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MEETINGS AND ABSTRACTS OF THE CZECHOSLOVAK BIOLOGICAL SOCIETY IN 2007 YEAR

SCHŮZE BRNĚNSKÉ POBOČKY ČESKOSLOVENSKÉ BIOLOGICKÉ SPOLEČNOSTI V ROCE 2007

Členská schůze 17. ledna 2007

(Schůze konaná ve spolupráci s Centrem pro cyanobakterie a jejich toxiny, Laboratoř cyanotoxinů, Společné pracoviště Botanického ústavu AV ČR a RECETOX Přírodovědecké fakulty MU na téma: Toxiny sinic v ČR: Nové poznatky o vlivech na lidské zdraví a ekosystémy)

L. Bláha, B. Maršálek: **Úvod a představení problému toxických sinic v ČR**

L. Bláhová, P. Babica, O. Adamovský, J. Kohoutek, E. Maršálková, B. Maršálek, L. Bláha: **Microcystiny a další cyanotoxiny v ČR a ve světě**

J. Kohoutek, P. Babica, B. Maršálek, T. Ocelka, D. Jančula, O. Adamovský: **Nové přístupy ke sledování microcystinů v úpravnách pitných vod**

O. Adamovský, R. Kopp, K. Hilscherová, P. Babica, L. Bláha: **Akumulace microcystinů v rybách a hodnocení souvisejících zdravotních rizik**

K. Hilscherová, M. Smutná, B. Burýšková, P. Babica, L. Ambrožová, B. Maršálek, L. Bláha: **Jsou toxické microcystiny opravdu toxické i pro vodní organismy?**

Členská schůze 14. února 2007

(Schůze konaná ve spolupráci Ústavem biochemie Přírodovědecké fakulty MU a Ústavem chemie a biochemie Agronomické fakulty MZLU Brno na téma: Je třeba studovat thioly?)

R. Kizek, V. Adam, S. Křížková, O. Zítka, K. Stejskal, J. Zehnálek, B. Sures, L. Trnková, M. Beklová, L. Havel: **Biologicky významné thiolové sloučeniny a jejich význam pro udržování homeostázy**

O. Zítka, V. Adam, S. Křížková, R. Kizek: **Analytické a molekulárně biologické nástroje pro stanovení metalothioneinu**

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S. Křížková, I. Fabrik, V. Adam, R. Kizek: **Vztah metalothioneinu k nádorovým onemocněním**

J. Baloun, V. Adam, L. Havel, J. Zehnálek, R. Kizek: **Fytochelatiny – jejich význam u rostlin**

Členská schůze 17. března 2007

J. Neradil (Biologický ústav Lékařské fakulty MU)*:* **Význam FGF-2 signalizace v lidských embryonálních kmenových buňkách**

Z. Holubcová (Biologický ústav Lékařské fakulty MU)*:* **Regulace buněčného cyklu v lidských embryonálních kmenových buňkách**

L. Eiselleová (Biologický ústav Lékařské fakulty MU)*:* **Kultivace lidských embryonálních kmenových buněk na různých typech fibroblastů**

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O. Šedo (Ústav experimentální biologie Přírodovědecké fakulty MU): **Charakterizace proteinů hmotnostní spektrometrií**

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(Seminář u příležitosti 80. narozenin prof. RNDr. Stanislava Rosypala, DrSc.; uspořádaly Genetická společnost Gregora Mendla, Československá biologická společnost, Československá společnost mikrobiologická a Ústav experimentální biologie Přírodovědecké fakulty MU)

J. Doškař (Ústav experimentální biologie Přírodovědecké fakulty MU): **Profesor Rosypal a 50 let výuky molekulární biologie na Přírodovědecké fakultě MU**

R. Pantůček (Ústav experimentální biologie Přírodovědecké fakulty MU): **Molekulární taxonomie stafylokoků**

V. Růžičková (Ústav experimentální biologie Přírodovědecké fakulty MU): **Molekulární diagnostika *S. aureus* a jejich virů**

J. Šmarda jr. (Ústav experimentální biologie Přírodovědecké fakulty MU): **Možnosti reaktivace diferenciačního potenciálu nádorových buněk**

Členská schůze 17. října 2007

(Schůze konaná ve spolupráci s Českou anatomickou společností a Ústavem histologie a embryologie Lékařské fakulty MU k výročí narození prof. MUDr. Jana Floriana a prof. MUDr. Karla Mazance, DrSc.)

S. Čech (Ústav histologie a embryologie Lékařské fakulty MU): **Vědecký odkaz Jana Floriana a Karla Mazance**

M. Sedláčková, J. Žáková, I. Crha, E. Lousová, P. Ventruba (Ústav histologie a embryologie Lékařské fakulty MU): **Ultrastruktura zárodečného epitelu mužů s azoospermií**

L. Krejčířová, I. Lauschová (Ústav histologie a embryologie Lékařské fakulty MU): **Distribuce olova, rtuti a kadmia v játrech fétu po jejich podávání březím samicím laboratorní myši – morfologická studie**

P. Chovanec, M. Nováková, D. Horký (Ústav histologie a embryologie a Fyziologický ústav Lékařské fakulty MU): **Haloperidol ako ligand sigma receptorov a jeho kardiotoxicita**

Členská schůze 14. listopadu 2007

(Téma: Epigenetické změny v dediferencovaných rostlinných buňkách)

B. Koukalová (Laboratoř molekulární epigenetiky Biofyzikálního ústavu AV ČR Brno): **Hypometylace genových jednotek kódujících ribosomální RNA**

M. Fojtová (Laboratoř molekulární epigenetiky Biofyzikálního ústavu AV ČR Brno): **Epigenetická nestabilita transgenů indukovaná dediferencovaným stavem**

11. prosince 2007
Symposium Aktuální otázky bioklimatologie zvířat 2007

(Uspořádala Česká bioklimatologická společnost při ČAV – Sekce bioklimatologie zvířat a Výzkumný ústav živočišné výroby v Praze ve spolupráci s Ústřední komisí pro ochranu zvířat Praha a Brněnskou pobočkou Československé biologické společnosti)

ABSTRACTS

L. Bláhová, P. Babica, O. Adamovský, J. Kohoutek, E. Maršálková, B. Maršálek, L. Bláha (Centre for Cyanobacteria and Their Toxins, Institute of Botany, Academy of Sciences of the Czech Republic and RECETOX, Faculty of Science, Masaryk University, Brno): **Microcystins in the Czech Republic and in the world**

Massive blooms of cyanobacteria have become a worldwide problem as a result of surface water eutrophication having several negative effects on the quality of water (changes in transparency, pH or biodiversity, production of chemical odours and toxins – neurotoxins, hepatotoxins, lipopolysaccharides, etc.). The most studied group of cyanotoxins are cyclic heptapeptides microcystins that were shown to be acute and chronic hepatotoxins and liver tumour promoters. World Health Organization (WHO) recommends a guideline value of 1.0 microgram per litre of drinking water for microcystin-LR, a representative of this class of hazardous toxins. The aims of the present study were to evaluate health risks of microcystins in the Czech Republic (about 80 reservoirs were monitored since 1993, and from 2004 repeated seasonal analyses were performed). We have assessed risks of (i) dissolved (extracellular) toxins present in the water column that are hazardous for drinking waters (microcystins analysed by ELISA), (ii) and the biomass-bound microcystins that may be hazardous during recreational activities (concentrations were determined by HPLC-DAD). Our study revealed that more than 80% of reservoirs contained microcystins with no apparent trends during 1993–2005. The levels of microcystins

were among the highest reported in the world. Concentrations of extracellular microcystins in the drinking water supplies are not predictable with substantial variability during a summer season (from nondetectable to hundreds μg/L). Calculation of hazard indexes using US EPA methodology showed high health risks (HI>1) from recreational exposures under certain scenarios (especially for children; HI>10), while the risks from MC-contaminated drinking waters seem to be minor when considering mean MC concentrations. Further research and regulatory actions should focus on prevention and remediation of massive cyanobacterial blooms and careful control and treatment of the quality of drinking waters.

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J. Kohoutek[1], P. Babica[1], B. Maršálek[1], T. Ocelka[2], D. Jančula[1], O. Adamovský[1] ([1]Centre for Cyanobacteria and Their Toxins, Institute of Botany, Academy of Sciences of the Czech Republic and RECETOX, Faculty of Science, Masaryk University, Brno, [2]Institute of Public Health in Ostrava): **New approaches to monitor cyanobacterial toxins in water**

With the increasing concerns about environmental and human health problems related to cyanobacteria, there is a need to improve monitoring of occurrence of cyanobacteria and their metabolites (cyanotoxins) in waters. Passive sampling could be used as an appropriate alternative to active sampling methods, and it also offers several advantages such as concentration of the ultra-trace amounts of the compound of interest and/or sequestration of residues from episodic events (that are usually difficult to be recorded with single-point active sampling). In our studies, we have investigated the ability of a passive sampler (modification of the Polar Organic Chemical Integrative Sampler – POCIS) to sequestrate a group of common and highly hazardous cyanobacterial toxins – microcystins, and we have also evaluated their ability to concentrate trace amounts of microcystins. Samplers were exposed to the water with cyanobacteria producing microcystins at variable concentrations. After the exposure (periods of several days were evaluated), samplers were extracted with methanol, concentrated by solid phase extraction and analysed by HPLC-DAD for microcystins. Our results have shown that the samplers are able to concentrate cyanotoxins, and that there is a good correlation between the amount of cyanobacterial biomass and retained microcystins. All structural variants of microcystins (MC-RR, MC-YR and MC-LR) were detected in the sampler in the same ratio as it was produced in the cyanobacteria. We have also shown the ability to concentrate ultra-trace amounts of microcystins, and our research is now focused on further optimalization of this novel approach for practical applications. In summary, we have demonstrated that a novel passive sampling technique has a potential to detect cyanotoxins in natural conditions and it may be used as an integral part for monitoring of the drinking water quality.

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O. Adamovský[1], R. Kopp[2], K. Hilscherová[1], P. Babica[1], L. Bláha[1] ([1]Centre for Cyanobacteria and Their Toxins, Institute of Botany, Academy of Sciences of the Czech Republic and RECETOX, Faculty of Science, Masaryk University, [2]Department of Fishery and Hydrobiology, Mendel University of Agriculture and Forestry, Brno): **Microcystin bioaccumulation in common and silver carp exposed to toxic cyanobacterial blooms and assessment of the human health risks**

Cyanotoxins produced by freshwater cyanobacteria may cause poisonings and deaths of wild and domestic animals and they pose a significant hazard to human health. The most studied groups of cyanotoxins – cyclic heptapeptide microcystins (MC) – can be accumulated in fish but the mechanisms of uptake and distribution in different tissues are not well understood. Further, only few reports focused on bioaccumulation under natural conditions from the complex toxic cyanobacteria biomass. In our study, two species of common edible fish (common carp *Cyprinus carpio* and silver carp *Hypophthalmichthys molitrix*) were exposed to *Microcystis* spp. dominated cyanobacterial water bloom. After 4 and 9 weeks, fishes were collected, weighted and measured, and the samples of tissues were immediately frozen and stored at -80°C for further analyses (5 fish per group). No mortalities

were recorded during experiments. Concentrations of microcystins in the fish muscle ranged from 1.4 to 29 ng of MC per g fresh weight (ng/g fw) in both species. Hepatopancreas accumulated more microcystins than muscle (29–226 ng/g fw), and there was an interspecies variability in accumulation kinetics. To evaluate health risks of microcystins accumulated in fish, we have used the methodology of US EPA and the World Health Organization (WHO) recommended tolerable daily intake for human ingestion of MC-LR (0.04μg . kg^{-1}body weight . day^{-1}). Calculated values of hazard indexes (HI>1 indicate significant risk) for maximum concentrations observed in the silver carp and common carp were 0.03 and 0.04, respectively. In summary, our results demonstrate that microcystins can be accumulated in the fish muscle but concentrations observed in present study are unlikely to significantly harm human health. Further monitoring of MC occurrence in the edible fish is needed to critically evaluate low risks suggested by experimental studies.

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K. Bártová, K. Hilscherová, P. Babica, B. Maršálek (Centre for Cyanobacteria and Their Toxins, Institute of Botany, Academy of Sciences of the Czech Republic and RECETOX, Faculty of Science, Masaryk University, Brno): **Are microcystins toxic for autotrophs?**

Cyanobacteria produce a wide range of biologically active compounds that may mediate interactions with other organisms. Metabolites that affect growth or development of other phytoplankton are called allelopathics, and some chemicals with identified structure as well as extracellular products (exudates) or extracts of various cyanobacteria have been shown to posses some allelopathic properties. We have studied effects of (i) purified microcystins MC-LR and MC-RR (heptapeptide cyanotoxins that were extensively explored before) and their mixtures, (ii) exudates of various cyanobacterial species and (iii) crude extract of microcystin-producing cyanobacteria *Microcystis aeruginosa* on the growth of selected phytoplankton species from both *Chlorophyta* (eukaryotic algae) and *Cyanobacteria*. A modified growth algal inhibition test according to ISO 8692 was used. Changes in selected oxidative stress biomarkers (content of glutathion, activities of glutathion S-transferase and glutathion reductase) were assessed with standard spectrophotometric methods. Microcystins caused weak growth inhibitions of 6 studied algal species, and the effects occurred only at extremely high (environmentally not relevant) concentrations 25000 μg/l. MC-RR was more potent inhibitor than MC-LR. Variable modulations of GSH, GST and GR were recorded demonstrating thus "xenobiotic-like" effects of microcystins. Dose-dependent growth stimulations were observed after exposures to the exudates from cyanobacterial genera *Cylindrospermopsis*, *Microcystis* and *Aphanizomenon*. On the other hand, complex extracts of cyanobacteria *M. aeruginosa* (up to 300 μg/l of microcystins) had no effect on any of the studied organisms. Our experiments do not support the hypothesis about allelopathy of microcystins, as the effective concentrations were high above those occurring in the environment 0.1–10 μg/l. Stimulatory effects of exudates indicate presence of some growth modulators that should further be studied.

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K. Hilscherová, M. Smutná, B. Burýšková, P. Babica, L. Ambrožová, B. Maršálek, L. Bláha (Centre for Cyanobacteria and Their Toxins, Institute of Botany, Academy of Sciences of the Czech Republic and RECETOX, Faculty of Science, Masaryk University, Brno): **Role of microcystin in the toxicity of cyanobacteria on aquatic organisms and in vitro cytotoxic effects**

Most of the previous research of cyanobacterial toxins focused on effects of a single group of metabolites – microcystins. However, recent reports document various toxic effects independent of microcystins. Ecotoxicity and in vitro cytotoxicity of six cyanobacterial biomasses with various species composition and different microcystin content was evaluated in a series of assays. Next to the complex cyanobacterial biomass also its separated fractions were studied (pellet – cell debris – containing lipopolysaccharides; crude aquaeous extract devoid of lipopolysaccharides; fractions obtained by SPE with C-18 cartridges, i.e. permeate (devoid of microcystins) and eluate (mostly microcystins). We have also studied toxicity of microcystin mixture and its two structural variants (microcystin-

LR, microcystin-RR) in selected models. The effects of cyanobacterial biomass and fractions were assessed in acute and chronic tests with water flea *Daphnia magna,* in the test for embryotoxicity and teratogenicity with *Xenopus laevis* frog embryos (test FETAX), and also using *in vitro* asays with two permanent cell lines. The complex biomass and crude aquaeous extract were the most toxic. On the contrary, eluates with microcystins had little effect in both FETAX and *Daphnia magna*. The sample with the highest concentrations of microcystins caused interestigly the least immobilization of *Daphnia magna,* while the sample without microcystin caused significant 100% immobilization. The experiments showed strongest toxic effects of complex samples and generally low toxicity of the microcystin fraction. The hepatoma cells H4IIE.luc as well as dermal cell line HaCaT showed significant toxicity of tested biomasses regardless of the microcystin content. The results document that microcystin alone cannot be responsible for the observed toxicity. In conclusion, the results from studied models show that other cyanobacterial components than microcystin contribute significantly to the cyanobacterial (eco)toxicity and they can have synergistic effects with microcystin present in the cyanobacterial biomass.

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R. Kizek[1], V. Adam[1], S. Křížková[1], O. Zítka[1,2], K. Stejskal[1,2], J. Zehnálek[1], B. Sures[3], L. Trnková[4], M. Beklová[5], L. Havel[6] ([1]Laboratory of Molecular Biochemistry and Bioelectrochemistry, Department of Chemistry and Biochemistry and [6]Department of Plant Biology, Faculty of Agronomy, Mendel University of Agriculture and Forestry in Brno, [2]Department of Biochemistry and [4]Department of Theoretical and Physical Chemistry, Faculty of Science, Masaryk University, [3]Applied Zoology/ Hydrobiology, Universität Duisburg-Essen, [5]Department of Veterinary Ecology and Environmental Protection, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Brno): **Biologically important thiols and their importance to maintain homeostasis**

Introduction. A regular function of a cell depends on stability or minimal changes in its intracellular space. The maintaining of equilibrium in an organism is needed to keep the organism alive. For these purposes various regulative mechanisms have been emerged. Ones of those mechanisms is to maintain balance of metal ions and, thus, very dangerous oxygen radicals. It is a common knowledge that thiols (-SH group rich compounds) play a crucial role in these processes.

Glutathione. The ubiquitous tripeptide called glutathione belongs to the most important thiols in an organism both plant and animal origin. Its level increases under a stress. Moreover, glutathione exists in two forms as reduced GSH and oxidized GSSG one. The ratio between these forms determines an oxidation state. Besides glutathione a cystein rich protein with molecular weight about 6–8 kDa (at mammals) plays crucial role at maintaining of metal ions homeostasis. Due to its properties it has been called as metallothionein.

Metalothionein. Metallothioneins (MTs) occurs through whole animal kingdom and have been found at higher plants, eukaryotic microorganisms and lots of prokaryotes. In animals they have been determined in liver, kidneys, pancreas and colon and changes have been described in MTs level among animal species. Moreover, the MTs levels are influenced by aging, stage of development, food and other factors, which are not clear yet. Metallothionein content in a cell, which is not stressed, is probably constant (to our knowledge there has not been published any paper to prove or disprove this hypothesis). In addition MTs have been found in cell nuclei. Their role in intracellular fixation of essential microelements like as zinc or cuprum, in controlling of free ions concentration, in regulation of free ions flow through cell has been published and discussed. This concern relates to the fact that metallothioneins are key molecules in intracellular exchanging of various both essential and non-essential metal ions. A scavenging of reactive oxygen species belongs to other very important functions of MTs. This phenomenon has been investigating in brain tissues (*R Kizek et al: Anal Chem 73, 2001, 4801*).

Conclusion. Metallothioneins are biologically important group of proteins occurring through whole animal kingdom. A regulation of level of essential metals (such as zinc, cuprum and others) is one of their most important roles, whereas all of their biological functions are not clear yet *(J Petrlová et al: Klinická onkologie 19, 2006, 138)*

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O. Zítka[1], V. Adam[2], S. Křížková[2], R. Kizek[2] ([1]Department of Biochemistry, Faculty of Science, Masaryk University, [2]Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University of Agriculture and Forestry in Brno): **Analytical and molecular biological instruments to detect metallothioneins**

Metallothioneins (MT) is a family of heavy metals binding proteins rich in cysteine residues, which represents nearly the 30% of its amino acidic residues. They can be found in animals, higher plants, eukaryotic microorganisms and prokaryotic ones. These proteins play several crucial biological roles such as scavenging of reactive oxygen species, detoxifying of various xenobiotics (heavy metals) and their very close associating with tumour diseases. Mammalian MTs have molecular weight within the range from 6 to 7 kDa. They are commonly composed from 60 to 68 aminoacids residues, where the most repeating motif in their primary structure is cystein(C)-serin(S)-cystein(C). In animals the highest content of MTs have been detected in kidneys, liver and pancreas. There have been described and discussed various effects of kind of a tissue, age of an animal, environment where the animal had lived and many others on content of MTs.

Metallothioneins are heat shock proteins. Therefore we can denature a sample of interest (e.g. cells, blood, tissues) to remove other proteins from the sample. The denatured sample is consequently centrifuged. The supernatant obtained can be consequently purified according to kind of the sample prepared or analytical method used for determination of MTs. To detect MTs can be utilized a battery of analytical methods including both "direct" ones based on spectroscopic detection of MTs after their separation (high performance liquid chromatography with mass spectrometry – HPLC-MS or with UV detection – HPLC-UV) and "indirect" ones, where content of MTs is determined by means detection of content of heavy metals. In addition, molecular biological methods, where MTs are determined via specific antibodies, belong to other techniques of such kind. These techniques can be divided on quantitative such as ELISA and RIA (limit of detection about units or tens of ng of MTs per ml) and semi-quantitative such as Dot-blot and Western blot (limit of detection about units or tens of ng per band or dot). Immunocytochemistry can be utilized for localization of MTs in a tissue of interest. In addition to detection of MTs directly, expression of them at nucleic acid level can be determined by RT-PCR.

On the other hand, electrochemical methods are capable for determination of MTs with similar results as time consuming and high cost methods mentioned above. Thanks to SH groups, MTs give well detectable electrochemical signal, which can be measured by both voltametric and chronopotentiometric methods. Adsorptive transfer stripping technique in connection with differential pulse voltammetry called Brdička reaction, where the detection limit is about 100 pM of MTs (0.64 ng/ml), belongs to the most sensitive methods from voltametric ones. Moreover chronopotentiometric stripping analysis measuring so-called peak H enable to us detecting MTs at attomolar level (detection limit is about 2.2 aM or16 pg/ml), but it is time consuming in comparison with voltametric techniques. In conclusion, Brdička reaction can be considered as suitable tool for routine analysis of MTs in various types of biological samples due to its robustness, high sensitivity, low cost and very low detection limits.

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V. Adam[1], S. Křížková[1], J. Petrlová[1], O. Zítka[1,2], K. Stejskal[1,2], J. Zehnálek[1], B. Sures[3], L. Trnková[4], M. Beklová[5], R. Kizek[1] ([1]Laboratory of Molecular Biochemistry and Bioelectrochemistry, Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University of Agriculture and Forestry in Brno, [2]Department of Biochemistry and [4]Department of Theoretical and Physical Chemistry, Faculty of Science, Masaryk University, [3]Applied Zoology/Hydrobiology, Universität Duisburg-Essen, [5]Department of Veterinary Ecology and Environmental Protection, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Brno): **Biosensors utilizing metallothionein**

The pollution of the environment with toxic metals is a result of many human activities, such as mining and metallurgy, and the effects of these metals on the ecosystems are of large economic and public health significance, because these substances are not biodegradable and retained by the ecological system. Besides "standard" toxic metals such as cadmium, lead and mercury, which have been monitoring for many years, following the introduction of automobile catalytic converters the platinum group metals (platinum and rhodium) gain on increasing interest in environmental research. Moreover, platinum complexes play an important role in the chemotherapy of various tumour diseases. As a consequence of the increasing employment of platinum for exhaust purification, in industry and tumour diseases treatment, it became necessary to determine not only "standard" toxic metals but also platinum compounds in a wide range of biological and environmental matrices. Suggestion of biosensors could be very suitable for these purposes instead of robust analytical techniques, because biosensors have the advantages of specificity, low cost, ease of use, portability and the ability to furnish continuous real time signals. The aim of this work was to suggest new heavy metal biosensors based on interaction of metals (cadmium and zinc) with heavy metal binding biological compound (metallothionein – MT), using adsorptive transfer stripping (AdTS) differential pulse voltammetry (DPV). After that we did proved that we were able to suggest biosensor through modification of hanging mercury drop electrode, we attempted to utilize metallothionein for determination of heavy metal ions. We studied the electrochemical behaviour of MT on the surface of hanging mercury drop electrode by AdTS DPV. Perfect coverage of the electrode surface – forming of the surface assembled monolayer – was probably reached at time about 240 s for 10 μM protein concentration. The quantification limits of the analysed heavy metals (cadmium(II) and zinc(II)), which were analysed in the presence of 0.5 M NaCl (pH 6.4), were 160 and 220 fmole in 5 μl drop, respectively. In addition, we applied the MT biosensor to analyse heavy metals in human body liquids (human blood serum and human urine) and to compare with differential pulse anodic stripping voltammetry. Moreover, we used MT biosensor to investigate interactions between cisplatin and dsDNA and to quantify the Pt(II)-DNA adducts. The average concentration of the drug bound to DNA was estimated as 8.1 ng of cisplatin per 500 ng of DNA by MT biosensor. It follows from the results obtained that the suggested MT biosensor could be a new useful tool for investigation of interaction of DNA with cisplatin, because give a response only in the present of Pt(II)-DNA adduct.

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S. Křížková[1], I. Fabrik[2], V. Adam[1], R. Kizek[1] ([1]Laboratory of Molecular Biochemistry and Bioelectrochemistry, Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University of Agriculture and Forestry in Brno, [2]Department of Biochemistry, Faculty of Science, Masaryk University, Brno): **Association of metallothionein with tumour disease diagnostics**

Metallothioneins (MT) are intracellular low-molecular proteins. Their molecular weight is about 6–10 kDa and cysteine content is about 30 %. MTs are assorted into three classes according their primary structure and origin. Mammalian metallothioneins are included into MT class I. Human metallothionein (hMT) gene family codes ten isoforms of MT I. MT I are composed from 61 aminoacid residues, from which twenty of them are cysteines and no aromatic aminoacids are present in their primary structure.

Metallothioneins play a crucial role in the process of cell protection against the oxidative stress in the organism. Besides the detoxification, due their high affinity to heavy metals one of their main functions is to maintain their homeostasis by chelating them into cysteine clusters. The expression of metallothionein increases not only under exposition of the organism to heavy metals, but also under stress of various origins, which leads to formation of free radicals.

There has been shown that the content of both heavy metals and metallothionein in tumours increased. During the last ten years their overexpression in human tissues in relation to tumour diseases, predominantly carcinomas originating both from surface and organ epithelium have been investigating. The overexpression of metallothionein is initiated more at malignant and more grading tumour types at breast carcinoma skin, hepatocellular carcinomas, melanomas, cervical

carcinomas, acute lymphoblastic leukaemia, and pancreas carcinoma. Based on the results obtained the higher content of MT is associated with progression and worse prognosis of tumour disease. The overexpression of hMT metallothionein isoforms hMT-1a, hMT-2a in these carcinomas is studied as new prognostic factor in disease progression, patients surviving, and the correlation with histological type, phase of disease, tumour grading or metastases appearance. It follows from our results that the monitoring of metallothionein level in full blood or in blood serum by using adsorptive transfer technique coupled with differential pulse voltammetry (AdTS-DPV) in the presence of cobalt (III) ions (Brdicka reaction) is very promising and could be used to evaluate MT as prognostic marker for tumour diseases (*J Petrlová et al: Electrochim Acta, 51, 2006, 5112*).

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J. Baloun[1,2], V. Adam[2], L. Havel[1], J. Zehnálek[2], R. Kizek[2] ([1]Department of Chemistry and Biochemistry and [2]Department of Plant Biology, Faculty of Agronomy, Mendel University of Agriculture and Forestry in Brno): **Phytochelatins – their roles in plants**

In the last century the markedly increase of heavy metals concentration in the environment has been observed. There have been described several ways ad hoc can pollute the environment, one of them is that the heavy metals come into the soils together with fertilizers. Toxic heavy metals influence the biota adversely. It is a common knowledge that among other effects the heavy metals compete for binding places with essential heavy metals in enzymes. That way the proper function of the enzyme is changed. Furthermore, they form Reactive Oxygen Species (ROS), which cause the oxidative damage of cell components (e.g. proteins, cell structures and DNA).

During the eighties of the last century, the plant peptide with high affinity to heavy metals was discovered. Later it was found out that this compound is synthesized by the cell in the presence of heavy metals and its detoxification function for heavy metals was observed. This compound was called phytochelatin. Phytochelatins (PCs), small peptides consists of 4–23 amino acids, participate in the detoxification of heavy metals. PCs have a basic formula $(\gamma\text{-Glu-Cys})_n\text{-Gly}$ (n = 2 to 11). This repetition is terminated by glycine in most cases. Except glycine the termination by alanine or no termination has been also published. Synthesis of PCs does not occur on ribosomes but it is catalyzed by phytochelatinsynthase enzyme. Phytochelatinsynthase (γ- glutamylcysteine dipeptidyltranspeptidase, EC 2.3.2.15) is activated by an increased concentration of the heavy metal (Cd, Cu, Hg, As or Pb) in the plant cell cytoplasm. The exact mechanism of phytochelatinsynthase expression is still unclear. The higher number of glutamate-cysteine repetition as well as, the higher number of heavy metals can be built in the phytochelatin structure. But the stability of phytochelatins decreases with increasing repetition number. The low-molecular complex formed by the reaction of phytochelatin and heavy metal is transported into the vacuoles where an immediate toxicity does not menace yet. In vacuoles this complex is changed into high-molecular structure, which is unable to pass the vacuole membrane. Moreover, the negative action of heavy metals can be eliminated by the reactions with organic acids in the vacuole.

Based on the mentioned facts phytochelatins are essential constituents of the plant cells due to protecting them against heavy metals toxicity. Because these peptides are synthesized only under heavy metals stress, they could be used as a marker of heavy metal contamination of the soils.

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S. Čech (Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno): **Scientific heritage of Brno embryologists Jan Florian and Karel Mazanec**

An age difference of 25 years stands in between the Professors and Heads of the Department of Histology and Embryology of the Masaryk University, Jan Florian (1897–1942) and Karel Mazanec (1922–1967). They did not know each other personally and they even never met. What puts them together is their interest in new methods and research topics, thanks to which they are credited for the incorporation of the Czech and Slovak embryology into the European context. Due to the effort of Professor Florian systematic microscopic study of trilaminar human embryos was developed, Professor

Mazanec initiated ultrastructural and ultrahistochemical investigations of fertilized and cleaving ova of mammals and the man.

Jan Florian, a pupil of F. K. Studnička – outstanding figure of Czech biology of the 20th century, started his work in embryology just in the time when early embryogenesis of the man became actual. In 1928 year, he published an extensive study of a 15-day human embryo "TF" with established primitive streak. The favourable response to this paper motivated Florian so much that he concentrated to this topic and described more than 10 next embryos called "Bi"(ttmann) to the half of thirtieth years. The call to the Department of Histology and Embryology in Bratislava (1933–1935) and excessive pedagogical and organisational work, as well as election in the Dean office (1939/1940) postponed publication of his findings in a monograph. He managed to use them in a textbook of embryology, which he wrote together with Zdeněk Frankenberger (1936) and in a popular-scientific book *Od prvoka k člověku (From protozoa to the man, 1939)*. In the autumn of 1941 Jan Florian was arrested by the Gestapo and on the 7th May 1942 shot in the concentration camp Mauthausen near Linz.

After the 2nd World War Karel Mazanec, a pupil of the embryologist Zdeněk Frankenberger in Prague reassumed Florian´s research. Mazanec started with study of early human embryos with uncommon sedulousness and enthusiasm, which resulted in a monograph *Blastogenesa člověka (Blastogenesis of the man)* published later in German (1953 and 1959). He gave in it a synthetic and still modern overview of the human development during the first 3 weeks, which was based on his own observations and findings of Florian and other embryologists. In the beginning of the sixties (of the 20th century) Karel Mazanec, as one of the first embryologists, started with purposeful investigation of preimplantation stages by TEM and described the course of cytodifferentiation of cleaving mammalian egg. In the year 1967 Professor Mazanec died unexpectedly in unattained 45 years.

M. Sedláčková, J. Žáková[1], I. Crha[1], E. Lousová[1], P. Ventruba[1] (Department of Histology and Embryology, Faculty of Medicine, Masaryk University and [1]Clinic of Gynaecology and Obstetrics, Faculty Hospital Brno): ***The ultrastructure of seminiferous epithelium of azoospermic men***

Introduction: Methods of assisted reproduction admit to treat also some forms of male infertility as asthenospermia, oligospermia and even azoospermia. In such case, the low number of spermatozoa may be usually obtained bioptically from epididymis or testis to perform fertilisation by intracytoplasmic sperm injection (ICSI) into the oocyte. Authors examined testicular biopsies of 3 patients of in vitro fertilisation (IVF) programme with ascertained azoospermia. The aim of the study was to determine the process of formation of spermatozoa (spermatogenesis) in seminiferous tubules and to assess the reasonability of repeated therapeutic procedure.

Methods. Samples of testes were processed for electron microscopy by standard way, the ultrastructure of the germinal epithelium as well as Leydig cells has been examined.

Results. Patient A: Diabetes mellitus and professional contact with oil products are given in anamnesis. All developmental stages of spermatogenesis were identified in the germinal epithelium with relatively high rate of structurally defective spermatids. *Patient B:* Varicocele was in anamnesis. The germinal epithelium comprised only Sertoli cells alone but sporadic spermatogonia were occasionally seen it. Remnants of extinct seminiferous tubules regularly occurred in the testicular interstitium in his form of concentrically arranged basal laminae. Azoospermia of genetic origin was verified in this patient later. *Patient C:* A man with vasectomy carried out 20 years ago. In the germinal epithelium, spermatogonia, primary spermatocytes as well as early and late spermatids were constantly identified. However, findings of abnormal bi- or trinucleated spermatogonia and of high incidence of binucleated spermatids seem to indicate faulty progress of the sperm maturation. Numerous mast cells were also found around seminiferous tubules. On the other hand, Leydig cells showed intact structure in all examined samples.

Conclusions. An obtaining of appropriate spermatozoa for ICSI is not excluded in patient A in contrast to the patient B whose germinal epithelium contained only Sertoli cells; probability to obtain spermatozoa in patient C is quite high but result of IVF will be precarious.

L. Krejčířová, I. Lauschová (Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno): **Distribution of heavy metals in the liver of mouse females and their fetuses – an experimental study**

Distribution and cumulating of some heavy metals have been studied mostly in liver of adults (*PB Hamilton et al, Poultry Sci. 61, 1982, 1832; LG Hansen et al, Am J Vet Res. 37, 1976, 711*, etc) or in cultured fetal liver under physiological condition (*L Bucio et al, Toxicology 102, 1995, 285; KK Gabis et al, Biochim Biophys Acta 21, 1996, 113*). Microscopic observations paid to visualization of them simultaneously in mother and fetal liver during pregnancy were not done. The aim of this paper is to identify binding sites for heavy metals in the fetal liver with a special view to hepatic lobule and to compare them with findings in liver of pregnant females as well as to study the filtering role of the placenta in the transportation of heavy metals between the mother and the fetus.

Authors used 40 pregnant mouse females that have been divided into 4 groups, each for 10 animals: a) control, b) fed by lead, c) fed by mercury and d) fed by cadmium. Animals of groups b), c), and d) have been fed with Pb, Cd or Hg salts in food in dose 0.03 mg of respective salt per day for a period of 12 days. On day 20 of pregnancy, samples of liver of both females and fetuses were taken and processed by standard way for the light and electron microscopy. Thick or thin sections were cut and stained for heavy metals. Histochemical procedure, based on conversion of metal onto specific sulphide that conjugates with silver according to Pearse (1972) and modified by Horký et al (2002), was used.

Light microscopy: Black granules of reaction product were found in the liver of both females and fetuses. In comparison with females, the fetal liver usually contained more deposits of all metals under study. The product was scattered throughout the hepatic parenchyma, however, the central area of liver lobule contained particles in higher density. Reaction product was also detected in Kupffer cells of the liver of fetuses.

Electron microscopy: In hepatocytes, the reaction product was observed freely in the ground cytoplasm, in small vesicles, lysosomes, and in the rough endoplasmic reticulum. Abundant deposits were especially typical of secondary lysosomes. In many cases, positive reacted cells showed marks of dilatation and fragmentation of the rough endoplasmic reticulum, increased density of ribosomes and even segregation of pars fibrosa and pars granulosa of the nucleolus. In contrast to controls, an increased staining of all membranous structures was found in animals fed with heavy metals. All mentioned findings were expressed more distinctly in the liver of fetuses.

It is concluded that heavy metals pass through the placenta and are cumulated by fetal liver more intensely than by liver of pregnant females. Predilection areae of accumulation of metals were the central zones of hepatic lobules. Reaction product particles were bound to secondary lysosomes of both hepatocytes as well as Kupffer cells. Heavy metals are supposed to "impregnate" plasma membrane and to penetrate through it into cell interior. Here they lead to disintegration of nuclear envelope, segregation of nucleoli, condensation of chromatin and in the final step to dilatation and fragmentation of the rough endoplasmic reticulum, and destruction of mitochondria.

P. Chovanec, M. Nováková[1], D. Horký (Department of Histology and Embryology, [1]Department of Physiology, Faculty of Medicine, Masaryk University, Brno): ***Antipsychotic Haloperidol as a Ligand of Sigma Receptors and its Cardiotoxicity***

The sigma receptors were identified in many tissues including a cardiac muscle tissue. At first, they were members of opioid receptors family, but later they were reclassified, due to their ability to bind ligands of nonopioid character. The aim of the study was to investigate submicroscopic and physiologic changes of cardiomyocytes throughout acute effect of haloperidol (H).

For this study, we chose two rats and two guinea pigs: One of each group as a control (C) and one as an experimental (E) animal. The animals were of male sex, because it is highly possible, that the endogenous ligand of sigma receptors is progesterone. It could interfere with H, and therefore induce false positivity. The animals were sacrificed by cervical dislocation in ether narcosis. We put out their hearts immediately and put them into the Langendorff apparatus. Simultaneously we scanned an electrocardiogram (ECG). At first each heart was perfused by Krebs-Henseleit solution (KHS) for 30 minutes, then washed the C by KHS, the E by H at the concentration of 10 nM for 30 minutes.

Afterwards the hearts were washed out by KHS and were perfused by 3% solution of glutaraldehyde for 10 minutes. The hearts were cut in 1x1x3 mm strips from both atria and both ventricles that were processed for electron microscopy by standard procedure.

The electronmicrographs showed that the ultrastructure of C was normal. In E, there were damages of Z discs. The mitochondria were elongated, many of them were swollen and rounded. We cannot leave out of consideration the agglomeration of mitochondria. We observed the increased amount of mitochondrial corpuscles. The diads were widened. ECG results in C revealed no pathology. In E, we could see alterations immediately after the perfusion of H. The QT interval was elongated followed by retarding of the heart rate. Then the ventricular arrythmias were discovered, both the free and the fixed arrythmias.

Analogous to other studies haloperidol is considered to be toxic, too. The reason of ECG pathology is perhaps caused by the activity of H at kalium channels. However, some changes we can not explain this way. It is possible, that the dilatation of diads and alterations of mitochondria manipulate the calcium (Ca^{2+}) homeostasis.

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