton Tachev

Session D.

Chairpersons:

Prof. Margarita Gabrashanska, DVM, PhD

*Institute of Experimental Morphology, Pathology and Anthropology with Museum,
Bulgarian Academy of Sciences*

Assoc. Prof. Diana Rabadjieva, PhD

Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences

Secretary: Boyka-Andonova-Lilova

*Institute of Experimental Morphology, Pathology and Anthropology with Museum,
Bulgarian Academy of Sciences*

13.30 – 13.45

**DO1. SR-MODIFIED DICALCIUM PHOSPHATE DIHYDRATE AS A
BONE GRAFT CANDIDATE**

*B. Andonova-Lilova, T. Zhivkova, L. Dyakova, M. Alexandrov
D. Rabadjieva, S. Tepavitcharova,, R. Alexandrova*

13.45 – 14.00

DO2. IN VIVO BEHAVIOUR OF CALCIUM PHOSPHATE COMPOSITES

*I. Vladov, M. Gabrashanska, M. Alexandrov, V. Nanev, P. Dimitrov, S. Tepavitcharova,
R. Ilieva*

14.00-14.30

**DO3. MANUFACTURING OF SCAFFOLDS WITH INTERCONNECTED
POROUS HYDROXYAPATITE CERAMICS IN POLY(L-LACTIC ACID)
MATRIX FOR BONE TISSUE ENGINEERING**

*S. Simeonova, M. Evstatiev, E. Dyulgerova, R. Aleksandrova, M. Gabrashanska, M.
Aleksandrov, D. Rabadjieva, St. Tepavitcharova*

14.30 14-50 COFFE BREAK

14.50 – 15.20

**DO4. DEVELOPMENT OF INJECTION AND ORAL AGENTS IN THE
TREATMENT OF MULTIPLE SCLEROSIS**

Vera Kolyovska, Denislava Deleva

15.20 - 15.50

**DO5. ALCOHOL BASED FIXATIVES PROVIDE EXCELLENT TISSUE
MORPHOLOGY, PROTEIN IMMUNOREACTIVITY AND RNA INTEGRITY IN
PARAFFIN EMBEDDED TISSUE SPECIMENS**

Rositsa Milcheva, Pavol Janega, Peter Celec, Russy Russev, Pavel Babál

15.50-16.05

DO6. CONNECTION BETWEEN ESTROGEN AND OPIOID RECEPTORS

Tim Vladimirov

Medical Faculty, Sofia University “St. Kliment Ohridski”

16.05 – 17.00

INVITED LECTURE

**DO7. CONSEQUENCES OF DIETARY INDUCED HYPOTHYROIDISM
FOR THE REPRODUCTIVE CAPACITY OF MALE AND FEMALE OFFSPRING**

Prof. Katja Teerds,

University of Wageningen, The Netherlands

Thursday, 29 November 2012

Session E.

Chairpersons:

Assoc. Prof. George Miloshev, PhD

Institute of Molecular Biology, Bulgarian Academy of Sciences

Assist. Prof. Delka Salkova, DVM, PhD

*Institute of Experimental Morphology, Pathology and Anthropology with Museum,
Bulgarian Academy of Sciences*

Secretary: Abdulkadir Abudalleh

*Faculty of Biology, Sofia University St. Kliment Ohridsky,
Institute of Experimental Morphology, Pathology and Anthropology with Museum,
Bulgarian Academy of Sciences*

10.00-10.30

**EO1. CHANGES OF BIOLOGICALLY ACTIVE AND
ANTICANCEROGENIC SUBSTANCES IN COW'S MILK OF THE
BULGARIAN RHODOPES CATTLE BREED**

Lyubomir Angelov, Silviya Ivanova, Tzonka Odjakova, Dimitar Gadjev

10.30 – 10.45

**EO2. CONTENT OF BIOLOGICALLY ACTIVE SUBSTANCES IN FLOUR
FROM
OAT AND RYE**

Silviya Ivanova, Nadka Mihalkova, Lyubomir Angelov

10.45 – 11.00

**EO3. ANTIOXIDANT CAPACITY OF DIFFERENT TOMATO LINES
DURING HOME STORAGE**

Elena Shopova, Sergey Ivanov, Iskren Sergiev, Elena Balacheva, Tania Kartzeva

11.00-11.20 COFFEE BREAK

11.20 – 11.50

**EO4. IMMUNOLOGICALLY ACTIVE PECTIC POLYSACCHARIDES FROM
ORANGE FRUITS**

Manol H. Ognyanov, Yordan N. Georgiev, Irina Z. Yanakieva, Veselin K. Kussovski,
Maria G. Kratchanova

11.50 – 12.05

**EO5. DAMAGING ACTIVITIES OF SEVERAL COMPOUNDS ON THE
GENOME OF TWO YEAST SPECIES - *S. CEREVISIAE* AND *K. LACTIS***
Ekaterina Peycheva, Dessislava Staneva, Milena Georgieva and George Miloshev

12.05 – 12.35

**EO6. GENETIC ENGINEERING, SEEN THROUGH THE LENS OF TWO
YOUNG LADIES...
POISON FOR THE AVERAGE PEOPLE=DOPING FOR THE
LABORATORY MUTANTS**
Veneta Dimitrova, Radostina Djaleva

Session F.

Chairpersons:

Assoc. Prof. Albena Alexandrova, PhD

Institute of Neurobiology, Bulgarian Academy of Sciences

Assist. Prof. Milena Georgieva, PhD

Institute of Molecular Biology, Bulgarian Academy of Sciences

Secretary: Veselin Nanev, DVM

*Institute of Experimental Morphology, Pathology and Anthropology with Museum,
Bulgarian Academy of Sciences*

13.45 – 14.15

**FO1. PROJECT 2007CB16IPO006-2009-1-101 “CREATION OF A DATABASE OF
POLLUTANTS FROM INDUSTRIAL WASTE IN A TRANS-BOUNDARY
ASPECT AND A LOGISTICAL FRAMEWORK FOR PROBLEM SOLVING” -
PRESENTATION OF THE RESULTS**

Radostina Hristova, Konstantin Mitov, Nesho Chipev, AlbenaAlexandrova

14.15 – 14.30

**FO2. IS THE COPPER ENVIRONMENTAL CONTAMINATION HARMFUL
FOR HUMAN BEINGS?**

AlbenaAlexandrova, Lubomir Petrov

14.30 – 14.45

FO3. ZINC DEFICIENCY AND INFERTILITY

Galina Nenkova, Institute of Neurobiology, Bulgarian Academy of Sciences

14.15 – 15.05 COFFEE BREAK

15.05 – 15.20

**FO4. COMPARATIVE EFFECTS OF Co AND Cd COMPOUNDS ON
TESTIS MORPHOLOGY AND SPERM COUNT**

Ekaterina Pavlova, Donika Dimova, Maria Madzharova, Yordanka Gluhcheva,
Juliana Ivanova, Nina Atanassova

15.20 – 15.35

FO5. EFFECT OF CO-EDTA ON IRON METABOLISM IN IMMATURE MICE

Y. Gluhcheva, E. Pavlova, I. Vladov, V. Atanasov,
Ju. Ivanova, M. Mitewa

15.35 -15.50

FO6. IRON AND BLUE PEOPLE

Dzhem Farandzha

15.50 – 16.20

**FO7. NEW FLUORESCENT DENDRIMERS AS DETECTOR FOR
BIOLOGICALLY IMPORTANT METAL IONS**

Stanislava Yordanova, Stanimir Stoyanov, Ivan Petkov, Ivo Grabchev

16.20 – 16.30

CLOSING REMARKS

ABSTRACTS

Session A.

Chairpersons:

Assoc. Prof. Radostina Alexandrova, PhD *Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences*

Assoc. Prof. Rumiana Tsoneva, PhD
Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences

Secretary: Lora Dyakova
Institute of Neurobiology, Bulgarian Academy of Sciences

AO1. THE EFFECT OF ERUFOSINE ON CYTOSKELETON REORGANIZATION AND CELL DEATH IN MALIGNANT AND NON-TUMORIGENIC ADHERENT BREAST EPITHELIAL CELL LINES

Veselina Uzunova*, Viktoria Pehlivanova*, Iana Tsoneva*, Martin Berger*, Iva Ugrynova** and Rumiana Tzoneva***

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***Institute of Molecular Biology, Bulgarian Academy of Science, Sofia 1113, bl. 21, e-mail:ugryiva2003@yahoo.com*

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Cell adhesion plays a key role in tumor progression and its control could diminish the tumor metastases. In the present study the action of erufosine on reorganization of actin cytoskeleton and apoptosis was analyzed in breast cancer and mammary epithelial cells. The influence of electroporation on cytotoxicity was studied also.

Breast cancer cell lines MDA-MB-231 and MCF-7 as well as non-transformed MCF-10A were treated with erufosine (5-15 μ M) or subjected to combine treatment with biphasic electrical pulses. MTS test, actin and DAPI staining were used. For MDA-MB-231 erufosine provoked apoptosis and actin reorganization, since MCF-7 and MCF-10A were less sensitive to the action of erufosine (Fig. 1). The combine treatment with erufosine and electrical pulses lead to stimulation of cell proliferation for MCF-7 and lack of additional effect for MDA-MB-231. The cytotoxic action of erufosine on breast cancer cells and epithelial cells is cell specific. The most sensitive is the high invasive MDA-MB-231 cell line since 15 μ M erufosine cause cytoskeleton reorganization and apoptosis.

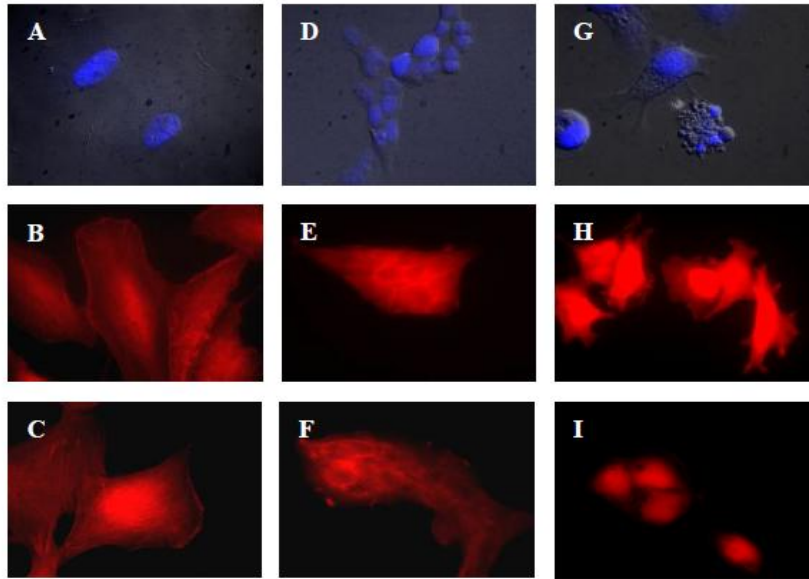


Fig. 1. Effect of erufosine on cell nuclei and actin cytoskeleton. MCF-10A (A and B), MCF-7 (D and E) и MDA-MB-231 (G and H) were treated with 15 μ M erufosine and stained with DAPI (upper panel) and BODIPY/Phalloidin (middle panel). Lower panel–non-treated MCF-10A (C), MCF-7 (F) and MDA-MB-231 (I). Bar is 50 μ m.

Keywords: erufosine, breast cancer cells, apoptosis, actin cytoskeleton

AO2. THE INFLUENCE OF ELECTROPORATION ON ADHESIVENESS OF FIBROBLASTS AND BREAST CANCER CELLS

V. Pehlivanova, V. Uzunova, I. Tsoneva and R. Tzoneva

*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences,
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Electroporation as a universal method applicable to different cell types is used for different purposes. In medicine it is used for electrochemotherapy (1). The purpose of the present work was to investigate the role of electric field (high – voltage direct current electrical pulses) alone on tumor cell adhesion in respect to tumor progression. We studied the influence of electroporation on cytoskeleton organization, initial cell adhesion and spreading of breast cancer cells and fibroblasts.

Cells: MDA-MB-231 - grown in RPMI 1640 medium, MCF-7 – cultivated in DMEM and 3T3 cells - cultivated in MEM with 10% FCS and supplements. All cells were cultivated at 37°C, 5 % CO₂ and humidified atmosphere. **Aparatus and conditions for cell electroporation:** Chemopuls III electroporation apparatus is produced in the Institute of Biophysics and Biomedical Engineering, BAS, Sofia, Bulgaria. Electrical parameters - 8 biphasic pulses; 50 + 50 μ s, 1ms interval between the phases, frequency of the pulses - 1 kHz, the voltage is between 250 - 1000 V. Two parallel stainless - steel electrodes with length – 22mm and 10 mm distance between them. **Electroporation of cells:** Cells were cultivated 24h prior to electroporation on the bottom of 24 well plates or on cover slides (18/18 mm). The electroporation was carried out in basal RPMI 1640 and the applied initial voltages were fixed to 200 V/cm, 500 V/cm and 1000 V/cm. **Determination of**

electro-permeabilization: The cellular uptake of propidium iodide (0.1 mM) was measured and visualized on fluorescent inverted microscope. **Colorimetric method for estimation of cell adhesion (crystal violet staining):** After electrotreatment cells were stained with 0.1 % solution of crystal violet. The number of adhesive cells was defined colorimetrically by the intensity of the solution of crystal violet extracted from cells with 0.1 M HCl by using microplate reader. **Actin staining:** Adhered cells after electrotreatment was stained for actin using BODIPY 558/568 phalloidin and visualized by inverted fluorescent microscope. **The results** revealed that at 1000V/cm cells were completely electroporated, since at 200V/cm and 500V/cm the electroporation was uncomplete. Application of 1000V/cm suppressed cell adhesion, but the applied lower electrical field (200V/cm and 500V/cm) stimulated cell adhesion. Electroporation provoked an increased proteolytic activity of 3T3 cells. Motility of the cancer cells after electrotreatment was diminished. Electrical field with 200V/cm and 500V/cm intensity formed tumor aggregates by MDA-MB-231 and MCF-7.

The work can provide an insight into putative mechanisms which could play role in the restriction of tumor invasion after application of appropriate electric pulses and suggests that changes in cytoskeleton organization and cell adhesiveness could contribute to the amplification of anti-tumor effect of electroporation-based tumor therapy

Keywords: electroporation, cell adhesion, breast cancer cells, fibroblasts

Reference:

1. M. Kanduser and D. Miklavcic, "Electroporation in Biological Cell and Tissue: An Overview" in E. Vorobiev, N. Lebovka (eds.), *Electrotechnologies for Extraction from Food Plants and Biomaterials*, DOI: 10.1007/978-0-387-79374-0 1, C _ Springer Science+Business Media, LLC 200

AO3. GROWTH INHIBITORY ACTIVITY OF SELECTED MICROALGAE AND CYANOBACTERIA TOWARDS HUMAN CERVICAL CARCINOMA CELLS (HELA)

Gergana V. Gacheva^{1*}, Liliana G. Gigova¹, Reneta A. Toshkova², Elena G. Gardeva², Natalia J. Ivanova¹, Liliya S. Yossifova², Georgi D. Petkov¹

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Although a large number of strains have been shown to possess anticancer activity, the microalgal and cyanobacterial potential is still largely unexplored. The present study was aimed to evaluate and compare the growth inhibitory activity of 33 extracts, 15 fatty acid mixtures and five exopolysaccharides from 19 species on HeLa cells, using MTT assay. All investigated strains showed growth inhibitory activity of at least one tested extract/constituent. More than 80% decrease in HeLa cells viability was

registered for the crude hot water extract of *Gloeocapsa* sp. and ethanol extracts of the new Bulgarian isolates *Gloeocapsa* sp. and *Clorella* sp. The most active cell-free culture liquids were these from *Anabaena* sp. and *Synechocystis* sp., while the exopolysaccharides from *Gloeocapsa* sp. exhibited the lowest IC₅₀ value (24.4 µg/ml). An interesting and useful finding of our investigation was that the isolated fatty acid mixtures from five out of eight cyanobacterial strains and from four out of seven microalgal strains studied had potent activity against HeLa tumor cells with the IC₅₀ values under 30 µg/ml and even under 15 µg/ml. High percentage of the active Bulgarian isolates among the strains tested fulfil the effort of screening cyanobacteria and microalgae of local habitats. In conclusion, the most promising strains for further study are the thermal cyanobacteria *Synechocystis* sp. and especially *Gloeocapsa* sp., which showed the strongest inhibition of tumor cell growth and produced wider range of active components.

Acknowledgements: Financial support from the Bulgarian National Science Fund (Grant DOO2-299/2008) is gratefully acknowledged.

AO4. ANTIPROLIFERATIVE ACTIVITY OF QUATERNIZED CHITOSAN-COATED NANOFIBROUS MATERIALS CONTAINING GOSSYPOL TOWARD HELA CELLS

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Nanofibrous polylactide-based materials loaded with a natural polyphenolic compound gossypol (GOS) with antitumor properties were prepared by electrospinning. The nanofibrous materials were coated with a thin film of crosslinked quaternized chitosan (QCh). GOS incorporated in the nanofibrous mats was in the amorphous state. GOS release was diffusion-controlled and its *in vitro* release profiles depended on the mat composition. The nanofibrous materials exhibited high cytotoxicity towards HeLa tumor cells. It was established particularly pronounced cytotoxicity in the case of fibrous materials, which contain both QCh and GOS. The observed strong antiproliferative effect of the nanofibrous mats was mainly due to induction of cell apoptosis. These results indicate that the obtained nanofibrous materials are promising candidates as systems for local delivery of antitumor drugs in the treatment of cervical tumors.

Acknowledgments: Financial support from the Bulgarian National Science Fund (Grant DO-02-164/2008) is gratefully acknowledged.

AO5. AMMONIUM VANADATE AFFECTS VIABILITY AND PROLIFERATION OF CULTURED CELLS

Abdulkadir M. Abudalleh^{1,2}, Tanya D. Zhivkova², Lora V. Dyakova³, Kalinka N. Popova^{1,2}, Anna B. Gancheva^{1,2}, Boyka D. Andonova-Lilova¹, *Radostina I. Alexandrova¹

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The interest in biological activity of vanadium and its compounds has increased significantly during the recent years. This fact could be explained by several reasons: i) vanadium is widely distributed in soils, water, plant and animal tissues; ii) it has considered to be among the 40 essential micronutrients that are required in small amounts for normal metabolism; iii) the daily human exposure to vanadium compounds due to the wide application of this metal in current industry; iv) 66 000 tons of vanadium are released into atmosphere each year (Alexandrova, 1999; Mukherjee et al., 2004). Among the most intriguing properties of vanadium are its insulin mimetic action and anticancer potential. Vanadium in several animal cancer models provides protection against all stages of carcinogenesis – initiation, promotion, and progression (Evangelou, 2002; Kostova, 2009; Bishayee et al., 2010). The aim of our study was to evaluate the influence of ammonium vanadate on viability and proliferation of cultured human and animal tumor and non-tumor cells using cytotoxicity assays with different cell targets and mechanism(s) of action.

Permanent cell lines obtained from some of the most common human cancers were used as model systems: MCF-7 (breast cancer), HeLa (carcinoma of the uterine cervix), HepG2 (hepatoma). The non-tumor cell lines established from human embryos (MRC-5 and Lep-3) and bovine kidney (MDBK) were also included in the experiments. The investigations were performed by thiazolyl blue tetrazolium bromide (MTT) test, neutral red uptake cytotoxicity assay (NR) and crystal violet staining (CVS). The results obtained revealed that applied at concentrations of 0.1, 0.5, 1, 5, 10 and 20 $\mu\text{g/ml}$ for 24 h, 48 h and 72 h, NH_4VO_3 decreased significantly (in a time- and concentration-dependent manner) the viability and proliferation of human tumor (MCF-7, HeLa, HepG2) and non-tumor (MRC-5, Lep-3) cells whereas bovine kidney MDBK cells seem to be relatively more resistant. A positive correlations between the data coming from MTT, NR and CVS methods were observed.

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AO6. METAL COMPLEXES OF ISOXICAM – EFFECTS ON VIABILITY AND PROLIFERATION OF TUMOR CELLS

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Nonsteroidal anti-inflammatory drugs (NSAID) are among the most consumed drug over the world. Their extending use is endorsed by their analgesic, antiinflammatory and antipyretic properties as well as other recent application. Some of them have proven to inhibit the proliferation of different cancer cell cultures, such as colorectal , and prostate cancer cells , non-small lung cancer cells and osteosarcoma cells , as well as the growth of animal tumors . However, these agents produce adverse effects (gastric mucosal damage including bleeding, ulceration and perforation in humans and animals) that may appear in around 25% of patients. These effects are related to the inhibition of the enzyme cyclooxygenase, which is the main established mechanism of action for these drugs . Therefore, it is essential to develop new antiinflammatory drugs, with a high rate of efficacy and less side effects. A method often used by some researchers consists in linking active drugs to metal ions (Daniela C. Culita et al, 2012). Many reports have demonstrated that non-steroidal anti-inflammatory drugs suppress malignant transformation and tumor growth, and some NSAIDs are expected to be new anticancer agents. In our past investigations we found that metal complexes with piroxicam (a non-selective COX inhibitor) and meloxicam (selective COX-2 inhibitor) decrease in a time and concentration dependant manner viability and proliferation of cultured human and animal tumor cells.

The aim of our study was to evaluate the influence of Isoxicam and its metal on viability and proliferation oncultured human and animal tumor cells.

Materials and Methods

We used in our investigations Isoxicam and its 4 metal complexes – Cu(II)-Isoxicam, Zn(II)- Isoxicam, Co(II)- Isoxicam and Ni(II)- Isoxicam. As an experimental model we used human tumor cell lines MCF-7 and Hela, virus-transformed animal cell line LSCC-SF (Mc29) and LSR-SF_SR , and non-tumor cell line Lep. We used cytotoxicity assays- MTT test, Neutral red uptake assay and double staining with acridine orange and propidium iodide.

Results:

The results obtained, that at concentration of 5-500 µg/ml for 24-72h , the compounds investigated, decrease in a time and concentration dependant manner viability and proliferation of treated cells. Among the compounds investigated Ni (II) and Co(II) complexes with isoxicam were shown the most promissing antiproliferative activity on virus-transformed tumor cell lines and Zn(II) complexes- on cultured human tumor cells. Among the cell lines used in our experiments virus-transformed tumor cells are more sensitive than cultured human tumor cells.

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Daniela C. Culita, Radostina Alexandrova, Lora Dyakova, Gabriela Marinescu, Luminita Patron, Reni Kalfin, Marin Alexandrov. Evaluation of Cytotoxic and Antiproliferative Activityof Co(II), Ni(II), Cu(II) and Zn(II) Complexes withMeloxicam on Virus – Transformed Tumor Cells. Revista de Chimie 2012, 63(4), 384 - 389.

AO7. AMMONIUM VANADATE INHIBITS THE IN VITRO AND IN VIVO GROWTH OF VIRUS-TRANSFORMED RAT SARCOMA CELLS

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Vanadium (atomic number 23) is a metal of physiological, environmental and industrial importance. As a micronutrient it is included in the list of 40 essential elements that are required in small amounts for normal metabolism. In recent years there is a

growing interest in biological behavior of vanadium, especially due to its insulin-mimetic and anti-cancer properties. According to the literature available, the antineoplastic activity of vanadium compounds in virus-transformed tumor cells has not been clarified yet.

The aim of our study was to evaluate the antitumor activity of ammonium vanadate in virus transformed tumor cells in vitro and in vivo.

The influence of NH_4VO_3 (0.1 – 20 $\mu\text{g}/\text{mL}$ for 24, 48 and 72 h) on viability of LSR-SF-SR cell line (transplantable rat sarcoma induced by SR-RSV) was examined by MTT test, neutral red uptake cytotoxicity assay, colony forming technique and cytology methods. For in vivo experiments one-month old Wistar rats were divided into three groups (n = 4) as follows: A – Control; B and C – transplanted s.c. with 3.7×10^6 viable LSR-SF-SR cells where in contrast to group B the animals from C were treated with NH_4VO_3 (0.5 ppm in the drinking water given ad libitum started from the implantation of tumor cells until the end of the experiment). The rats were sacrificed 20 days after the sarcoma cells transplantation.

Results: NH_4VO_3 expressed significant cytotoxic and cytostatic activities in cultured sarcoma cells ($\text{CC}_{50} = 1 \mu\text{g}/\text{mL}$ and $\text{CC}_{90} = 4.9 \mu\text{g}/\text{mL}$, MTT test, 72h). Applied at concentrations $> 5 \mu\text{g}/\text{mL}$ the compound completely inhibited colony-forming ability of LSR-SF-SR cells in semisolid medium. In vivo tumor growth was observed in 100% (4/4) of the rats from groups B and C with no difference in latent periods. The volume of tumors in group C was found to be smaller (by ~10%) as compared to group B (p > 0.05).

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AP1. METAL COMPOUNDS IN THE FIGHT AGAINST GLIOBLASTOMA

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Gliomas are the most frequent primary neoplasms of the central nervous system (CNS) in adults, consisting of 63% of all primary CNS tumors. The majority of them remain difficult to cure because of the infiltrative growth of cancer cells and their resistance to standard therapy. Survival of patients affected by GBM has reminded

virtually unchanged during the last decades (i.e. 6-12 months post-diagnosis) despite advances in surgery, radiation, and chemotherapy [1-3]. In the present study we present data about the effect of some metal (Zn, Cu, Co, Ni, Fe, La, Au, Ag) complexes with various ligands (Mannich bases, Schiff bases, cholic acids, non-steroidal anti-inflammatory drugs) on viability and proliferation of cultured 8 MG BA human glioblastoma multiforme cells. The experiments were performed using MTT test, neutral red uptake cytotoxicity assay, trypan blue dye exclusion technique, crystal violet staining, double staining with acridine orange and propidium iodide and colony-forming method.

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AO8. DIFFERENT INCIDENCE OF PROSTATE CANCER INDUCTION AFTER TESTOSTERONE-N-METHYL-N- NITROSUREA TREATMENT IN TWO MODELS OF METABOLIC SYNDROME

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Objectives: Prostate cancer is a common human malignancy, thought to be related at least in part with a cluster of compounds of the metabolic syndrome (obesity, hyperglycemia, dyslipidemia, arterial hypertension). Treatment of rats with testosterone and N-methyl-N-nitrosurea is a well established model of prostate cancer.

Our aim was to test two different models of metabolic syndrome in rats – fructose feeding and high fat diet and to reveal the impact of those two types of diet for prostate cancerogenesis.

Methods. 75 rats were divided in 3 groups - group C (n=25), group L (n=25), and group F (n=25). Group L was fed during the whole experiment with a high fat diet (mixture of nuts), and group F received for the whole period 10% fructose instead of drinking water. Group C received only standard laboratory show. All were treated with testosterone-N-methyl-N-nitrosurea and the incidence of prostate cancer was recorded for approximately 1 year of observation.

Results. The incidence of the prostate cancer observed is as follows: 20 rats - from the group C, 22 rats - from the group L (p=NS) and only 12 – from the group F (p<0.05) .

Conclusion. Our results indicate that different models of metabolic syndrome in rats impact differentially prostate cancerogenesis. High fat diet tends to increase prostate cancerogenesis in rats, whereas fructose feeding could have a protective effect. Further evaluations are needed in order to elucidate underline mechanisms.

Session B.

Chairpersons:

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BO1. ANTIOXIDANT ACTIVITY OF NOCICEPTIN AND ITS NEW-SYNTHEZIZED STRUCTURAL ANALOGUES IN RAT LIVER AND PAW TISSUE

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Nociceptin (N/OFQ) and its receptor (NOP) were discovered in 1995. Their role in central and peripheral nerve system has been extensively studied. Nowadays it is well known that they modulate the nociception. However the data about their effects on cell pro/antioxidant status are scanty. For that reason the aim of this work was to investigate the effect of nociceptin and its new – synthesized structural analogues on cell pro/antioxidant status in norm and pathology, and to classify their presumptive antioxidant capacity. The structural analogues of nociceptin used in the present work differ in amino acid lysine in position 9, which is substituted respectively with ornithine [Orn⁹]N /OFQ (1–13)NH₂, diaminopropanoic acid [Dap⁹]N/OFQ(1–13)NH₂, diaminobutanoic acid [Dab⁹]N/OFQ(1–13)NH₂ and canavanine [Cav⁹]N/OFQ(1–13)NH₂. Lysine was chosen for substitution, because it seems to be crucial for receptor occupation, most probably interacting with the amino acids present in the second extracellular loop of the NOP-receptor. The tested peptides (20 mkg/ml) were injected intracerebroventricularly. The pathological conditions were induced by carrageenan (CG), which induced peripheral inflammation and is widely used as proper method for examination of different substances. CG (1%/100µl) was administrated subplantary in the right hind paw. In the study the following indexes of the cell pro/antioxidant status were determined: lipid peroxidation (LP), total glutathion, antioxidant enzyme activities of superoxide-dismutase, glutathion reductase, glutathion peroxidase, and glucose-6-phosphate dehydrogenase. The experiments were conducted in vivo and in vitro and the rat liver and paw tissue were analyzed.

The obtained results showed that:

1. The in vitro experiments showed that the peptides had distinct activity in liver. [Dab⁹]N/OFQ(1–13)NH₂ and [Dap⁹]N/OFQ(1–13)NH₂ tested alone lead to cell's damage

(increased LP and decreased GSH); cytoprotective effect was obtained only in presence of N/OFQ and [Orn⁹]N /OFQ (1–13)NH₂. All peptides did not change the other tested parameters.

2. The results of the in vivo experiments showed that N/OFQ and [Orn⁹]N/OFQ(1–13)NH₂ applied alone did not change the parameters of LP and GSH in liver and paw tissue. The CG injection led to increased levels of LP and decreased levels of GSH compared with the controls. A tendency toward an increased GSH level after injection of [Orn⁹]N /OFQ (1–13)NH₂ was observed in the liver of CG-treated animals.

3. Four hours after GC-injection the activities of SOD decreased. The CG-treatment did not change the activity of GSSG-Red, but increased GSH-Px activity and especially the Glu-6-P-DH activity in tissue of right paw. The peptides alone did not change significantly the measured parameters. Neither N/OFQ nor [Orn⁹]N /OFQ (1–13)NH₂ modified CG-induced changes in the antioxidant status.

Recently the interest on nociception and oxidative stress, which underlines a number of diseases increased. Many investigations were conducted with nociceptin and wide range of nociceptin analogues in order to understand the mechanism of action and pharmacological and pharmacokinetic properties of them. Our results contribute to reveal other aspect of their characteristics - the pro/antioxidant capacity. These data are necessary prerequisite for their further use in laboratory and clinical practice application.

BO2. POTENTIAL ROLE OF VASPIN FOR OBESITY AND APPETITE REGULATION

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Obesity is the most prevalent nutritional problem worldwide which in the long run predisposes to development of diabetes mellitus, cardiovascular diseases, carcinomas, osteoarthritis and gall stones. Multiple factors are involved in the development of obesity. These may be social, environmental, behavioral and genetic. Obesity develops when energy intake exceeds energy expenditure over time.

Feeding and energy expenditures are modulated by interplay of hormones, adipokines, neuropeptides and neurotransmitters in the central nervous system. The arcuate nucleus of the hypothalamus plays a key role in those processes controlling the energy homeostasis and integrating neural and blood-borne signals due to its privileged location near the median eminence, a region lacking a complete blood-brain barrier. Two populations of neurons within the arcuate nucleus integrate peripheral-derived adiposity and nutritional signals. Neuropeptide Y (NPY) and agouti-related peptide (AgRP) co-expressing neurons stimulate food intake, while cocaine- and amphetamine-regulated transcript peptide (CART) and proopiomelanocortin (POMC) co-expressing neurons inhibit food intake. Energy homeostasis is controlled by peripheral signals from adipose tissue, pancreas, and the GIT. Gut-derived peptides and adiposity signals influence central circuits in the hypothalamus and brain stem to produce a negative (–) or positive (+) effect on energy balance. Recent advances indicate that adipose tissue is not simply an energy storage

organ, but also a secretory organ, producing a variety of bioactive substances and plays key roles in the regulation of food intake and energy balance, insulin action, and glucose and lipid metabolism. In the last decade, vaspin (visceral adipose tissue-derived serine protease inhibitor) was identified as an adipokine with insulin-sensitizing effects. Vaspin is predominantly secreted from the visceral adipose tissue in rat model of type 2 diabetes. Evidence from animal experiments, suggests that vaspin may play a role in the regulation of food intake. The role of vaspin in the regulation of human metabolism is unclear at present, but it appears that vaspin might represent a novel marker of obesity and insulin resistance.

Keywords: appetite regulation, obesity, vaspin

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BO3. ANGIOTENSIN II RECEPTOR BLOCKADE – IMPORTANCE FOR BLOOD PRESSURE CONTROL AND SOME NEW ASPECTS

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It is well known that the renin-angiotensin system (RAS) is a general regulator of blood pressure and volume, as well as body fluid and electrolyte balance. The main effector of the RAS – Angiotensin II (Ang II), is a highly active octapeptide that is generated in the circulation or locally in many tissues. Today, the requisite components of the RAS, as renin and angiotensin-converting enzyme (ACE) are studied and can be controlled by various pharmacological products, thus ultimately preventing the formation of Ang II. One therapy for blood pressure regulation is administration of beta-blockers, which reduces the synthesis of renin. A second way for down regulation of Ang II formation is the application of ACE inhibitors. The most recent and accurate method is a direct blockade of Ang II receptors. Ang II has been implicated in a wide range of

physiological processes by the activation of two receptor subtypes. For many years, most of the effects of Ang II were attributed to the activation of the AT₁ receptors, especially concerning the smooth muscle (SM) contractile activity. Recently, more often is discussed the significance of AT₂ receptors. They have been also described in the SM cells, but the importance of AT₂ receptors for SM motility has not been established yet. It is supposed that they mediate the opposite effect of AT₁ receptors, but as a factor for the SM relaxation they had been proved only in few research works.

For the maintenance of adequate SM tone and normal blood pressure, it is important not only the contraction, but also the relaxation and the development of the whole SM contraction process in the time. This suggests the involvement of both Ang II receptors in the contractile process. The better study of the effects mediated by the different Ang II receptor subtypes requires specific blockers to be created. Losartan is the first approved non-peptide AT₁ antagonist. Except for the treatment of hypertension, losartan has additional beneficial effects: regression of myofibrosis and reduction of atherosclerosis, increase in microcirculation flow, reducing the levels of uric acid. By AT₂ receptor blockade with the selective blocker PD 123319 was found that these receptor subtype largely modulate or even counteract the effects, mediated by activation of AT₁ receptors. Moreover, PD 123319 directly affects placental vascular SM cell, apoptosis in IgA nephropathy, ventricular tachycardia and others. Our experience with study of Losartan and PD 123319 showed that they affect the motility of the gastro-intestinal system, which may be another area that requires attention in the study of the effects of these drugs.

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BO4. NEW CHOLINESTERASE REACTIVATORS AND THEIR BIOLOGICAL ACTIVITY

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Organophosphorus compounds (OPC) are organic compounds containing phosphorus which can be directly bonded to carbon in molecules or which can be bonded to the organic part of the molecules by a heteroatom, mainly oxygen. OPC are used in technology, agriculture, medicine, scientific research, etc. Therefore poisonings with OPC are frequent. The mechanism of toxic effect of OPC is based on acetylcholinesterase (AChE) inhibition in the nerve system. The intoxication of OPC is treated with different drug combinations consisting of cholinesterase (ChE) reactivators and cholinolytics. Commonly used antidotes in the treatment of OPC poisoning are the oxime reactivators of ChE which break the bond between AChE and OPC and restore enzyme activity.

The reason for the synthesis of new oximes as possible reactivators of ChE is the high variability of the chemical structure of inhibitors. The new compounds may have an ability to reactivate sufficiently the inhibited enzyme.

The aim of this study is focused on synthesis of new symmetrical bisquaternary pyridinium aldoximes with methylene bridge consisting of three (BT-07 - 1,3-bis (2-hydroxyiminomethyl-pyridinium)-propane dibromide) and four (BT-08 - 1,4-bis (2-hydroxyiminomethyl-pyridinium)-butane dibromide) methylene groups, while oxime groups are in position two in pyridinium rings.

The compounds were structurally characterized using IR, UV-Vis and NMR spectroscopies; in addition retention time (R_f , TLC) and melting point were determined. The reactivation of inhibited AChE (achieved by Paraoxon, DDVP and Tabun) was carried out on male albino "Wistar" rats and the activity of the enzyme was assessed by Ellman's method. The percentage of reactivation (%R) was calculated from the measured activities of the intact enzyme (A_o), tabun-inhibited enzyme (A_i) and reactivated enzyme (A_r). The experimental results showed that BT-08 possess higher reactivation activity on the enzyme inhibited by Paraoxon.

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BO5. PRELIMINARY STUDY ON *IN VIVO* TOXICITY OF MONENSIN, SALINOMYCIN AND THEIR METAL COMPLEXES

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The polyether antibiotics are applied in veterinary medicine as coccidiostatic and antimicrobial agents. For long time they were known as monovalent polyether ionophores for their ability to bind monovalent metal cations. Recently it was shown that they complex with divalent metal ions forming new compounds of various composition and different stoichiometry. The new metal(II) complexes possess generally better antimicrobial and anticancer activity than initial antibiotics. These results arose the question whether the inclusion of metal(II) cations could influence their *in vivo* activity.

The aim of the present research was pointed out to the evaluation of acute toxicity (24 h) of polyether ionophores and their metal complexes, as well as to the assessment of some biochemical indices upon 72 h treatment of laboratory animals.

The acute toxicity of monensin, salinomycin and their derivatives with ions of Na(I), Mg(II), Ca(II), Mn(II), Co(II), Zn(II) was evaluated in ICR mice. The experimental data revealed that Ca(II) and Mg(II) complexes of salinomycin showed the highest toxicity among the compounds tested with LD₅₀ values of 20.5 mg/kg b.w (13 µmol/kg b.w.) and 25.8 mg/kg b.w (17 µmol/kg b.w.), respectively. The preliminary evaluation of biochemical indices of survived animals had shown that no significant changes occur within 72 h treatment with ionophorous antibiotics and their complexes. From the clinical signs of toxicity observed and based on the preliminary biochemical data obtained it can be suggested that the lethal outcome is associated with CNS toxicity and breath insufficiency.

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BO6. ADIPOSE TISSUE - AN IMPORTANT PARTICIPANT IN THE ENVIRONMENTAL MOLECULAR TOXICOLOGY

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Homo sapiens recens is exposed to an overwhelming number of chemical contaminants circulating every day in the air, water, food, and general environment. The body is a well-equipped entity with capabilities to excrete water-soluble pollutants, but not as well-equipped to excrete some of the lipid-soluble xenobiotics. Adipose tissue is an important participant, even a master, in this process. Adipose tissue nowadays is seen not only as a lipid store, but as a secretory – endocrine and paracrine – organ, synthesizing, storing, and releasing more than 100 signaling proteins collectively termed adipokines. Human adipose tissue is partitioned into two large depots (subcutaneous and visceral) and many small depots associated with internal organs, e.g. heart, blood vessels, major lymph nodes, pancreas, prostate gland, mammary gland, and ovaries. Adipotoxicology connotes the study of storage, release, and metabolism of exogenous substances (xenobiotics) including so-called “environmental obesogens”, as related to toxicology and pathogenesis of disease. Recent data demonstrate that chlorinated pesticides and polychlorinated biphenyls as well as other pollutants (organotins, phthalates, bisphenol A, fungicides, heavy metals) accumulated within adipose tissue are linked to the development of cardiometabolic diseases (atherosclerosis, obesity, type 2 diabetes, metabolic syndrome) and some neurodegenerative diseases. Altogether, such an adipocentric approach underlines the need for human biomonitoring of xenobiotics accumulation in adipose tissue. Here we present data that adipose tissue may be a new bridge between environment and health - an important participant in the environmental molecular toxicology.

Session C.

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CO1. FRACTIONING OF PROTEIN EXTRACTS OF SIX TRICHINELLA ISOLATES BY THE METHODS OF ISOELECTROFOCUSING AND SILVER STAINING

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Comparative electrophoretical studies on protein extracts from six *Trichinella* isolates have been carried out with the purpose of obtaining novel data about their species characterization. The methods of isofocusing and silver staining have been used. The electrophoretical analysis of the water-soluble proteins of the six isolates: ISS03, ISS13, ISS10, ISS02, ISS029, ISS035 has yielded as a result a heterogeneity both of number and electrophoretical mobility of the individual protein fractions.

The method of electrophoresis in PAAG is a reliable biochemical method by which differences between the helminth species under study can be established and it is recommended as a diagnostics tool especially in cases of species whose individuality is disputable [^{1,2}]. According to certain authors PAAE, isoelectrofocusing and silver staining [³] represent an objective feature for the differential diagnostics of helminthes especially with the view that hosts do not exert influence on the protein composition of the studied helminth.

The different numbers of the polypeptide fractions, the different electrophoretic mobility, different pI, as well as the studies of Pozio et al.1992 [⁴] give as grounds to assume that the water-soluble proteins of the isolates: ISS03, ISS13, ISS10, ISS02, ISS029, ISS035 belong to the species: *T. spiralis*, *T. pseudospiralis*, *T. nativa*, *T. britovi*, *T. nelsoni* and *T. murrelli* and display both common protein components as well as typical ones for the individual isolates and can be successfully implemented in the identification of various strains and isolates from the *Trichinella* genus.

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CO2. ALTERNATIVE METHODS FOR CONTROL OF HELMINTH PARASITES

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During the last 10-15 years it has been observed an increasing need of development of new alternative for chemical control of parasitic nematodes in animals. The major reason is the serious development of anthelmintic resistance in parasitic populations. The global tempo of development and extent of anthelmintic resistance in helminthes indicates that the numerous anthelmintics and strategies developed and implemented over the period of last 40-50 years have been incorrectly applied. Biological control of animal parasites could become a strong arm for integrated parasite control in the very near future. Biological control is defined as the action of natural enemies which maintain a parasitic population at levels lower than would occur in the absence of enemies. This not only includes classical unexploited organisms but also those that are genetically modified to enhance these properties (Waller and Faedo, 1996). Biological control is divided into two major categories - natural and applied. Natural biological control is affected by native or co-evolved natural enemies in the environment without human intervention. A number of organisms have been identified to exploit the free-living stages of parasites as food source. That organisms are micro-arthropods, protozoa, predacious nematodes, virus, bacteria and fungi. Fungi that exhibit anti-nematode properties have been known for a long time. They are divided into three major groups based on their morphology and types of nematode-destroying apparatus – predaceous fungi, endoparasitic fungi and egg-parasitic fungi (Barron, 1997). Paraud and Chartier (2003) have tested the effect of nematophagous fungus *Duddingtonia flagrans* on third stage larvae of *Teladorsagia circumcincta* and its influence on the survival of first stage larvae of *Muellerius capillaris*. Another method for biological control of helminthes is the use of some plant extract. For example, Lalchandama et al. (2009) have tested ethanol extract of *Acacia oxyphylla* against *Ascaridia galli*. Many authors have studied the use of allicin from garlic against experimentally induced *Ascaridia galli* infection in chickens

(Velkers et al., 2011). The use of phytogetic bioactive compounds to control poultry helminthes is increasing in different production systems. In vitro and in vivo anthelmintic activity of citrus peels ethanolic extracts against *Ascaridia galli* was investigated (Abdelqader et al., 2012).

Acknowledgements: This work is connected with a co-project between the BAS and RAS (2012-2014).

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CO3. MACROPHAGE ACTIVITY IN MICE WITH INFLUENZA INFECTION, TREATED WITH NATURAL COMPOUNDS

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Seasonal influenza viruses are a major cause of respiratory infection in humans and result in substantial illness, death and economic burden worldwide. Current vaccination strategies and antiviral drugs provide limited protection, therefore, new strategies are needed. The application of natural products with antiviral and immunostimulating activity is a promising approach for the treatment of influenza. In the present study we investigated separately and combined treatment with polyphenol-rich extract from *Geranium sanguineum* L. (PC) and protease inhibitor (PI).

In *in vivo* experiments, mice with lethal experimental influenza infection were treated with both natural compounds in different combinations. The infection was induced intranasally (i.n.) under slight ether anaesthesia with A/Aichi/2/68 (H3N2) influenza strain, adapted to murine lungs.

The effect of the therapy was studied on 2, 6 and 8th day post infection in *in vitro* experiments. Stimulation of the immune system is a needed therapeutic approach for the

treatment of influenza, so we investigated the effect of a combination of plant polyphenol extract and protease inhibitor on the functions of alveolar macrophages (aMa) and peritoneal macrophages (pMa), isolated from treated healthy and influenza virus infected mice. The results obtained, clearly showed that macrophage functional activity (spreading and phagocytosis) was stimulated in different degree and it was established a beneficial effect of the combination of both compounds (PC + PI) on the spontaneous NO production and myeloperoxidase activity in macrophages, derived from treated animals. Based on results obtained we could suggest that the combination of polyphenol-rich extract from *Geranium sanguineum* and protease inhibitor is a promising therapeutic approach, useful for the treatment of influenza-virus infection. Further studies are needed to elucidate their biological effects in *in vivo* conditions.

Acknowledgements: This study was supported by a research grant DO 02 188/2008 from The National Science Fund, Bulgaria

CO4. ANTIVIRAL AND VIRUSTATIC ACTIVITIES *IN VITRO* OF MASTIC GUM EXTRACT, OBTAINED FROM THE TRUNK AND LEAVES OF *PISTACIA LENTISCUS VAR. CHIA*

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All the Mediterranean countries are extremely rich in native plants, whose medicinal properties have known since ancient times. The southern part of Chios, a Greek island in the Aegean is the only place in the world where the mastic tree (*Pistacia lentiscus var. Chia*) exudes its aromatic resin, hence mastic's Greek nickname “tears of Chios”. Mastic gum and its essential oils displays beneficial action against various gastrointestinal disorders, *Helicobacter pylori*, *Escherichia coli*, contributes to oral hygiene, providing teeth protection against plaque formation, induces apoptosis and possess anti-proliferative, anti-inflammatory, cardio- and hepatoprotective and cosmetic abilities.

In this study we aimed to detect a possible inhibitory and virustatic effect of the Chios mastic gum, extracted from oleoresin exudates on the replication of Herpes Simplex type 1 and type 2 (HSV-1 and HSV-2), human cytomegalovirus (HCMV) and influenza virus type A (Inf A). Viruses assayed herein were chosen for their different structural characteristics, replication mechanisms and pathogenesis. The herpes viruses have one very important property – latency and under the influence of stress factors infection can be reactivated. The antiviral substances known so far (e.g. acyclovir) exhibit their virustatic activities only in the proliferative phase and not in the latent phase thus

they are unable to eradicate the virus. However, the widespread use of nucleoside-based drugs, especially among HIV positive patients, selects resistance. The high prevalence of HSV-resistant strains among these populations suggests that new antiviral drugs should be discovered. HCMV has a high level of contamination (30 – 40% of the population). Trough placenta transmission in the carrier, the HCMV can cause malformation. Moreover, infection with this virus leads to fatal pneumonia, myocarditis or hepatitis in immunocompromised hosts with HIV-infection or organ transplantation. From the other hand, influenza continues to be a major public health problem and much efforts have been extended towards the discovery and development of this significant viral disease.

To assess the cytotoxic effect of extract of Chios mastic gum *in vitro*, we tested human lung fibroblasts (MRC-5) and Madin-Darby canine kidney cell line (MDCK). The maximal nontoxic concentrations (MNC) as well as concentrations required reducing cell viability by 50% (CC_{50}) at the 24h and 48h was determined using the MTT assay. Commercially available antiviral drug aciclovir served as positive control. To determine the antiviral activity of mastic gum extract, preliminary screening by the CPE inhibition assay was performed against HSV-1, HSV-2, HCMV and Inf A. The data, which show 2 log of the viral CPE at the MNC concentration, were considered active. Their activity was confirmed by the plaque reduction assay (HSV-1, HSV-2) or MTT method (Inf A). Viral stocks of HSV-1, HSV-2 and HCMV were prepared in MRC-5 cells and MDCK cells for Inf A. The selective indexes (SI) of the active mastic extract against HSV-1, HSV-2, HCMV and Inf A virus, respectively, justify a further analysis. SI values higher than eight contain potentially useful effect against some viruses (Hayashi *et al.*, 1998).

Comparable CC_{50} and MNC (24h and 48h) data were obtained for mastic gum resin extract (100 $\mu\text{g/ml}$, 1500 $\mu\text{g/ml}$) and aciclovir (100 $\mu\text{g/ml}$, 580 $\mu\text{g/ml}$) for MRC-5 cell line. The MDCK cells were less sensitive to tested extract than other cell line. The mastic gum extract was active against HSV-1 and Inf A, but not to effective to HSV-2 infection. Tested compound was not active to HCMV. This oleoresin extract presented high SI values, especially against HSV-1 (SI = 110,7), HSV-2 (SI = 58,05) and Inf A (SI = 29,5). These data confirm that the existing HSV resistance to acyclovir can be overcome using appropriate extract.

This native specie from Aegean island area is interesting because its popular uses support its efficacy in treating of HSV and strains of influenza viruses type A. The development of experimental strategies to isolate and characterize the biological activities of some Mediterranean herbals will provide new potential antiviral agents to threat these relevant viral infections.

CP1. NEWLY SYNTHESIZED ZINC/SILVER COMPOUND: PRELIMINARY INVESTIGATIONS ON ITS CYTOTOXIC AND ANTIVIRAL ACTIVITIES

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The Schiff-bases are widely employed as ligands in coordination chemistry. These ligands are versatile and allow a good control over the stereochemistry of the metallic centers, as well as over the nuclearity of the complexes, by the appropriate selection of the starting materials (carbonyl precursors and primary amines). All these advantages make Schiff bases suitable candidates in the effort to synthesize metal complexes of interest in bioinorganic chemistry, catalysis, encapsulation, transport and separation processes, etc.. In a recent paper, Marinescu and coworkers showed that the self-assembly process between binuclear $[Zn_2L_1]^{2+}$ complex cation and complex anion $[Ag(CN)_2]^-$ generates one-dimensional coordination polymer with the following formula: $1 \infty [\{ L_1 Zn_2 (\mu_3-OH) \} 2 (H_2O) \{ \mu - [Ag(CN)_2] \}] (ClO_4)_3 \cdot THF \cdot 0.5 MeOH$ (H_2L_1 is bicompartamental Schiff-base ligand resulting from condensation reaction of 2,6-diformyl-p-cresol with 2-aminomethyl-pyridine) [1-4].

The aim of our study was to evaluate the effects of this newly synthesized zinc/silver compound (noted as Zn-ampy-Ag) on viability and proliferation of bovine kidney MDBK cells and replication of bovine herpes virus type 4 (BHV-4) strains Movar 33/63 and DN 599. The investigations were carried out by thiazolyl blue tetrazolium bromide (MTT) test and method of Reed and Muench. The viability of MDBK cells cultured for 144 h in the presence of the compound tested applied at concentrations of 1.6; 3.1; 6.3; 12.5; 25.0 and 50.0 $\mu g/ml$ was found to be $> 92\%$ ($p > 0.05$). At concentrations of 25.0 and 50.0 $\mu g/ml$ the Zn-ampy-Ag did not decrease the titer of BHV-4.

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CO5. MICROBIOLOGICAL ASSESSMENT OF NEW COSMETIC PRODUCT BASED ON NATURAL INGREDIENTS

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Natural products are a subject which presents a great interest in the modern way of life and are a part of natural orientated trends in cosmetics. In answer to these trends the purpose of this research was to create a new natural cosmetic product (hand-made cosmetics), which is a mixture of bioactive compounds with natural and animal origin with a revitalizing effect on the skin and an antimicrobial activity.

The preparation of this product, named "Ksenoleum", includes a set of classical technologies: gradually adding of the raw materials, mixing and dissolving until a stable and homogenized product is created. The order in which the compounds were added is based on their physical chemistry properties. The product has been subdued to standard cosmetics quality tests and microbiological analysis in two directions-Agar Diffusion test and determination of Minimal Bactericidal Concentration (MIC).

"Ksenoleum" includes in itself ingredients which are traditionally used in bulgarian medicine and some others which are novel compounds in the national cosmetic industry but still have a good perspective. For the base of the mixture Ostrich oil (*Oleum Struthio*) and Beeswax (*Cera flava*) were used. The healthy effect of the unsaturated fatty acids like omega 3,6 and 9 and vitamin A (retinol) was enforced with Grape seed oil (*Vitis Vinifera Oleum*) and Wheat germ (*Oleum Triticigerminis*) which are rich in antioxidants. The characteristic aroma was achieved with the use of Cinnamon oil (*Oleum Cinnamoni*) which also strengthens the bioactive effect. The nature of the used substances does not require the additional use of chemical improvers: emulators and preservatives.

In view of the non-specific purposefulness of the components the vaseline is expected to have a wide-array of effect."Ksenoleum" moisturizes and protects the skin from atmospheric influences, has anti-wrinkling effect, prevents skin irritations, removes scars and also has an antioxidant and aromatherapy effect. The results of the given microbiological test represent antimicrobial influence against pathogenic species as follows: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Escherichia coli*. By comparing the assembled data it was concluded that Gram-positive microorganisms are more sensitive to the antibacterial compounds of the vaseline. An expectable reason for this is found in the specificity of architecture of cell wall and the difference in the diffusion of analyzed product.

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Session D.

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DO1. SR-MODIFIED DICALCIUM PHOSPHATE DIHYDRATE AS A BONE GRAFT CANDIDATE

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The aim of the present study was to evaluate the effect of strontium-modified dicalcium phosphate dihydrate ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, brushite) on viability and proliferation of cultured human Lep 3 cells using various cytotoxicity assays: thiazolyl blue tetrazolium bromide (MTT) test, neutral red uptake cytotoxicity assay (NR), crystal violet staining (CVS) and double staining with acridine orange and propidium iodide (AO/PI). The results obtained reveal high survival rate of the cells after 72 h cultivation in a $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ -modified medium - $81.5\% \pm 3.4$ (CVS), $98.3\% \pm 4.6$ (MTT) and $110.3\% \pm 4.6$ (NR). No cytopathological changes were observed in the treated cells after staining with AO/PI.

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DP1. VIABILITY AND PROLIFERATION OF MURINE BONE MARROW CELLS CULTURED IN THE PRESENCE OF NEWLY SYNTHESIZED COMPOSITE MATERIALS FOR BONE IMPLANTS

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Calcium phosphate bioactive materials have been used in the medicine and dentistry due to their chemical and structural similarity with bone tissue mineral composition. The aim of our study was to evaluate the influence of three newly synthesized composite materials for bone implants on viability and proliferation of cultured murine bone marrow cells (BMC). BMC were obtained according to the standard procedure from femurs of ICR mice (2 months old) from both sexes. The cells were grown in 24 well/plates in the presence of the composite materials. The BMC cultured in non-modified medium served as a control. The investigation was performed after 72 h of treatment by trypan blue dye exclusion test using a Cell counter (Invitrogen). The percent of viable cells was found to vary between 46.67% and 67.16% (after 3 day treatment period) and to be 14.58% - 79.4% (on the 6th day) as compared to the control. Additional experiments are underway to clarify better the biocompatibility of the examined composite material as well as its osteoconductivity and osteoinductivity.

Acknowledgement: This study was supported by Grant DTK 02-70/2009, National Science Fund, Bulgaria; European Social Fund and Republic of Bulgaria, Operational Programme "Development of Human Resources" 2007-2013, Grant № BG051PO001-3.3.06-0048 from 04.10.2012.

DO2. IN VIVO BEHAVIOUR OF CALCIUM PHOSPHATE COMPOSITES

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Aim. The ability of composite scaffold on the base of Zn-modified-tricalcium phosphate and mixtures of xanthan gum, caragenan and gelatin was studied in vivo. Histological and biochemical studies were done on the rats with the implants.

Material and methods. Preparation of the composite scaffold. The hydrogels from gelatin (20 mass.%), xanthan gum (1 mass %) and karagenan (1mass %) were preliminary prepared and mixed. The powder of Zn- β -TCP and saccharose were added and homogenized. Then, the dense pastes were molded as cylinders and lyophilized. The samples were washed with the water and hardened by 1% glutaraldehyde.

In vivo experiment. Bone defects were created in rat tibia. A longitudinal holes were created on the medio-proximal region of the right tibia. The rats were divided into 3 groups – 1st -control (with autologous implant), 2nd gr.– defects filled with Zn-modified-tricalcium phosphate and mixtures of xanthan gum and gelatin (W21) and 3rd gr. defects filled with Zn-modified-tricalcium phosphate and mixtures of xanthan gum, caragenan and gelatin (W23). The biochemical studies were done prior operation, at the end of the 2-nd week and 14th week. The serum bone markers Ca, P, total alkaline phosphatase (TAP) and bone alkaline phosphatase (BAP) were measured using Human diagnostic kits by the Screen master 588 LiHD 111. After 14 weeks the rats were euthanized and the tibia retrived for the histological evaluation.

Results. Composite scaffold. Natural bioresorbable polysacharide xanthan gum and karagenan as well as gelatin were used for composite scaffold preparation. Dissolution of the powder saccharose ensures preliminary porosity while the glutaraldehyde increases the hardness.

Biochemical studies. At the beginning of the experiment the level of Ca, P and Zn in all rats as well as the activity of the enzymes were similar. At the end of the experiment the values of Ca, P and Zn were slightly increased in the rats from the gr. 2 and 3 in comparison with the controls. At the 14th week the activity of the BAP and TAP were reduced in the rats from gr. 2 and 3 compared to those from the gr. 1. The levels of Ca, P, Zn, TAP and BAP were in physiological values during the experiment.

Histological studies. The control defects of the tibia were repaired with a bridge like osseous tissue considerably thinner than the bone walls before defect creation. The tibial defects with W21 implants were with similar morphological features with slightly bended osseous tissue grown under the implanted material. These implants lost their cylindrical form and were fragmented in different in size pieces ingrown with a new formed fibrous tissue in a reticular pattern. Following 14th week after the implantation procedure the W23 implants remained their cylindrical forms. They were surrounded with cylindrically formed thin osseous tissue flattening the bone marrow against the opposite inside part of the tibial wall.

The new material did not have a negative influence on biological response of organism. The data showed good tissue tolerance to the new materials.

DO3. MANUFACTURING OF SCAFFOLDS WITH INTERCONNECTED POROUS HYDROXYAPATITE CERAMICS IN POLY(L-LACTIC ACID) MATRIX FOR BONE TISSUE ENGINEERING

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Introduction: Tissue engineering was defined as an interdisciplinary field that applies the principles of engineering and the life sciences toward a development of biological substitutes that restore, maintain, or improve tissue function. Currently, the approaches to provide these requirements involve a provisional scaffold or matrix in combination with cells. These scaffolds should be biodegradable, but also stable enough to function as three-dimensional (3-D) constructs. The aim of this study was to develop a way to obtain an interconnected three-dimensional (3D) porous polymer-ceramics composite material as well as to investigate the morphology, thermal and biological properties of the hybrid scaffolds.

Materials and methods: A commercial PLLA grade Natur 2002D was supplied by NatureWorks®, USA. Calcium hydroxyapatite (HA) ceramics have been synthesized and used clinically as bone substitutes with PLLA matrix. NaCl particles of 350 to 400 μm particle sizes were hand-sieved. The milled PLLA and HAP powders were individually mixed in **65/35 wt %** ratio; then the mixture was blended with the sieved NaCl particles. The compression-moulded (CM) plates from PLLA/HAP/NaCl mixture (40/20/40 by wt. %) and PLLA/NaCl mixture (60/40 by wt. %) were manufactured. The scaffolds have 0,6 mm thickness, 50 mm length and 10 mm width. The composite materials were prepared by hydraulic press on a steel mould at 175 °C with constant load of 15 kPa for 5 min. The leaching of the NaCl from the CM plates was performed in beakers filled with distilled water. The poly (L-lactic acid) scaffolds were biomineralisation whit simulated body fluid (SBF).

Results and discussion: Upon immersion of the PLLA/HAP/NaCl and PLLA/NaCl plates into water, the NaCl was leached out and the 3-D micropores open cellular structure was created. The topography and crystallinity of the scaffolds could play an important role in regulating cell behaviour and the time of bioresorption of the implant in the living tissue.

The Scanning electron micrograph of the surface and cross-sections of the hybrid composite showing a uniform distribution of the HAP nanoparticles within the PLLA matrix. After 30 days biomineralisation of the scaffolds in simulated body fluid at 37 °C layers of calcium phosphates (CaP) nucleus over the PLLA plate were observed. By X-ray diffraction (WAXS) and differential scanning calorimeter (DSC) methods were investigated: the degree of crystallinity, the thermal properties and the role of hydroxyapatite (HAP) and calcium phosphates (CaP) nucleus in poly (L-lactic acid) matrix. The manufactured polymer-composite scaffolds were implanted in the field bone

tissue defect (calvaria). The *in vivo* investigations showed forming of a thin fibrous capsule without any inflammation response around the implant.

Acknowledgement: The authors gratefully acknowledge the financial support of the Bulgarian Ministry of Education and Science, Fund “Scientific Investigation” (Project DTK 02-70/09)

DO4. DEVELOPMENT OF INJECTION AND ORAL AGENTS IN THE TREATMENT OF MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is the most common autoimmune inflammatory demyelinating disease of the central nervous system (CNS). Many young individuals are afflicted. Although the basic pathology of MS was already recognized in the 18th century (145 years), its causes and pathogenesis remain elusive. The clinical progress can be characterized by a relapsing-remitting MS (RRMS) or a chronic progressive course, and the pathological features of MS consist of inflammation, demyelination, gliosis and axonal degeneration. Several therapies for MS exist, although there is no known cure. Based on this understanding, the initial therapeutic strategies were directed at immune modulation and inflammation control. Currently available MS therapies have shown significant efficacy throughout many trials, but they produce different side-effect profiles in patients. Since they are well known and safe, they require regular and frequent parenteral administration and are associated with limited long-term treatment adherence. One of the most common ways to manage MS is by taking an injectable therapy. There are several types of injection therapies which are administered differently — either into the muscle or under the skin (corticosteroids, interferon beta, copaxone, mitoxantrone, natalizumab etc.). These injection agents designed to help reduce the frequency of relapses — some even slow disability progression.

Oral medicaments are possibly more effective than, current injectable agents. It is believable that improved outcomes will translate into higher real and perceived efficacy rates and contribute to improved adherence. The decision to switch established patients from injectable to oral medications will be made on balancing the efficacy and tolerability of the patient’s existing therapy and their compliance history, even though safety is likely to become the most important factor in the future development of MS drugs.

Most often **corticosteroids** (methylprednisolone, prednisone, and dexamethasone) are administered with immunosuppressive or anti-inflammatory effect.

Interferon beta-1a (also interferon beta-1-b) is a drug in the interferon family used to produce about a 18–38% reduction in the rate of MS relapses, and to slow the progression of disability in MS patients.

Copaxone (glatiramer acetate) is a random chain (polymer) of amino acids. It has been shown in clinical trials to reduce the average relapse rate in people with RRMS.

Mitoxantrone is an immunosuppressant. It is used to treat several forms of advancing MS, including secondary progressive MS, progressive relapsing MS, and advanced RRMS.

Natalizumab (tysabri) is a monoclonal humanized antibody often used in the treatment of multiple sclerosis. It is another immunosuppressive drug and has been administered in combination with interferon beta-1a.

Immunosuppressive treatments are frequently associated with suboptimal response in terms of efficacy and several side effects leading to poor patient adherence. Thus, there is an important need for new therapeutic strategies, especially those that may offer greater patient satisfaction in order to optimize therapeutic outcomes.

Currently, oral therapies with cladribine and fingolimod have been approved for the treatment of RRMS. **Cladribine** (litak) is an oral drug used to treat MS. In 2011 the FDA has rejected cladribine for MS and requiring more data on its safety and risk-benefit.

Fingolimod (gilenya) is an oral immunomodulating drug with a novel mechanism of action and unique immunological and neurobiological properties. It has reduced the rate of relapses in RRMS by over half, but has serious adverse effects.

In October 12, 2012 (Lyon, France) Susan Jeffrey reports full results of the **Teriflunomide** oral use in people with RRMS. Results showed a significant but smaller annualized reduction in relapse rate but not in sustained accumulation of disability in the disease.

The salts and esters of the **Fumaric acid** are known as fumarates. Dimethyl fumarate significantly reduces disability progression in patients with RRMS.

Laquinimod is an experimental immunomodulator investigated as an oral treatment for MS. Teriflunomide, fumaric acid and laquinimod are in a phase 3 trial.

The recent studies found that over 60% of MS patients use complementary and alternative medicine, possibly because conventional treatments lack effectiveness.

Combination therapy (polytherapy) in MS is the use of more than one medication or other therapy. The studies that we have now are all single-agent studies, other than this one. There are hardly any combination trials. But, moving forward and thinking about what we have learned from this design, it would be of interest to take an anti-inflammatory agent and combine it with a neuroprotective agent, so you cover more of the pathologic spectrum of multiple sclerosis.

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DO5. ALCOHOL BASED FIXATIVES PROVIDE EXCELLENT TISSUE MORPHOLOGY, PROTEIN IMMUNOREACTIVITY AND RNA INTEGRITY IN PARAFFIN EMBEDDED TISSUE SPECIMENS

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Fixation techniques preserving morphological fidelity, protein antigenicity and integrity of nucleic acids can have a high impact on both basic and applied biomedical sciences and diagnostic pathology. Different types of mouse tissues were fixed with neutral buffered formalin, ethanol supplemented with acetic acid and modified methacarn (methanol-Carnoy) fixative. The alcohol-fixed samples were processed in an Autotechnicon tissue processor or in an incubator. The preservation of tissue morphology was assessed in all specimens and the immunoreactivity was evaluated with antibodies specific for proteins with nuclear, membrane or cytoplasmic localization. RNA was extracted from all groups of fixed hind limb skeletal muscle specimens and was assessed versus unfixed tissue for preservation of its quantity and quality by amplification of gene-specific fragments of different lengths. Both alcohol-based fixatives preserved the tissue architecture and the specificity of immunoreactivity in excellent quality; the trimming approach did not result in detectable differences. Oligonucleotide fragments of length between 108 and 577 base pairs were amplified from all groups of alcohol-fixed skeletal muscle specimens in amounts comparative to the unfixed muscle tissue. We conclude that both alcohol-based fixatives are an excellent tool for storage of tissue samples designed for immunohistochemical and mRNA expression studies when the access to fresh samples is limited.

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DO6. CONNECTION BETWEEN ESTROGEN AND OPIOID RECEPTORS

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Liu NJ, Chakrabarti S, Schnell S, Wessendorf M and Gintzler AR from State University of New York, Downstate Medical Center, Brooklyn, New York, USA in 2011 published an article in “The Journal of Neuroscience”. The name of the article is “Spinal Synthesis

of Estrogen and Concomitant Signaling by Membrane Estrogen Receptors Regulate Spinal κ - and μ -Opioid Receptor Heterodimerization and Female-Specific Spinal Morphine Antinociception” and it is about that men and women experience pain in different ways. Using Sprague Dawley rats they demonstrate that spinal synthesis of estrogen is critical to these processes (of forming and using KOR/MOR heterodimers), and blockade of either estrogen receptor (ER) α -, β -, or G-protein-coupled ER1 or progesterone receptor (PR) substantially reduces KOR/MOR and eliminates mediation by KOR of spinal morphine antinociception. KOR/MOR could serve as a novel molecular target for pain management in women.

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Liu NJ, Chakrabarti S, Schnell S, Wessendorf M, Gintzler AR. Spinal synthesis of estrogen and concomitant signaling by membrane estrogen receptors regulate spinal κ - and μ -opioid receptor heterodimerization and female-specific spinal morphine antinociception. *J Neurosci.* 2011 Aug 17;31(33):11836-45.

Session E.

Chairpersons:

Assoc. Prof. George Miloshev, PhD

Institute of Molecular Biology, Bulgarian Academy of Sciences

Assist. Prof. Delka Salkova, DVM, PhD

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

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EO1. CHANGES OF BIOLOGICALLY ACTIVE AND ANTICANCEROGENIC SUBSTANCES IN COW'S MILK OF THE BULGARIAN RHODOPES CATTLE BREED

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The fatty acid composition of the milk fat of milk from cows of the Bulgarian Rhodopes cattle breed (dairy breed) during stable and the transition to pasture-stable rearing in the period April-July was investigated. An increase of the saturated fatty acids as a result of the stable feeding from 68,95 to 75,32 g/100 g fat and a decrease of their quantity up to 69,66 g/100g fat after the transition to pasture-stable rearing breeding with adding of combined forage and lucerne were established.

The content of mono- and polyunsaturated acids decreases during barn breeding. The quantity of vaccenic, oleic, omega-6 fatty acids decreased, while for CLA (conjugated linoleic acid) an increase of the quantity was observed. After the transition to barn/pasture breeding the quality of the milk in respect to biologically active substances (mono- and polyunsaturated, omega-3 and CLA) improved.

Keywords: cow's milk, Bulgarian Rhodopes cattle, milk fat, fatty acid composition, CLA, omega-3 and omega-6 fatty acids.

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EO2. CONTENT OF BIOLOGICALLY ACTIVE SUBSTANCES IN FLOUR FROM OAT AND RYE

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The fatty acid profile and the mineral composition of oat, rye and a mix of rye, oat and white flour has been investigated with the objective to establish the biologically active substances and to improve the mixes for bread production.

The fatty acid composition of oat, rye and a mix of rye, oat and white flour is presented mainly by linoleic acid(C18:2)- respectively 41,60, 48,31 и 52,75 g/100g fat, γ - linolenic acid (C18:3n6)- 6,63 g/100g fat in the mix, α - linolenic acid (C 18:3n3)- 1,30, 5,26 и 5,19 g/100g fat and dihomogamma- linolenic acid (C20:3n6)- 11,29 g/100g fat in the rye flour and 0,1 g/100g fat in the mix. The total content of the monounsaturated fatty acids is e 37,57 g/100g fat in the oat, 18,60 g/100g fat in the rye flour and 19,39 g/100g fat in flour mix, and of polyunsaturated fatty acids 43,11 g/100g fat, 65,07 g/100g fat и 64,69 g/100g fat, respectively. The oat and rye flours are rich in omega- 6 (41,75 g/100g fat in the oat and 59,77 in the rye) and poor in omega- 3 (oat- 1,36 g/100g fat and rye flour- 5,28 g/100g fat) fatty acids.

The analyzed oat flour for macro and trace elements is with the following composition: calcium- 865 mg/kg, potassium- 3924 mg/kg, manganese- 1815 mg/kg, sodium- 17,7 mg/kg, phosphorus- 7173 mg/kg, iron- 54,2 mg/kg, copper -7,85 mg/kg and zinc- 42,60 mg/kg. The rye flour is characterized by a relatively high content of calcium- 350,6 mg/kg, potassium- 4069,5 mg/kg, sodium- 7,47 mg/kg and low content of copper - 2,71 mg/kg, zinc- 13,89 mg/kg and iron- 15,29 mg/kg. The combining of the three flour types leads to an increase of the content of calcium with 50,59% and sodium with 21,75% and a decrease of the content of potassium with 21,75%, while the trace elements keep their quantity except for iron, where is recorded an increase with 52,65%,and a decrease of zinc with 24,05%.

Keywords: oat, rye and white flour, fatty acids, macro elements, trace elements.

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EO3. ANTIOXIDANT CAPACITY OF DIFFERENT TOMATO LINES DURING HOME STORAGE

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The aim of this study is to compare the antioxidant capacity of ripe tomato fruits whit various color during home storage. Six different tomato lines were investigated: At16 (pink fruits, high anthocyanins content), Xp6 (pink fruits, high lycopene content), X2 (red fruits pink fruits, high lycopene content), Bcr 8-11 (yellow fruits), H13 (orange

fruits, high beta carotene content), J2-11 (orange fruits, lower beta carotene content than H13), as well as combination of equal parts of (Xp6) +(Bcr8-11)+(H13)+(J2-11). In our investigation the following methods were used: the ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) assays after two step procedure of processing and extraction of water-soluble and lipo-soluble antioxidants. Three measurements were made - immediately after picking of the fruits and three and six days later. On the basis of the fresh weight of the fruits, At 16 line had the highest TEAC activity and FRAP (respectively expressed as micromoles of Trolox equivalents per gram and as micromoles ferric reducing antioxidant power) during the whole experimental period. Our analyses of water soluble fraction showed an increase of TEAC values during storage in all experimental groups (excluding Xp6 line). Highest activity in FRAP assay (both water and lipo soluble antioxidants) was detected three days after the picking in all variants. It is worthy to note that the antioxidant activity of combination of (Xp6)+(Bcr8-11)+(H13)+(J2-11) (measured by TEAC and FRAP assays) showed a higher antioxidant capacity as compared to the values obtained from the individual samples.

EO4. IMMUNOLOGICALLY ACTIVE PECTIC POLYSACCHARIDES FROM ORANGE FRUITS

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Water- and acid-soluble orange pectic polysaccharides (WEOP_(c) and AEOP_(c)) with significant immunological activity, were isolated from dried orange peels by sequential extraction with hot distilled water and hot (0.5%) HCl. Fresh orange peels were microwave (MW) pretreated for 10 min (2450 MHz, 0.63 kW), and used after drying for isolation of water- and acid-soluble pectic polysaccharides (WEOP_(mw) and AEOP_(mw)). MW pretreatment was used for a better extraction of pectin, resulting in an increase (96 %) in the yield of water-soluble orange pectins. Chemical and physicochemical characterization of all pectins was accomplished. All extracted pectins were high-methoxylated (> 50 %), and with high anhydrouronic acid content (> 70 %). Degree of acetylation was varying between 1.52 – 2.07 %. Ester-linked ferulic acid (degree of feruloylation) was presented in minor amounts in all pectins. The neutral sugar HPLC profile of all investigated pectins was as common recognized: L-Ara, D-Gal, L-Rha, D-Xyl and L-Fuc. High D-Gal and L-Ara presence showed that pectic side chains were predominantly from arabinan and galactan type or presumably arabinogalactans. Pectic polysaccharides differed in molecular weight and homogeneity. All investigated pectins showed *in vitro* immunomodulating activity by complement activation in the classical and alternative pathway, at four different concentrations between 1.25 – 5.0

mg/mL on sensitized mutton red blood cells. It was made a comparison between some biologically active pectic polysaccharides from different plant sources isolated previously in our lab. WEOP_(mw) and AEOP_(mw) were modified with endo-polygalacturonase (PGI, *Asp. aculeatus*). Carbohydrate profile of AEOP_(mw) and its enzyme modified product, obtained after alcohol precipitation were investigated by size-exclusion chromatography on Sephacryl S-300 HR.

Keywords: modified citrus pectin, microwave irradiation, immunostimulating pectic polysaccharides.

EO5. DAMAGING ACTIVITIES OF SEVERAL COMPOUNDS ON THE GENOME OF TWO YEAST SPECIES - *S. CEREVISIAE* AND *K. LACTIS*

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Genotoxins are physical or chemical agents that induce damages in DNA molecule. They can be chemical compounds or physical agents like UV light, X- and gamma rays, etc. A large number of methods have been developed to detect genotoxicity. The usefulness of these tests depends on many parameters such as sensitivity, cost, time, versatility and feasibility. One of these methods is the Comet Assay which is widely applied for investigation of the DNA damaging effect of diverse types of compounds. For the very first time the method has been developed for use on yeast cells *Saccharomyces cerevisiae* and as a result it has been named *Yeast Comet Assay*.

Two yeast species *Saccharomyces cerevisiae* and *Kluyveromyces lactis* are generally used as model organisms. In this study, the method of *Yeast Comet Assay* has been applied for the first time on the yeast *Kluyveromyces lactis*. Specific modifications of the method have been developed and the standard genotoxins hydrogen peroxide, methyl methanesulfonate and the food additive – sodium nitrite (E 250) were tested.

A comparison between the sensitivity of both yeast species by *Yeast Comet Assay* will be discussed.

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EO6. GENETIC ENGINEERING, SEEN THROUGH THE LENS OF TWO YOUNG LADIES... POISON FOR THE AVERAGE PEOPLE=DOPING FOR THE LABORATORY MUTANTS

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Did science cross the border that should have remained untouched?!- that was the question which we dived with into the infinite science. Everything started when our country was shaken of discussions either for or against the genetic engineering (GE). Like most, we also were not very informed on this matter. Though, our curiosity and all the opportunities we are provided by the 21st century urged us to understand the topic scientifically, but not the way it was presented by all the media.

While **searching** the Net, looking for publications on this quite new branch of science, we came across the words of Erwin Chargaff, an Austrian- American biochemist. These words, after rephrasing them, we have chosen for the initial question. After that, step by step, we began to puzzle out the problem.

The first particle from it gave us the information about Dolly, the sheep, the cold resistant tomato, the pomato...and more, and more **examples**. However, how does it all happen?! It is hard to believe that this process could be presented in, at first sight, simple **stages**: isolating DNA fragment, finding an appropriate vector, using restrictases and ligases, introducing the DNA fragments in the host cell.

We included a brief **overview of the GE’s history heretofore** in our work in order to arouse society’s interest on it. As we have already mentioned the society, our purpose was to be helpful in any way- that is the reason why we started up this challenge. That is why we covered **the involvement of GE in our food**- the thing we have not been able to live without, yet. And what is the actual meaning of food?! It is different for everybody- for Asia means rice, America- corn; Bulgaria- wheat. Because of this, there had no way for us to miss the plants, occurring to be vital for all the people around the world. We focused on these that have already been produced by genetic manipulation- wheat, oat, rice, corn, soybean.

To have a clear view over the situation in Bulgaria, we have made some **references on how common genetically-modified organisms (GMO) are in our country**- both at the retail network and in nature, comparing the results with global and European data

Everything has two sides. On the one hand, there are a lot of **problems and dangers**, connected with GE- using it for acceleration of evolution, increasing the yields of many cultures, we, though don’t know neither what we can exactly expect, nor whether

the result will be only positive or we will self-destroy because of our lack of information, causing a dangerous mutation or a lethal allergy. Another disadvantage is gaining the so called **terminator seeds** which alter the balance in nature. GE is one of the main reasons for exacerbation of **bio prospecting** and **world's famine**. One more negative feature is that the effect of genetically modified products (GMP) is tested on people in the Third World, although, that is an open secret. These products contain antibiotics, which increase human's resistance to different medicines and then these meds cannot be effective while used for treating diseases. GMPs contain also industrial enzymes which cause different **allergies**. As an alternative of GE in agriculture, we offer **organic farming**, though, it is financially disadvantageous.

On the other hand, we find the other face of **GE**- these technologies are irreplaceable **in medicine**- for diagnosing, prevention and even for synthesizing essential hormones, used as medicines (insulin, interferon, relaxin). Maybe, it sounds naive and impossible for somebody, but by changing a "wrong" gene with a "new" one, scientists can give people with genetic diseases a chance for a normal live- achievement of the **gene therapy**, a branch of GE.

We do really hope to have succeeded in **presenting objectively** enough both the **pros and cons for GE**, because our aim is not to change society's opinion, but to give the opportunity each of them to choose what is right for him on their own. This is the reason why we have chosen such a title for the project "**GMP- it's our choice!**", where "our" means personal.

After all, we can boldly announce ourselves at the team of the optimists, but with the clear consciousness of not passing through the border which should stay untouched, i.e. we say "Yes!" to GE if it is controlled by the competent institutions.

A lot of people, who are not informed enough, are against GE, because they think that GMPs are **poisonous for the average person and a doping for the laboratory mutant**. Don't we receive our everyday dose of poison with each food product (like different additives) from the shop on the street?! Yes, we do!, although our awareness again is low. That's why we designed a **handbook of the informed European consumer** in order to help people to decide what to buy at the hypermarket. Here the advice is simple- if there are more than 5 **E- additives** on the label, do not buy!! Probably, few people have asked themselves about these Es, but actually they can be really dangerous, causing a number of pathological conditions in human body. Don't know what about you, but if we have to choose, we prefer eating GM corn than a product, coloured with an insect's wings!

It is high time for us to tell you what is hidden behind the mysterious E numbers. These different additives are added to food in order to improve its taste and extend the shelf line. There are intervals of numbers for each group, every group combines additives classified according to their effects- colouring agents, preservatives, antioxidants, acids and regulators, emulsifiers, thickeners, stabilizers, raising agents, mineral salts, flavourings, and others that cannot be listed to a group. It occurred that it is not easy to understand what lies behind these innocent indexes. Here we come to the aid of the website: http://www.vegebg.org/materiali/razumniat_vegetarianec.html , where we have found a lot of information. However, still a lot of questions remained unanswered. We have understood that a great number of them are forbidden in many countries. After **conducting a poll**, we found out that people in Bulgaria do not even suspect about all of the above. Our activity was to **organize the information** in way, suitable for the person, who goes to the shop every day. Some of the E- additives are safe, but most of them are risky to consume, especially for babies and small children, because they may cause cancer, intestine upset, skin disorders, allergies.

As for the Es, we are pessimistic and because of this, we tried to offer their alternative. Has anybody heard about the **anthocyanins**? These organic pigments do wonders! Anthocyanins (in Greek "anthos" means flower; "kyanos"- blue) are the largest group of water-soluble colored compounds from the family of flavonoids. They are also interesting for those keen on chemistry, because they can change their color depending on the acidity of the cell in plants: when increasing the number of-OH groups, blue colour dominates; methylating –OH groups, leads to the occurrence of the red one.

Some research of the Institute of Feed Psychology, at the Federal Food Searching Center, shows that anthocyanins have a health effect on human body. They slow blood coagulation, catch free radicals (ROS), protect the cell and prevent against cancer; they are natural **antioxidants**. They also improve eyesight and have some anti inflammatory effect; anthocyanins help people suffering from diabetes.

In conclusion, we consider that we have already given you enough food for thought. The purpose of the project is not for you to accept our point of view. It's all in your hands. It's your choice! You decide whether to say **Yes!** or **No!**; or you may also choose the middle answer **Yo!** or **Nes!**☺

We have used various sources of information such as the books: 'Genetic engineering- yesterday, today and tomorrow' (Todor Evrev,1989), 'Genetic engineering- present and future' (Markov, Sveshtarov, 1989), '17-th chapter – Genetic engineering and genetic modified food from the 'The world's conspiracy against human health' (Atanas Gylybov), the Internet, the films: 'The World according to Monsanto' (2008), 'David vs. Monsanto', 'Future of Food.

We would like to thank the oraganisation 'Obedinen detski kompleks-Blagoevgrad' for their unstinting support for creating and developing our project (both financial and moral).

EP1. PROJECT BASED EDUCATION ON FOOD, NUTRITION AND HEALTH AS AN IMPACT FACTOR REGARDING THE MOTIVATION OF THE STUDENTS

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Contemporary pedagogical practice offers different variants of models of quality teaching and learning. One of them is related to the development of student projects. Project - Based Learning (PBL) stimulates reflection on their own cognitive activity, a process that clearly leads to the formation of metacognitive skills. The latter are pointed to be a reliable basis for the formation of personality and self-controlling their own learning, personality, able to improve throughout their lives.

Centre in the article is project-based learning. It motivates students by engaging them in the process of their own learning, allowing them to integrate knowledge from different areas of science and implement them in developing specific educational project. Based

on this, students are active participants and partners in the learning process. The aim of this study is to present specific educational projects on food, nutrition and health in the learning process in preparation to teach biology.

The experiment was conducted with students with specialization of "Geography and Biology" and "Biology and Chemistry" in the Faculty of Biology, Sofia University "St. Kliment Ohridski".

An investigation was conducted after the training. The students told us that they feel extra motivated because they have the opportunity to plan, predict, design and carry out their own plans /82% of respondents/; said that they feel like artists in the selection process, restructuring, use of multiple data resources /74%/; expressed their positive attitude by the fact that they can create their own product and can protect its purpose and meaning /82%/. 88% of students approved the use of PBL as a medium of creating individual knowledge, rather than copying others knowledge.

On the basis of this study, we can say that educational technology called PBL can function as a flexible integrated learning environment. This is an environment structured around learning rather than teaching, environment, provocative discussions and negotiations on the objectivity, accuracy, usefulness of individual knowledge, opinions, assumptions. PBL allows for individual expression of each student, according to his knowledge, skills and values in an environment of mutual cooperation and tolerance for other positions, and hence overall motivation for the learning process in biology.

Session F.

Chairpersons:

Assoc. Prof. Albena Alexandrova, PhD

Institute of Neurobiology, Bulgarian Academy of Sciences

Assist. Prof. Milena Georgieva, PhD

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FO1. PROJECT 2007CB16IPO006-2009-1-101 “CREATION OF A DATABASE OF POLLUTANTS FROM INDUSTRIAL WASTE IN A TRANS-BOUNDARY ASPECT AND A LOGISTICAL FRAMEWORK FOR PROBLEM SOLVING” - PRESENTATION OF THE RESULTS

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The Municipality of Bor in Republic of Serbia has been a major center for mining and processing of copper and other precious metals for almost a century (mining production started in 1903). Copper content of the mined material in Bor mines is in general less than 0.5%. This means that practically 99.5% is waste, which has to be separated from the copper to produce a useable product. The waste material contains many toxic components, which include large amounts of bound sulphur and metallic elements including copper, arsenic, lead etc. Mine drainage water and wastewater from mining industry can affect the natural and human environments. However by this time there were no specialized system studies to evaluate the quality and quantity of pollutants. So the aim of this project was to estimate the state of environment in cross border regions between Serbia and Bulgaria in terms of surface and groundwater, to determine the present pollutants, to evaluate the environment damages and define the environmental problems and to suggest a remediation strategy.

Analyses of water, soil and sediment samples were conducted. The results indicated that drastic pollutant concentrations cannot be found in the Timok river because of multiple dilutions of drainage solutions to them, but in the stagnant water of the old river bed the copper concentrations exceed the benchmark up to 215 times, the iron concentration – up to 324, the manganese concentration - up to 13, sulfates – up to 16.2, lead – up to 1.5 and arsenic – up to 2.4 times. All extracts of soil samples taken in the sampling points were with weak-to strong-acid reaction with relatively high conductivity

and salt content, which is probably the main reason for the presence of sterile (devoid of vegetation) terrains. Almost in all soil samples the content of sulfur, copper, zinc and lead are above their background and over the maximum allowable concentrations (MAC). The copper content in soils is from 2.26 to 37.93 times over the background and from 4.35 to 32.24 times over the MAC, and sulfur from 4.25 to 22.37 times over the average content for soils. The mechanism of uptake of contaminants in soil is by flooding them and by irrigation with river and borehole water. All tested sediments were anthropogenically loaded with products from the processing of mineral raw materials containing heavy metals and arsenic. The sediments were in process of oxidation and leaching as the mobile toxicants pollute the environment. There were conditions for their accumulation in aquatic organisms and vegetation. According to the toxic response factor provided by Hakanson (1980) there is a moderate to high risk for the environment from pollution with heavy metals – mainly copper. In its magnitude the copper contamination placed the area of the Timok River on the second place in Bulgaria - after Srednogorie (Pirdop) area.

FO2. IS THE COPPER ENVIRONMENTAL CONTAMINATION HARMFUL FOR HUMAN BEINGS?

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Copper is one of the major metals causing environmental contamination. However the copper toxicosis in the general population is remarkably low. This reflects the efficiency of the homeostatic control at the level of intestinal absorption and biliary excretion to keep tissue copper concentrations within narrow range. Nevertheless copper poisoning can develop under certain conditions, depending of many factors incl. genetics, age and diet. The principal sources of copper contamination of humans are copper water-pipes (Graedel et al., 2004; Dietrich et al., 2004), copper sprays (Bacon and Hudson, 2001) used in agriculture against fungi and algae, copper or brass cooking vessels (Tanner et al., 1983; Bhave et al., 1987; Muller et al., 1996), seafood (Bragigand et al., 2004), metallurgy and mining (Koptsik et al., 2003).

Copper can be absorbed by oral, inhalation and dermal routes of exposure. In humans ingestion of gram quantities of copper salts may cause gastrointestinal, hepatic and renal damage with symptoms such as severe abdominal pain, vomiting, diarrhea, hemolysis, hepatic necrosis, hematuria, proteinuria, hypotension, tachycardia, convulsions, coma and death (U.S. AF, 1990). Gastrointestinal disturbances and liver toxicity have also been registered after long-term exposure to drinking water containing 2,2-7,8 mg Cu/L (Mueller-Hoecker et al., 1988).

Inhalation exposure to copper fumes or fine dust at concentration of 0.075 – 0.12 mg Cu/m³ may cause so-called metal fume fever with symptoms such as cough, chills and muscle ache (U.S. AF, 1990). It has been reported for gastrointestinal disturbance, headache, vertigo, drowsiness and hepatomegaly in workers exposed to copper dust

(Suciu et al., 1981). Vineyard workers chronically exposed to Bordeaux mixture (copper sulfate and lime) exhibit degenerative changes of the lungs and liver.

Dermal exposure to copper may cause contact dermatitis in some individuals (ATSDR, 1990).

Many of the toxic effects of copper are related to its role in the generation of reactive oxygen species such as superoxide, hydrogen peroxide, the hydroxyl radical. These damage proteins, lipids and DNA (Brewer, 2010).

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FO3. ZINC DEFICIENCY AND INFERTILITY

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Zinc was discovered to be essential to human health in the early 1960s. It is required for more than 200 enzyme activities in the body including enzymes involved in methylation and prevention of oxidative stress - Zn superoxide dismutase (Cu-Zn SOD) and metallothioneins capturing superoxide and hydroxyl radicals.

The role of zinc in men reproduction is well documented. During spermiogenesis (when the compaction of the nucleus starts), zinc is incorporated in the sperm nucleus. The earliest sign of zinc deficiency is an arrest at spermiogenesis with lack of elongated spermatozoa. Zinc is involved in the formation of a very stable nucleoprotamine complex in which DNA is protected from the adverse effects of extrinsic and intrinsic factors. Zn deficiency could lead to decreased compaction which would increase the likelihood of DNA damage. This in turn reduces the fertility of sperm.

As regards female fertility most of the zinc transporters, metallothioneins and metal regulatory transcription factors are expressed in oocytes and not in cumulus cells. This may indicate an important role for zinc, in particular with potential linking to genome stability during early embryogenesis because zinc is involved in numerous DNA repair enzymes that are active at this time.

Fully-grown but immature oocytes display an intact germinal vesicle. They proceed through the maturation period and establish metaphase II arrest before undergoing fertilization and preimplantation embryo development. In mouse oocytes zinc levels increase significantly during meiotic maturation. Compared to normal eggs many of zinc insufficient oocytes undergo symmetric meiotic division and extrude significantly larger polar body. Moreover fertilized zinc-insufficient mouse oocytes fail to form blastocysts. This confirms the importance of zinc for fertility and early embryo development.

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FO4. COMPARATIVE EFFECTS OF Co AND Cd COMPOUNDS ON TESTIS MORPHOLOGY AND SPERM COUNT

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Introduction: Cobalt (Co) is an essential trace element for mammals but when applied chronically it accumulates in different organs and tissues inducing pathological alterations¹. Cadmium (Cd) is a heavy metal and a major environmental pollutant². Our study is focused on the comparative effects of chronic exposure to cobalt chloride (CoCl₂·6H₂O) or subacute cadmium intoxication (Cd(CH₃COO)₂·2H₂O) on testis morphology and sperm count in adult mice. **Methods:** Both compounds were given via water. CoCl₂ was applied chronically with daily doses of 75- or 125 mg/ kg/bw from prenatal period to sacrifice of animals on day 60 whereas Cd(II) acetate was given for two weeks to adult mice with daily dose of 20 mg/kg/bw. Testes and epididymides were sampled, weighed and embedded in paraffin. Spermatozoa were isolated from both vasa deferentia and counted. **Results and discussion:** Despite different experimental design we established obvious changes in testis morphology after high dose of CoCl₂ and after Cd intoxication, namely depletion and retardations in germ cell development and disorganization of seminiferous epithelium, findings supported by data of other authors^{3,4}. Changes in the testes weight (TW) are indicative for altered spermatogenesis. High dose of CoCl₂ induced significant reduction of TW (30% lower than control) whereas low dose of CoCl₂ as well as Cd acetate fail to affect this parameter. Sperm count demonstrated a wide deviation in the values in all of the experimental groups and significant reduction was not achieved. We did not establish significant alterations of Cd acetate or CoCl₂ on gonado-somatic index (ratio of testis weight to body weight) or sperm count in adult mice although CoCl₂ -125 mg reduced the sperm count with 25% compared to control.

Conclusion: Despite different mechanism of action and different duration of treatment of both investigated metals we found similar effect of their compounds on testis morphology in adulthood. Regarding quantitative evaluation of the testis we found that CoCl₂ induced more severe changes compared to Cd acetate, especially on TW.

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FO5. EFFECT OF CO-EDTA ON IRON METABOLISM IN IMMATURE MICE

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Cobalt (Co) is an essential trace element and its accumulation in the organism affects the concentrations of other elements [5]. The wide use of cobalt alloys in medical devices requires full elucidation of its biological role in cells, tissues and organs after long-term exposure [2,4]. Spleen and liver are the main sites of iron (Fe) storage in the body. The aim of the present study is to investigate the effect of Co-EDTA on the iron metabolism in immature mice. Pregnant mice in late gestation were subjected to Co-EDTA treatment at a daily dose of 75 mg/kg and the exposure continued until days 18 and 25 of the newborn pups. The compound was dissolved and obtained via regular tap water. Age-matched mice drinking tap water were used as controls. The newborn pups (n=3 per group) were sacrificed on days 18 and 25. Serum iron and Fe content in spleen and liver were measured. Preliminary results showed elevated serum Fe in d18 mice and significantly reduced in d25 animals compared to the untreated controls. Fe content in the spleen and liver was affected as well. Significantly increased concentrations of Fe ions were measured in the livers of treated mice. Cobalt is a hypoxia inducing agent and this could explain the increased Fe content in the liver due to suppressed hepcidin levels [3]. On the other hand, hypoxia increases iron storage in the spleen [1]. The increased Fe content in the spleen of day 25 mice may be due the prolonged treatment and the effect of hypoxia induced by Co-EDTA. The results indicate that Co-EDTA treatment affects early postnatal mouse development altering Fe homeostasis.

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FO6. IRON AND BLUE PEOPLE

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FO7. NEW FLUORESCENT DENDRIMERS AS DETECTOR FOR BIOLOGICALLY IMPORTANT METAL IONS

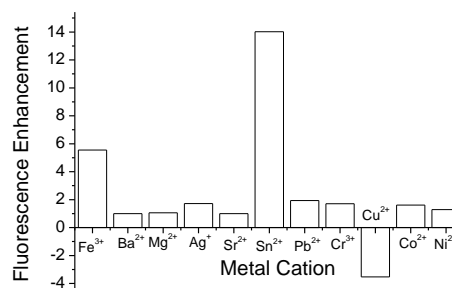
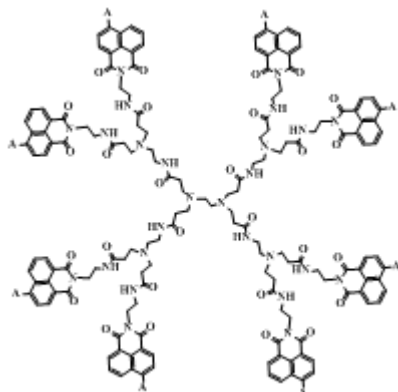
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Being microelements metal ions are of great importance for the plants and living organisms. However, accumulation of such ions at concentrations higher than the one needed for the development of plants organisms leads to pathological changes. The crucial fact is that the heavy metal ions accumulated in the plants do not cause visible changes in their organisms but having become a link in the chain 'soil-plants-animals-humans' they can have serious toxic effects on the latter two links. It is known that the doses inducing negative changes in the animal and human organisms are rather lower than those in the case of plants. Therefore the design and preparation of novel materials with tailored properties at a molecular level has been an eminent challenge for specialists from various fields.

Dendrimers are a relatively new class of organic compounds used as sensors for detection of metal ions and proton in different media. With this regard there have been ongoing studies on their functionalization with photoactive groups and receptor fragments able to recognize and coordinate metal ions, hence to change the photophysical characteristics of the dendrimers.



PAMAM dendrimer chemical structure Fluorescence enhancement for dendrimer 4

Here we present the photophysical characteristics of the new fluorescent dendrimers. The influence of different metal cations on the dendrimer fluorescence has been investigated viewing its sensor potential. It has been shown that the fluorescence intensity depends strongly on the nature of metal cations. Their effectiveness as metal ion sensors is evaluated by photoinduced electron transfer. The results show a good detective sensitivity even at low ion concentrations ($c = 10^{-4} \div 10^{-6} \text{ mol l}^{-1}$).

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FP1. UROV KASHIN-BECK DISEASE: BIOGEOCHEMICAL STUDY

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In connection with the supposition of significance of geochemical factors in etiology of Urov Kashin-Beck disease the biogeochemical researches were conducted. The field studies were carried out in the summer seasons of 1998 to 2012 in the landscapes of Eastern Transbaikalia with collecting rock, soil, plant, natural water, hair and cattle blood samples, as well as dairy products. Determination of macro- and trace elements in most of the samples was carried out using AAS, NAA, X-ray and ICP-mass-spectrometry (model 7500 CE manufactured by "Aglient Technologis", USA) following standard procedures.

It was found that the tested waters are slightly alkaline or neutral, bicarbonate-calcium-magnesium type. For number wetlands of endemic foci characterized by the decrease Eh and the presence of manganese, iron, and sometimes zinc. In most of the river waters tested content areas of ortho-phosphate in filtered water varies from 0.02 to 0.09 mg/l, and in areas where the urov disease phosphate concentration increased to 0.30 -0.34 mg/l. In some samples the levels of total phosphorus (in the form of phosphates) reaches 0.50-0.54 mg/l. For almost all the tested water characteristic low content of vital trace element selenium.

Enrichment of soils of Urov biogeochemical provinces by manganese, phosphorus, iron and to a lesser degree strontium that reflects landscape-geochemical contrast of an examined territories and features of migration of macro-and trace elements in conditions of a permafrost and bogging of territories is discovered. Concentration of calcium in soils of endemic villages in some cases surpass, and in most cases do not exceed a level of its content in soils of control sites. The fact of sharp enrichment of soil by strontium in flood-lands of the river Urov is obvious, that is connected to structure of soil-forming of rocks [1]. The most part of endemic villages is dated to gold-fields to displays and characterized by higher concentration of arsenic, fluorine and low concentrations of copper in flood-lands landscapes. Coefficient of biological absorption of calcium and strontium in system the plant - soil grows in endemic areas, and phosphorus - decreases. Manifestation of Urov bone-articulate pathology in East Transbaikalia occurs on a background of deficiency of selenium in all parts of a biogeochemical food chain [2], a low level of sulfates in natural waters with weak mineralization and fungous invasion of grain crops. Nevertheless, on a number of parameters the areas of spreading of Urov Kashin – Beck disease in Russia differ from those in China, except for the common deficiency of selenium and iodine [3].

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FP2. GEOCHEMICAL ECOLOGY AND ITS ROLE IN PRESENT-DAY BIOSPHERE STUDIES

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Fundamental concepts, major provisions and the role of geochemical ecology of organism as a new scientific area in the biogeochemistry of general ecology are elucidated. The formation and development of this scientific area and its role in dealing with ecological problems is considered. Geochemical ecology addressed the regularities in interactions between individual organisms and their associations with the natural-technogenic environment due to formation and running of migration processes of chemical element atoms in the biosphere and transformation of solar energy. The core of geochemical ecology comprises: specific features of chemical elemental composition of organisms and geochemical environment, biogeochemical food chains and parameters, biogenic migration of chemical elements and their biogeochemical cycles, different biological response of organisms, including ferment adaptations, threshold or critical concentrations of chemical elements in organisms and environment, biogeochemical model of homeostatic regulation processes in organisms, associations, biogeocenoses and their sustainable development, evolution of the chemical composition of living matter and biosphere as a representation of planetary and cosmic processes. The central place in geochemical ecology is occupied by the geochemical factor of influence (chemical elements or their associations). The problems of geochemical ecology are getting particularly pressing today. Studying technogenic provinces is a new scientific problem that needs to be solved for the general ecological assessment of biosphere development in the present-day Psychozoic era and search for more efficient technologies, for the assessment of the interaction between technogenic and natural factors. The complexity of the problem consists in the necessity of differentiating between technogenic and natural flows and forms of chemical element migration, in the assessment of interaction between technogenic and natural factors and manifestation of unforeseen biological response in organisms [2]. However, except for toxicosis, caused by an excess of minerals, there is a large group of hypo-microelementoses. Deficiency in trace elements – copper, zinc, manganese, cobalt, iodine, selenium – in fodder, especially in the winter stabling period, makes 30 to 70 percent of respective requirements of animals on all farms of the Central Chernozem zone. As a result, their levels in the animal body (in organs, tissues, milk) are 5 to 10 times lower than the normal level. The said trace elements determine the intensity of all metabolism processes and performance of all organs and systems of the body. Long-term deficiency, or even short-term reduction in the intake of the said trace elements by animals with forage results in pathological conditions – chronic complex hypomicroelementosis. In all kinds of animals, it manifests itself through severe disorders and does much harm to the health of animals and their productivity. In addition, the invasion by parasites of animals, including wild animals, results to the hypomicroelementoses [1].

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