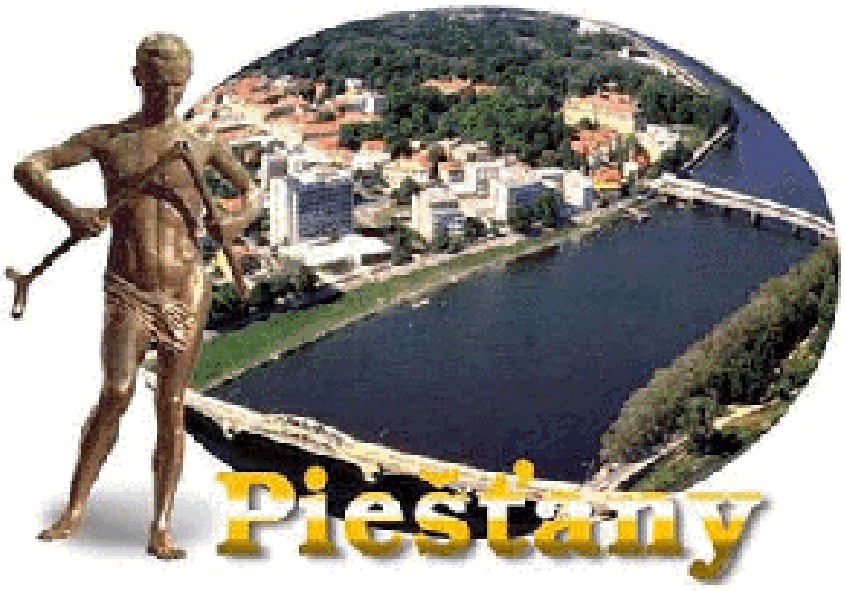
XX. BIOCHEMICKÝ ZJAZD

Piešťany

12. – 16. september 2006



Slovenská spoločnosť pre biochémiu a molekulárnu biológiu, člen IUMBM a FEBS
Česká společnost pro biochemii a molekulární biologii, člen IUMBM a FEBS
Biotechnologická spoločnosť na Slovensku
a
Ústav molekulárnej fyziológie a genetiky SAV

Proceedings

from *XX. Biochemický zjazd*
held in Piešťany 12. – 16. September 2006

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ISBN 80-969532-6-5

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všechny informace
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08:00	09:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00	20:00
T U E S D A Y												
Plenary Lecture Hall												
							Opening ceremony		PL1, 2			
W E D N E S D A Y												
Plenary Lecture Hall												
	PL3					PL4						
Lecture Room L1												
		Xenobiochemistry I					Xenobiochemistry II					
Lecture Room L2												
		Membrane Biochem. and Bioenergetics I.					Membrane Biochem. and Bioenergetics I.					
Lecture Room L3												
		Glycobiochemistry I					Glycobiochemistry II					
Lecture Room L4												
		Genomics					Free Radicals I					
Poster Evaluations I: posters in sections I, II, III, IV, IX												
T H U R S D A Y												
Lecture Room L1												
		Xenobiochemistry III				**Excursion to Castle Beckov**						
Lecture Room L2												
		Pathobio-chemistry I.				**Vine Testing in Sokolovce**						
Lecture Room L3												
		Cell regulations and signal transfer I.				**Excursion to Castle Beckov**						
Lecture Room L4												
		Free Radicals II				**Vine Testing in Sokolovce**						
Lecture Room L5												
		Biotechnology									**Concert**	
Poster Evaluations II: posters in sections V, VI, VII, VIII, X, XI, XII, XIII												
F R I D A Y												
Plenary Lecture Hall												
	PL5											
Lecture Room L1												
		Proteomics and Enzymology I.					Proteomics and Enzymology II.					
Lecture Room L2												
		Pathobioche-mistry II.					Patho-biochem. II.					
Lecture Room L3												
		Teaching biochemistry and molecular biology					Cell regulations and signal transfer II.					
Lecture Room L4												
		New Methods I.					New Methods I. and II.					
Poster Evaluations I: posters in sections I, II, III, IV, IX												

Tuesday, September 12, 2006

PLENARY LECTURE ROOM

12:00-20:00 REGISTRATION

15:00-15:45 Opening Ceremony

15:45-17.00 50 Years of Biochemistry Development in Czech and Slovak Republic

Radim Černý: FIFTY YEARS OF CSBMB

Jozef Čársky: DEVELOPMENT OF BIOCHEMISTRY IN SLOVAKIA

17:00-17:30 Coffee Break

PLENARY LECTURES
Chairs: Václav Pačes, Ján Turňa

17:30-18:00 **PL.1** Plenary Lecture I: **J.V. Koštíř prize for the best publication of Czech authors in 2004-2005:** *Šárka Pospíšilová*: GENOMIC AND PROTEOMIC APPROACHES TO THE ANALYSIS OF P53 ACTIVITY IN TUMOR CELLS.

18:00-18:30 **PL.2** Plenary Lecture II: **Winner of *"Drobnicov memoriál"* in 2005:** *Monika Baráthová*: ALTERNATIVE SPLICING PRODUCES TRUNCATED VARIANT OF CARBONIC ANHYDRASE IX THAT INHIBITS CA IX-MEDIATED EXTRACELLULAR ACIDIFICATION IN HYPOXIA

19:00-24:00 Get Together Party

Wednesday, September 13, 2006

07:00-08:30 Breakfast

PLENARY LECTURE ROOM

09:00-10:00 **PL.3** Plenary Lecture III: *Richard Kvetňanský*: CATECHOLAMINES IN STRESS: FROM GENE EXPRESSION OF BIOSYNTHETIC ENZYMES TO METABOLIC DEGRADATION

14:30-15:30 **PL.4** Plenary Lecture IV: **EMBO Lecture:** *Pico Caroni*: MEMBRANE-ASSOCIATED PROTEINS TO REGULATE THE CELL CORTEX IN MOTILITY AND MORPHOGENESIS

LECTURE ROOM L1

Section 5 XENOBIOCHEMISTRY I
Chairs: Miroslav Barančík, Jiří Hudeček

10:00-10:10 *Eva Kvasničková:* PROFESSOR I. M. HAIS - SCIENTIST AND INTERESTING PERSONALITY

10:10-10:50 K**L5.1** *Albert Breier:* ABC-TRANSPORTERS – PROTEINS WITH DIVERSE FUNCTIONS

10:50-11:15 **L5.2** *Miroslav Barančík, Vierka Boháčová, Ján Sedlák, Zdenka Sulová, Albert Breier:* LY294,002, AN INHIBITOR OF PI3K/AKT KINASE PATHWAY, REVERSES THE P-GLYCOPROTEIN-MEDIATED MULTIDRUG RESISTANCE IN L1210/VCR CELLS

11:15-11:35 Coffee Break

11:35-11:50 **L5.3** *Zdenka Sulová Annamaria Kovárová, Zuzana Vajcnerová, Branislav Uhrík, Danica Mislovičová, Albert Breier:* OVEREXPRESSION OF P-GLYCOPROTEIN IN L1210 CELLS IS ASSOCIATED WITH ALTERATION IN CELL SURFACE GLYCOSIDES

11:50-12:20 **L5.4** *Viktor Horváth, Karel Souček, Lenka Švihálková-Šindlerová, Jiřina Hofmanová, Petr Sova, Alois Kozubík:* DIFFERENCES IN CELL CYCLE REGULATION AFTER PLATINUM DERIVATIVES TREATMENT IN SENSITIVE AND CISPLATIN RESISTANT OVARIAN CANCER CELL LINES

12:20-12:35 **L5.5** *Barbora Szotáková, Veronika Křížová, Jiří Lamka:* THE ROLE OF CARBONYL REDUCING ENZYMES IN BIOTRANSFORMATION OF XENOBIOTICS

12:35-13:00 **L5.6** *Helena Kaiserová, Gertjan J.M. den Hartog, Tomáš Šimůnek, Ladislava Schröterová, Aalt Bast, Eva Kvasničková:* IRON CHELATORS IN ANTHRACYCLINE-INDUCED CARDIOTOXICITY

13:00-14:30 Lunch

Section 5 XENOBIOCHEMISTRY II

Chairs: Miroslav Machala, Albert Breier

15:30-16:10 K**L5.7** *Július Brtko, Dana Macejová, Slavomíra Ondková, Josef Thalhamer:* RETINOIC ACIDS-INDUCIBLE TRANSCRIPTION FACTORS AND THEIR ROLE IN THERAPY OF SELECTED MALIGNANT DISEASES

16:10-16:25 **L5.8** *Dana Macejová, Zdeněk Dvořák, Jitka Ulrichová, Július Brtko:* EFFECTS OF MICROTUBULES INTERFERING AGENTS AND/OR ALL-TRANS RETINOIC ACID ON EXPRESSION OF NUCLEAR RECEPTORS IN PRIMARY RAT HEPATOCYTES

16:25-16:55 **L5.9** *Miroslav Machala, Pavel Krčmář, Lenka Skálová, Barbora Szotáková, Martina Plíšková, Martin Bunček,*

Šárka Holasová, Lenka Dostálová, Jiřina Zatloukalová, Jan Vondráček: EXPRESSION OF ENZYMES OF METABOLIC ACTIVATION OF PAHs IN RAT LIVER CELLULAR MODELS

16:55-17:25 Coffee Break

17:25-17:40 **L5.10** *Zdeněk Dvořák, Radim Vrzal, Jitka Ulrichová, Martin Modrianský:* ROLE OF MICROTUBULES IN CELLULAR SIGNALING BY ARYL HYDROCARBON AND GLUCOCORTICOID RECEPTOR – CONSEQUENCES IN REGULATION OF DRUG METABOLIZING ENZYMES.

17:40-17:55 **L5.11** *Lenka Skálová, Martina Gavelová, Viktor Cvilink:* BIOTRANSFORMATION ENZYMES OF PARASITES AND THEIR ROLE IN DRUG-RESISTANCE DEVELOPMENT

17:55-18:10 **L5.12** *Silvia Letašiová, Soňa Jantová, Milan Miko:* BERBERINE–POSSIBLE POTENTIAL ANTICANCER COMPOUND AND ITS MECHANISM OF ACTION

18:10-18:30 **L5.13** *Viktor Viglaský, Patrik Danko:* IMPACT OF DNA BINDING INTERCALATORS ON ALTERNATIVE STRUCTURAL MOTIFS IN DNA

LECTURE ROOM L2

Section 3 MEMBRANE BIOCHEMISTRY AND BIOENERGETICS I

Chairs: Hana Rauchová, Miloslav Greksák

10:00-10:30 K**L3.1** *Ivan Hapala, Lucia Hronská, Martin Valachovič, Zuzana Mrózová*: LIFE WITHOUT OXYGEN: ADAPTATION OF YEAST LIPID METABOLISM TO ANAEROBIOSIS

10:30-11:00 K**L3.2** *Ľubica Lacinová, Bohumila Tarabová, Jutta Engel*: VOLTAGE GATED L-TYPE CALCIUM CHANNELS AND THEIR ROLE IN ORGANISM

11:00-11:30 Coffee Break

11:30-12:00 K**L3.3** *Alan Majerník, James Chong, Peter Šmigáň*: DNA REPLICATION INITIATION IN ARCHAEA - IS THERE A SPACE FOR FUNCTIONAL COUPLING TO CYTOPLASMIC MEMBRANE?

12:00-12:15 **L3.4** *Jiří Mašín, Radovan Fišer, Marek Basler, Jan Krůšek, Veronika Špuláková, Ivo Konopásek, Peter Šebo*: MEMBRANE TRANSLOCATION OF *BORDETELLA* ADENYLATE CYCLASE TOXIN PROMOTES CALCIUM ENTRY INTO $CD11b^+$ J774A.1 MACROPHAGE CELLS

12:15-12:30 **L3.5** *Bohumila Tarabová, Zdenka Sulová, Ľubica Lacinová*: EFFECT OF METHYLMERCURY ON NEURONAL T-TYPE CALCIUM CHANNEL

12:30-14:30 Lunch

Section 3 MEMBRANE BIOCHEMISTRY AND BIOENERGETICS II

Chairs: Eva Kutejová, Ivan Hapala

15:30-16:00 K**L3.6** *Peter Šmigáň, Zuzana Nováková, Stanislav Šurín, Alan Majerník*: Na^+ CYCLE AND ITS FUNCTION IN BIOENERGETICS OF METHANOARCHAEA

16:00-16:30 K**L3.7** *Anton Horváth, Petra Dunajčíková, Vladislava Benkovičová, Zdeněk Verner, Julius Lukeš*: RESPIRATORY CHAIN OF TRYPANOSOMATIDS

16:30-17:00 K**L3.8** *Gabriela Ondrovičová, Natalya Parkchomenko, Jiří Janata, Eva Kutejová:* MITOCHONDRIAL PROTEASES AND THEIR SUBSTRATES

LECTURE ROOM L3

Section 9 **GLYCOBIOCHEMISTRY I**
Chairs: *Peter Biely, Karel Bezouška*

10:00-10:45 K**L9.1** *Michaela Wimmerová, Martina Pokorná, Charles Sabin, Stephanie Perret, Jan Adam, Edward P. Mitchell, Anne Imberty:* BACTERIAL LECTINS AND RECOGNITION OF HOST GLYCANS: STRATEGIES FOR HIGH AFFINITY BINDING.

10:45-11:15 K**L9.2** *Karel Bezouška, Jan Sklenář, Ondřej Plíhal, Petr Pompach, Petr Man, Petr Novák, Vladimír Havlíček, Anna Fišerová, Luca Vannucci, Miloslav Pospíšil, Thisbe K. Linhorst, Joachim Thiem, Pavel Krist, Vladimir Křen:* MIMETICS OF THE CARBOHYDRATE LIGANDS FOR NK CELL RECEPTORS ARE EFFECTIVE IN EXPERIMENTAL TUMOR THERAPIES

11:15-11:40 Coffee Break

11:40-12:20 K**L9.3** *Michal Navrátil, Petr Pompach, Karel Bezouška:* PREPARATION OF ANTENNARY OLIGOSACCHARIDE LIGANDS FROM OVOMUCOID AND THEIR BINDING TO CD69 RECEPTOR

12:20-12:50 K**L9.4** *Vladimír Farkaš:* PLANT XYLOGLUCAN ENDOTRANSGLYCOSYLASE (XET) – PROPERTIES AND FUNCTION

12:50-13:10 **L9.5** *Mária Vršanská, Katarína Kolenová, Peter Biely:* MODE OF ACTION OF ENDOXYLANASES OF GLYCOSIDE HYDROLASE FAMILY 10, 11 AND 5 ON GLUCURONOXYLAN AND ACIDIC XYLO-OLIGOSACCHARIDES

13:10-14:30 Lunch

Section 9	**GLYCOBIOCHEMISTRY II**
Chairs:	*Jozef Čársky, Vladimír Farkaš*

15:30-16:00 K**L9.6** *Vladimír Puchart, Peter Biely*: UNIQUE TRANS-ALPHA GALACTOSYLATION TO INTERNAL SUGAR RESIDUES OF OLIGOSACCHARIDES CATALYZED BY *ASPERGILLUS FUMIGATUS* IMI 385708 ALPHA-GALACTOSIDASE

16:00-16:30 K**L9.7** *Jiří Liberda, Tichá Ivana, Tomáš Dráb, Lucie Prelovská, Marie Tichá, Pavla Maňásková*: FORMATION OF THE SPERM RESERVOIR IN REPRODUCTIVE TRACT

16:30-16:50 **L9.8** *Katarína Mikušová, Martina Beláňová, Petronela Dianišková, Zuzana Svetlíková, Jana Korduláková:* MYCOBACTERIAL GLYCOSYL TRANSFERASES - CHALLENGE AND EXPECTATIONS

16:50-17:10 Coffee Break

17:10-17:40 K**L9.9** *Grigorij Kogan, Eva Miadoková, Darina Slameňová, Viera Vlčková, Melánia Babincová, Peter Rauko:* ANTIOXIDANT, ANTIGENOTOXIC, AND IMMUNOMODULATING PROPERTIES OF YEAST CELL WALL POLYSACCHARIDES

17:40-18:10 K**L9.10** *Peter Biely, Gregory L. Côté:* CYCLIC OLIGO-SACCHARIDES – INTERMEDIATES OF STARCH UTILIZATION IN SOME BACTERIA

18:10-18:40 K**L9.11** *Jan Halámek, Ulla Wollenberger, Walter Stőcklein, Frieder W. Scheller:* ELECTROCHEMICAL AFFINITY SENSORS FOR GLYCATED HEMOGLOBIN

LECTURE ROOM L4

Section 1 GENOMICS
Chairs: Jaromír Pastorek, Ján Turňa

10:00-10:30 **L1.1** *Luboš Kľučár, Gabriela Bukovská*: BACTERIOPHAGE BFK20 GENOME ANOTATION

10:30-11:00 **L1.2** **Winner of Arnold Beckman award for the best publication in genomics:** *Tomáš Vacík, Michael Ort, Soňa Gregorová, Petr Strnad, Radek Blatný, Nathalie Conte, Allan Bradley, Jan Bureš and Jiří Forejt*: SEGMENTAL TRISOMY OF CHROMOSOME 17: A MOUSE MODEL OF HUMAN ANEUPLOIDY SYNDROMES

11:00-11:30 Coffee break

11:30-12:00 **L1.3** *Radka Václavíková, Miluše Hubáčková, Jana Stříbrná, Roman Kodet, Marcela Mrhalová, Ivan Gut and Pavel Souček:* EXPRESSION OF CYTOCHROMES P450 mRNA IN HUMAN CARCINOMA BREAST PATIENTS

12:00-12:30 **L1.4** *Anna Ohraďanová, Monika Baráthová, Miriam Zaťovičová, Juraj Kopáček, Silvia Pastoreková and Jaromír Pastorek*: FUNCTIONAL ANALYSIS OF AN UPSTREAM REGULATORY REGION OF THE GENE CODING FOR TUMOR ENDOTHELIAL MARKER (TEM1)/ENDOSIALIN

12:30-13:00 **L1.5** *Jozef Adamčík, Guillaume Witz, Dmitry V. Klinov, Sergey K. Sekatskii and Giovanni Dietler*: OBSERVATION OF SINGLE STRANDED DNA AND EXONUCLEASE III ACTIVITY BY ATOMIC FORCE MICROSCOPY

13:00-13:30 **L1.6** *Jana Jakubíková, Ján Sedlák*: QUANTIFICATION OF SPECIFIC ABC TRANSPORTER GENES EXPRESSION BY NOVEL MULTIPLEX ANALYSIS ON LUMINEX PLATFORM

13:30-14:30 Lunch

LECTURE ROOM L4

Section 6	**FREE RADICALS IN BIOLOGY AND MEDICINE I**
Chairs:	*Zdeňka Ďuračková, Ján Lehotský*

15:30-16:05 **KL6.1** *Zdeňka Ďuračková:* OXIDATIVE STRESS IN PATHOGENESIS OF DISEASES AND ITS MONITORING

16:05-16:30 **L6.2** *Zuzana Chovanová, Monika Dvořáková, Petra Högger, Zdeňka Ďuračková*: COMPARISON OF ANTIOXIDANT ACTIVITIES OF NATURAL EXTRACTS DISSOLVED IN DIFFERENT SOLUTIONS

16:30-16:55 **L6.3** *Ivana Márová , Simona Macuchová, Renata Mikulíková, Rostislav Kotrla*: INFLUENCE OF SEVERAL TYPES OF COMPLEX ANTIOXIDANT PREPARATIVES ON METABOLIC AND ANTIOXIDANT STATUS: A COMPARATIVE STUDY

16:55-17:20 **L6.4** *Ľubica Horáková, Miriam Štrosová, Janka Karlovská, Tilman Grune, Pavol Balgavý*: OXIDATIVE INJURY OF SARCOPLASMIC RETICULUM(SR) FROM RABBIT SKELETAL MUSCLE AND EFFECTS OF ANTIOXIDANTS

17:20-17:45 **L6.5** *Eva Babušíková, Miloš Jeseňák, Jozef Hatok, Peter Račay, Zuzana Tatarková, Anna Drgová, Ján Lehotský, Dušan Dobrota, Peter Kaplán*: OXIDATIVE CHANGES IN RAT BRAIN AND HEART DURING AGEING

POSTER SESSION I

POSTERS IN SECTIONS I , II , III , IV , IX

POSTER EVALUATIONS
08:00 – 09:00
13:00 – 14:30
18:00 – 20:00

19:00-20:00 Dinner

20:00-24:00 Disco Party

Thursday, September 14, 2006

07:00-08:30 Breakfast

LECTURE ROOM L1

Section 5	**XENOBIOCHEMISTRY III**
Chairs:	*Pavel Anzenbacher, Július Brtko*

10:00-10:45 K**L5.14** *Pavel Anzenbacher, Eva Anzenbacherová, Michal Otyepka*: CYTOCHROMES P450: FROM HISTORY TO STRUCTURE

10:45-11:30 K**L5.15** *Jiří Hudeček, Petr Hodek, Marie Stiborová:* CYTOCHROME P450: FROM STRUCTURE TO FUNCTION

11:30-11:50 Coffee Break

11:50-12:05 **L5.16** *Dagmar Aimová, Tereza Somolová, Helena Dračínská, Radka Václavíková, Marie Stiborová:* MECHANISM OF CYTOCHROME P450 1A1 AND 1A2 INDUCTION BY THE ANTICANCER DRUG ELLIPTICINE

12:05-12:20 **L5.17** *Jan Vondráček, Soňa Marvanová, Eva Hrubá, Pavel Krčmář, Jiřina Zatloukalová, Zdeněk Andrysík, Alois Kozubík, Miroslav Machala:* CONTROVERSIES IN THE ROLE OF p53 PROTEIN IN CELLULAR RESPONSE TO POLYCYCLIC AROMATIC HYDROCARBONS

12:20-12:35 **L5.18** *Jitka Poljaková, Jan Hraběta, Tomáš Eckschlager, Eva Frei, Marie Stiborová:* ANTICANCER DRUG ELLIPTICINE IS CYTOTOXIC TO HUMAN LEUKEMIA AND NEUROBLASTOMA CELL LINES

12:35-12:50 **L5.19** *Ladislava Schröterová, Zuzana Filarová, Pavlína Hašková, Emil Rudolf, Miroslav Červinka:* INOSITOL

HEXAPHOSPHATE – A NOVEL ANTI-CANCER AGENT

12:50-13:00 Information about XVII. Symposium of Xenobiochemistry

12:50-14:00 Lunch

LECTURE ROOM L2

Section 7 PATHOBIOCHEMISTRY I
Chairs: *Oľga Križanová, Marie Nováková*

10:00-10:35 K**L7.1** *Marie Nováková, Hana Bochořáková, Hana Paulová, Eva Táborská:* ISCHEMIC-REPERFUSION INJURY OF MYOCARDIUM: FROM CALCIUM PARADOX TO FREE RADICALS

10:35-10:55 **L7.2** *Oľga Križanová:* INOSITOL 1,4,5-TRISPHOSPHATE RECEPTORS AND THEIR MODULATION

10:55-11:15 **L7.3** *Dana Jurkovičová, Juraj Kopáček, Peter Štefánik, Lucia Kubovčáková, Silvia Pastoreková, Oľga Križanová:* HYPOXIA MODULATES GENE EXPRESSION OF THE IP_3 RECEPTORS IN MOUSE CEREBELLUM

11:15-11:40 **L7.4** *Ján Lehotský, Peter Kaplán, Martina Pavlíková, Peter Urban, Radovan Murín, Eva Babušíková, Zuzana Tatarková, Dušan Dobrota:* DIVERSE FORMS OF INTRACELLULAR CALCIUM STORES: IMPLICATION FOR THE DISEASED CELLULAR STATES

11:40-11:55 **L7.5** *Martina Pavlíková, Peter Urban, Radovan Murín, Dušan Dobrota, Ján Lehotský:* EXPRESSION OF GOLGI SPCA1 CA^{2+} PUMP IN RAT NEURONAL TISSUE: EFFECT OF ISCHEMIC/REPERFUSION INSULT.

11:55-14:00 Lunch

LECTURE ROOM L3

Section 4 **CELL REGULATIONS AND TRANSFER OF SIGNALS I**

Chairs: *Ján Kormanec, Silvia Pastoreková*

10:00-10:45 K**L4.1** *Silvia Pastoreková, Juraj Kopáček, Jaromír Pastorek*: HYPOXIA SIGNALING IN HEALTH AND DISEASE – BOTH SIDES OF STORY

10:45-11:10 **L4.2** *Radana Vrzáková, Radim Černý*: ECTOPIC EXPRESSION OF ENAMEL MATRIX PROTEINS ?

11:10-11:35 **L4.3** *Vladimír Mikeš**:*** SYSTEMIC RESISTANCE OF LANTS, THEORY VS. PRACTICE

11:35-12:00 **L4.4** *Martin Fusek, Jana Větvičková, Václav Větvička*: SECRETION OF CYTOKINES IN BREAST CANCER – MOLECULAR MECHANISM OF PROCATHEPSIN D MITOGENICITY

12:00-12:30 **L4.5** *Renata Nováková**,** Jana Bistaková, Ľubomíra Fecková, Ján Kormanec:* THE REGULATION OF PRODUCTION OF POLYKETIDE ANTIBIOTIC AURICIN IN *S. AUREOFACIENS*

12:30-14:00 Lunch

LECTURE ROOM L4

Section 6 **FREE RADICALS IN BIOLOGY AND MEDICINE II**

Chairs: *Lubica Horáková, Attila Ziegelhöffer*

10:00-10:25 **L6.6** *Attila Ziegelhöffer, Miroslav Ferko, Iveta Waczulíková, Jana Mujkošová, Terézia Holotňáková, Jaromír Pastorek, Silvia Pastoreková, Jozef Čársky:* FUNCTIONAL REMODELING OF THE DIABETIC MYOCARDIUM: THE ROLE OF FREE RADICALS

10:25-10:50 **L6.7** *Lukáš Kubala, Stephan Baldus, Antoni Lojek, Jason P. Eiserich*: MYELOPEROXIDASE BINDING TO EXTRACELLULAR MATRIX

10:50-11:15 **L6.8** *Martin Vejražka, Marek Grega, Stanislav Štípek*: URIC ACID STIMULATES SUPEROXIDE PRODUCTION BY NAD(P)H OXIDASE OF VASCULAR SMOOTH MUSCLE CELLS

11:15-11:45 Coffee Break

11:45-12:10 **L6.9** *Slávka Kaščáková, Anton Mateašik, Matthieu Refregiers, Daniel Jancura, Jean-Claude Maurizot, Pavol Miškovský:* CELLULAR INTERNALIZATION MECHANISMS AND INTRACELLULAR LOCALIZATION OF HYPERICIN IN GLIOMA CELL LINE U-87 MG

12:10-12:35 **L6.10** *Alexey Kondrashov:* THE DIVERSE MECHANISMS OF PLANT POLYPHENOL EFFECTS IN THE PREVENTION OF VASCULAR DISEASES

LECTURE ROOM L5

Section 8 BIOTECHNOLOGY
Chairs: Jaroslav Spížek, Jozef Timko

10:00-10:50 K**L8.1** *Jaroslav Spížek, Jiří Janata, Markéta Jelínková, Jan Kopecký, Jitka Novotná*: BIOTECHNOLOGY FOR PRODUCTION OF SECONDARY METABOLITES

10:50-11:15 **L8.2** *Juraj Gašperík, Ingrid Čipáková, Eva Hostinová*: PRODUCTION OF HUMAN RECOMBINAT ANTIMICROBIAL PEPTIDES

11:15-11:45 Coffee break

11:45-12:10 **L8.3** *Gabriela Borošová***:** A KEY ROLE OF HIGHPRODUCING STRAINS OF MICROORGANISMS IN BIOTECHNOLOGY

12:10-12:35 **L8.4** *Radka Podlipná, Zuzana Vavříková, Tomáš Vaněk*: FYTOREMEDIATION OF NITROESTERS

12:35-13:00 **L8.5** *Hana Drahovská, Eva Mikasová, Tomáš Szemes, Milan Sásik, Viktor Majtán, Ján Turňa*: MOLECULAR METHODS FOR DETECTION OF DNA VARAIBILITY IN CLOSELY RELATIVE SALMONELLA STRAINS

POSTER SESSION II

POSTERS IN SECTIONS V , VI , VII , VIII , X , XI , XII , XIII

POSTER EVALUATIONS
8:00 – 10:00

12:30-14:00 Lunch

14:00-18:00 Excursion in Castle Beckov or Vine Testing in Sokolovce

18:00-19:00 Dinner

19:00-21:00 Concert

Friday, September 15, 2006

07:00-08:30 Breakfast

PLENARY LECTURE ROOM

09:00-09:45 **PL5** **Plenary Lecture V:** *Peter Šebo*: LEARNING AND GETTING HELP FROM BACTERIAL TOXINS: FROM THE MECHANISM OF ADENYLATE CYCLASE TOXIN ACTION TO ANTIGEN DELIVERY FOR VACCINATION AND TUMOR IMMUNOTHERAPY

LECTURE ROOM L1

Section 2 PROTEOMICS AND ENZYMOLOGY I
Chairs: Lenka Hernychová, Ľudovít Škultéty

10:00-10:35 K**L2.1** *Lenka Hernychová, Ľudovít Škultéty*: CHARACTERIZATION OF SELECTED BIOLOGICAL WARFARE AGENT PROTEOMES USING MASS SPECTROMETRY

10:35-10:55 **L2.2** *Lucie Vojtová, Tomáš Zima, Vladimír Tesař, Markéta Kazderová*: CHANGES IN THE URINARY PROTEOMES IN PATIENTS WITH NEPHROTIC SYNDROM

10:55-11:15 **L2.3** *Hana Pančuchárová, Ingrid Krejnusová, Magdaléna Bystrická, Hana Blaškovičová, Gustáv Russ*: ANTIBODIES SPECIFIC FOR INFLUENZA A VIRUS PROTEIN PB1-F2 ARE PRESENT IN HUMAN CONVALESCENT SERA

11:15-11:45 Coffee Break

11:45-12:15 **L2.4** **Winner of Arnold Beckman award for the best publication in proteomics:** *Petr Man, Petr Novák, Marek Cebecauer, Ondrej Horváth, Anna Fišerová, Vladimír Havlíček, Karel Bezouška:* MASS SPECTROMETRIC ANALYSIS OF THE GLYCOSPHINGOLIPIDENRICHED MICRODOMAINS OF RAT NATURAL KILLER CELLS

12:15-12:35 **L2.5** *Ľuboslav Mihók, Daniel Kavan, Ondřej Vaněk, Petr Pompach, Jan Bílý, Petr Novák, Kateřina Hofbauerová, Vladimír Kopecký, Monika Nálezková, Lukáš Žídek, Vladimír Sklenář, Karel Bezouška*: OPTICAL SPECTROSCOPY, MASS SPECTROMETRY AND NMR ARE ESSENTIAL TOOLS IN THE PRODUCTION OF SOLUBLE RECEPTORS OF NATURAL KILLER CELLS

12:35-12:55 **L2.6** *Karel Mazanec, Karel Šlais, Josef Chmelík*: INVESTIGATION OF POSTTRANSLATIONALLY MODIFIED (MALTED) PROTEINS BY COMBINATION OF IEF AND MALDI-TOF/TOF MS

13:00-15:00 Lunch

Section 2 PROTEOMICS AND ENZYMOLOGY II

Chairs: Štefan Janeček, Ľudovít Škultéty

15:00-15:35 K**L2.7** *Štefan Janeček:* AMYLOLYTIC ENZYME FAMILIES IN THE POST-GENOME ERA

15:35-15:55 **L2.8** *Nina Davidová, Pavla Maňásková, Věra Jonáková:* STUDY OF PROTEINASE INHIBITORS IN BOAR EPIDIDYMAL FLUID

15:55-16:15 **L2.9** *Eva Cibulková, Pavla Maňásková, Věra Jonáková, Marie Tichá:* STUDY OF HYALURONIDASE IN BOAR REPRODUCTIVE TRACT.

16:15-16:45 Coffee break

16:45-17:05 **L2.10** *Oldřich Janiczek, Blanka Pokorná, Martin Mandl*: ENZYMES OF SULFUR METABOLISM IN *ACIDITHIOBACILLUS FERROOXIDANS*

17:05-17:25 **L2.11** *Oskar Markovič:* STRUCTURAL-FUNCTIONAL DIFFERENCES IN THREE CLASSES OF PECTIC ESTERASES

17:25-17:45 **L2.12** *Helena Ryšlavá, Karel Muller, Noemi Čeřovská:* REGULATION OF THE PHOSPHOENOLPYRUVATE CARBOXYLASE UNDER STRESS CONDITIONS

17:45-18:05 **L2.13** *Michal Otyepka, Pavel Banáš, Jiří Damborský, Alessandra Magistrato, Paolo Carloni:* THE IMPORTANCE OF THE OXYANION HOLE IN ESTER HYDROLYSIS OF ENZYMATIC DEHALOGENATION CATALYZED BY HALOALKANE DEHALOGENASE REVEALED BY QM/MM CALCULATIONS

LECTURE ROOM L2

Section 7 **PATHOBIOCHEMISTRY II**
Chairs: *Nadežda Lukáčová, Peter Račay*

10:00-10:35 K**L7.6** *Peter Račay, Zuzana Tatarková, Peter Kaplán, Dušan Dobrota:* MITOCHONDRIAL DYSFUNCTION INDUCED BY BRAIN ISCHEMIA

10:55-11:15 **L7.7** *Andrej Tillinger, Viliam Fischer, Ivan Gabauer, Barbara Grantnerová, Oľga Križanová, Richard Kvetňanský:* GENE EXPRESSION OF ADRENERGIC RECEPTORS IN FAILING HEART OBTAINED BY TRANSPLANTATION

11:15-11:45 Coffee Break

11:45-12:05 **L7.8** *Oľga Uličná, Oľga Vančová, Peter Božek, Jozef Čársky, Katarína Šebeková, Peter Boor, Miloslav Greksák:* MODULATION OF OXIDATIVE STRESS AND GLYCATION BY PLANT ANTIOXIDANTS IN EXPERIMENTALLY INDUCED DIABETES

12:05-12:25 **L7.9** *Nadežda Lukáčová, Jozef Maršala:* THE PARTICIPATION OF NITRIC OXIDE IN ANTEROGRADE SIGNALING

12:25-15:00 Lunch

Section 7 PATHOBIOCHEMISTRY III

Chairs: Juraj Kopáček, Michal Svoboda

15:00-15:35 K**L7.10** *Juraj Kopáček, Jaromír Pastorek, Silvia Pastoreková:* ROLE OF TUMOR MICROENVIRONMENT IN THE MODULATION OF CARBONIC ANHYDRASE IX EXPRESSION

15:35-15:55 **L7.11** *Jozef Hatok, Tatiana Matáková, Mária Franeková, Dušan Dobrota, Peter Račay:* COMPARISON OF ABC TRANSPORTERS EXPRESSION WITH CLINICAL OUTCOME IN ACUTE LEUKAEMIA

15:55-16:15 **L7.12** *Michal Svoboda, Ondřej Blaštík, Pavlína Šobrová, Richard Průša, Jiří Kukačka, Hana Binková, Zuzana Horaková, Vojtěch Adam, René Kizek:* METALOTHIONEIN AS A POTENTIAL TUMOR DISEASE MARKER

16:15-16:35 **L7.13** *Ivo Juránek, Ladislav Bačiak, Eduard Ujházy, Svatava Kašparová:* MATURATION OF ATP YIELDING IN NEONATAL RAT BRAIN IN RELATION TO ITS SENSITIVITY TO HYPOXIA – AN *IN VIVO* ^{31}P-MRS STUDY

LECTURE ROOM L3

Section 10 **TEACHING BIOCHEMISTRY AND MOLECULAR BIOLOGY**

Chairs: *Katarína Mikušová, Anton Horváth*

10:00-10:20 **L10.1** *Milan Kodíček, Miloslav Nič, Jiří Jirát, Jiří Znamenáček*: BIOCHEMICAL ENCYCLOPAEDIA: NEW DEVELOPMENT OF THE STUDYING MATERIAL

10:20-10:40 **L10.2** *Viktor Viglaský*: TRENDS IN EDUCATION OF BIOCHEMISTRY ON THE ŠAFARIK UNIVERSITY

10:40-11:00 **L10.3** *Vladimír Mikeš*: TEACHING BIOCHEMISTRY AT FACULTY OF SCIENCE IN BRNO

11:00-11:20 **L10.4** *Branislav Líška, Marta Brechtlová, Ján Podhradský, Lukáč Halčák, Monika Ďurfinová*: TEACHING OF MEDICAL BIOCHEMISTRY FOR STUDENTS FROM FACULTY OF NATURAL SCIENCES AND FACULTY OF PHYSICAL EDUCATION AND SPORT, COMENIUS UNIVERSITY, BRATISLAVA

11:20-11:40 Coffee break

11:40-12:00 **L10.5** *Zdenka Gálová, Želmíra Gregáňová*: TEACHING OF MOLECULAR BIOLOGY AT THE FACULTY OF BIOTECHNOLOGY AND FOOD SCIENCES SAU IN NITRA

12:00-12:20 **L10.6** *Danka Valková, Elena Hlinková, Anton Horváth, Marta Kollárová, Ján Turňa*: THE NEWEST ACHIEVEMENTS OF MOLECULAR BIOLOGY IN PRACTICE – STRUCTURE AND CONTENT OF THE SUBJECT: BIOTECHNOLOGY1, 2, 3 AND 4

12:20-12:40 **L10.7** *Miroslava Slaninová, Eliška Gálová, Andrea Ševčovičová, Katarína Mikušová, Gabriela Gavurníková, Jozef Nosek, Ľubomír Tomáška*: LABORATORY WORKSHOPS AS MEANS OF

MOTIVATION OF HIGH SCHOOL STUDENTS FOR EXPERIMENTAL BIOLOGY

13:00-15:00 Lunch

LECTURE ROOM L3

Section 4 CELL REGULATIONS AND TRANSFER OF SIGNALS II

Chairs: Ján Kormanec, Radim Černý

15:00-15:30 **L4.6** **Winner of Arnold Beckman award for the best publication in cell biology:** *Vlada V. Philimonenko, Jian Zhao, Sebastian Iben, Hana Dingová, Katarína Kyselá, Michal Kahle, Hanswalter Zentgraf, Wilma A. Hofmann, Primal de Lanerolle, Pavel Hozák and Ingrid Grummt*: NUCLEAR ACTIN AND MYOSIN I ARE REQUIRED FOR RNA POLYMERASE I TRANSCRIPTION

15:30-15:55 **L4.7** *Vladislava Mazuráková, Beatrica Sevčíková, Ján Kormanec*: THE ROLE OF TWO ALTERNATIVE SIGMA FACTORS, SigB AND SigH, IN REGULATION OF DIFFERENTIATION AND STRESS RESPONSE IN *STREPTOMYCES COELICOLOR* A3(2)

15:55-16:20 **L4.8** *Klára Hoyerová, Václav Motyka, Petre Dobrev, Miroslav Kamínek:* MECHANISMS OF HORMONAL HOMEOSTASIS IN PLANTS

16:20-16:45 **L4.9** *Boris Lakatoš, Jana Slováková, Karin Kaiserová, Jozef Orlický, Ľudovít Varečka:* BASAL CALCIUM INFLUX IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES

16:45-17:10 Coffe Break

17:10-17:35 **L4.10** *Jana Kamanová, Oľga Kofronová, Harald Genth, Jana Vojtová, Irena Linhartová, Ingo Just, Peter Šebo:* CAMP-MEDIATED SIGNALING OF *BORDETELLA*

ADENYLATE CYCLASE TOXIN INDUCES MEMRANE RUFFLING OF MYELOID MONOCYTES

17:35-18:00 **L4.11** *Iveta Bártová, Michal Otyepka, Zdeněk Kříž a Jaroslav Koča:* THE ROLE OF PHOSPHORYLATION IN CYCLIN DEPENDENT KINASE 2 AND 5 REGULATION. A COMPUTER SIMULATION STUDY

LECTURE ROOM L4

Section 11 NEW METHODS I
Chairs: Zdenka Sulová, Attila Ziegelhöffer

Commertial presentations

10:00-10:45 **CP 1** *Adam Andráško, Lucia Babjaková, Soňa Bernátová, Miloš Čebík :* MERCK LIFE SCIENCE INTRODUCE PRODUCTS FOR RESEARCH IN GENOMICS & PROTEOMICS.

10:45-11:30 **CP2** *Lucinda Gedge :* SNAP-TAG: USE OF A SINGLE PROTEIN TAG FOR PROTEIN DETECTION, LABELING AND IMMOBILIZATION

11:30-11:45 Coffee break

11:45-12:30 **CP 3** *Dalimil Žůrek :* ROCHE APPLIED SCIENCE SOLUTIONS FOR REAL-TIME PCR

12:30-13:15 **CP4** *Martin Janitor :* PROMEGA CELL BASED ASSAY SYSTEMS – HOW TO SEE STILL INVISIBLE PROCESSES?

13:15-15:00 Lunch

15:00-15:45 **CP 5** *Heidi Onderkova :* AUTOMATED CELL COUNTING AND FLOW CYTOMETRY ANALYSIS-MICROCYTOMETRY USING GUAVA TECHNOLOGIES PLATFORMS

15:45-16:30 **CP6** *Roman Vlček :* CELL LAB QUANTA, A COMBINATION OF FLOW CYTOMETRY AND THE COULTER PRINCIPLE

16:30-16:50 Coffee break

LECTURE ROOM L4

Section 11 New Methods II
Chairs: Ľudovít Varečka, Ladislav Bumba

16:50-17:20 **L11.1** *Katarína Dubayová, Jaroslav Kušnír*: FLUORESCENCE SPECTRAL MATRICES

17:20-17:50 **L11.2** *Ladislav Bumba, František Vácha*: TOWARDS STRUCTURAL AND FUNCTIONAL ASPECTS OF PLANT CHLOROPLAST AND CHLOROPLAST INNER MEMBRANES

17:50-18:20 **L11.3** *Michaela Vítková, Ján Polák, Eva Klimčáková, Michaela Kováčiková, Jindra Hejnová, Zuzana Kováčová, Magda Bajzová, Vladimír Štich:* MICRODIALYSIS AS A NEW TECHNIQUE FOR CYTOKINES DETECTION

18:20-18:50 **L11.4** *Pavel Banáš, Martin Petřek, Michal Otyepka, Pavlína Košinová, Jiří Damborský*: CAVER: A PROGRAM FOR MOLECULAR SPELEOLOGY.

POSTER SESSION II

POSTERS IN SECTIONS V , VI , VII , VIII , X , XI , XII , XIII

POSTER EVALUATIONS
8:00 – 9:00
13:00 – 14:30

19:00-24:00 Farewell Party

Saturday, September 16, 2006

07:00-08:30 Breakfast

Departure from Piešťany

POSTER SECTION

POSTER SESSION I

Posters should be exhibited between Tuesday 15:00 and Wednesday 20:00

Section 1 GENOMICS

PO 1.1 *Petra Belvončíková, Iveta Vančová Iveta, Valéria Hájnická, Marcela Kúdelová, Ingeborg Režuchová :* HEMOKINE BINDING PROTEIN ENCODED BY M3 GENE OF MURINE HERPESVIRUS 72

PO 1.2 *Jana Blaškovičová, Juraj Kopáček, Jela Mistríková, Miroslava Šúpolíková :* COMPARATIVE GENOMICS OF MOUSE HERPESVIRUS STRAINS ŠUMAVA AND MHV68

PO 1.3 *Jarmila Farkašovská, Luboš Kľučár, Andrej Godány***:** THE GENOME OF μ1/6, A STREPTOMYCES AUREOFACIENS INFECTING PHAGE

PO 1.4 *Mária Franeková, Pavol Žúbor, Andrea Štanclová, Tatiana Bohušová, Dušan Dobrota, Martin Péč , Peter Račay:* POLYMORPHISMS AND MUTATIONS IN TUMOR SUPPESSOR GENES *P53* AND *BRCA* IN BREAST CANCER.

PO 1.5 *Jozef Grones and Miroslava Kretová* **:** PLASMID pAG20 FROM *Acetobacter aceti* 3620.

PO 1.6 *Dita Královcová* **:** QUANTITATIVE ANALYSIS OF GENE EXPRESSION LEVELS IN HEP-2 CELLS AFTER INDUCTION OF APOPTOSIS.

PO 1.7 *Irena Linhartová, Marek Basler, Jeffrey Ichikawa, Vladimír Peličic, Radim Osička, Stephen Lory, Xavier Nassif and Peter Šebo :* MENINGOCOCCAL ADHESION SUPPRESSES PRO-APOPTOTIC GENE EXPRESSION AND PROMOTES EXPRESSION OF GENES SUPPORTING EARLY EMBRYONIC AND CYTOPROTECTIVE SIGNALING OF HUMAN ENDOTHELIAL CELLS.

PO 1.8 *Zuzana Marčeková, Eva Kosinová, Ivan Pšikal, Peter Šebo :* RECOMBINANT NUCLEOCAPSID PROTEIN OF PORCINE CIRCOVIRUS TYPE 2.

PO 1.9 *Daniel Mihálik, Edita Gregová, Michal Šajgalík :* BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF NOVEL *Glu-1Dy* LOCI ALLELE IN HEXAPLOID WHEAT (*Triticum aestivum* L.)

PO 1.10 *Jaroslava Ovesná, Katarína Mitrová, Ladislav Kučera :* SEQUENCE VARIABILITY OF S-alk(en)yl-L-CYSTEINE SULFOXIDE LYASE FROM GARLIC (*Allium sativum* L.) CLONES.

PO 1.11 *Róbert Seliga, Peter Pristaš, Peter Javorský :* „BIOLOGICAL COST" OF ACQUIRED ANTIBIOTIC RESISTANCE

PO 1.12 *Monika Sivoňová, Tatiana Matáková, Dušan Dobrota, Ján Kliment jr. and Ján Kliment :* GENETIC POLYMORPHISM OF GLUTATHIONE-S-TRANSFERASE P1 AND PROSTATE CANCER

PO 1.13 *Ondřej Slabý, Ingrid Garajová, Marek Svoboda, Miroslav Svoboda, Rostislav Vyzula :* MOLECULAR CHARACTERIZATION OF PRIMARY COLORECTAL CANCERS WITH PROGRESSIVE METASTATIC PHENOTYPE BY OLIGONUCLEOTIDE MICROARRAYS

PO 1.14 *Tomáš Szemes, Hana Drahovská, Eva Mikasová, Ján Turňa :* ASSESMENT AND DESIGN OF MOLECULAR GENOTYPING METHODS FOR DISCRIMINATION OF SALMONELLA STRAINS

PO 1.15 *Jana Tomášková, Martina Labudová, Silvia Pastoreková & Jaromír Pastorek :* PRIMARY STRUCTURE OF THE GENOME OF LYMPHOCYTIC CHORIOMENINGITIS VIRUS STRAIN MX

PO 1.16 *Tímea Tóthová, Peter Pristaš, Peter Javorský :* MACRONUCLEAR DNA ORGANIZATION IN SOIL PROTOZOAN *OXYTRICHA* SPP.

PO 1.17 *Danka Valková, Silvia Vávrová, Lenka Valkovičová, Elena Kováčová,Jozef Mravec and Ján Turňa:* TELLURITE

RESISTANCE GENES AND FITNESS OF THE UROPATHOGENIC *E. coli* CELLS

PO 1.18 *Monika Valovičová, Katarína Reinhardt, Petra Belvončíková, Ingeborg Režuchová, Ján Matis and Marcela Kúdelová :* ANALYSIS OF MURINE HERPESVIRUS STRAIN 72 AND 4556 (MHV72 AND MHV4556) GENOMES REVEALS GENES DIFFERENT IN PRIMARY STRUCTURE: PARTIAL RESULTS

PO 1.19 *Monika Valovičová , Vladimír Krivoš , Hana Rašlová , Ján Matis and Marcela Kúdelová :* COMPARISON OF HUMAN PAPILLOMAVIRUS 16 DISTRIBUTION IN SMEARS FROM TWO DIFFERENT CERVIX REGIONS OF ONCO-GYNAECOLOGICAL PATIENTS FROM WEST SLOVAKIA

Section 2 PROTEOMICS AND ENZYMOLOGY

PO 2.1 *Markéta Laštovičková, Karel Mazanec and Josef Chmelík :* THE IDENTIFICATION OF ALBUMIN AND GLOBULIN FRACTION OF PROTEINS AND GLYCOPROTEINS ISOLATED FROM BARLEY GRAINS

PO 2.2 *Jiří Šalplachta, Janette Bobáľová and Josef Chmelík :* STUDY OF WATER-SOLUBLE PROTEINS FROM BARLEY BY GEL ELECTROPHORESIS AND MALDI MASS SPECTROMETRY

PO 2.3 *Zuzana Vavříková, Radka Podlipná, Tomáš Vaněk :* XENOBIOTIC INFLUENCE ON *ARABIDOPSIS THALIANA* PROTEOME

PO 2.4 *Tatjana Stojnev, Jana Harichová, Edita Karelová, Katarína Chovanová, Domenico Pangallo, Peter Ferianc :* A NOVEL CADMIUM-BINDING PROTEIN FROM *Escherichia coli*

PO 2.5 *Denisa Petráčková, Lenka Šemberová, Petr Svoboda, Jaroslava Svobodová :* STRESS PROTEINS IN THE CYTOPLASMIC MEMBRANE OF BACILLUS SUBTILIS

PO 2.6 *Barbora Vidová , Michal Chotár, Andrej Godány :* BIOLOGICAL VARIABILITY OF SURFACE IMMUNOGENIC PROTEINS IN *Streptococcus agalactiae*

PO 2.7 *Eva Beregházyová, Ľudovít Škultéty, Rudolf Toman :* DIFFERENTIAL EXPRESSION OF *COXIELLA BURNETII* PROTEINS IN VIRULENT PHASE I AND AVIRULENT PHASE II

PO 2.8 *Barbora Jankovičová, Šárka Rösnerová, Josef Královský, Zuzana Bílková :* ISOLATION OF IMMUNOGLOBULIN E (IgE) FOR DETECTION OF ALLERGOGENIC EPITOPES USING BIOAFFINITY MAGNETIC REACTORS

PO 2.9 *Martina Labudová, Jana Tomášková, Anna Ohraďanová, Ľudovít Škultéty, Silvia Pastoreková and Jaromír Pastorek :* SEARCH FOR THE PROTEINS INTERACTING WITH THE NUCLEOPROTEIN OF LYMPHOCYTIC CHORIOMENINGITIS VIRUS STRAIN MX

PO 2.10 *Alžbeta Hulíková, Anna Ohraďanová, Adriana Gibadulinová, Eliška Švastová, Miriam Zaťovičová, Juraj Kopáček, Claudiu Supuran, Silvia Pastoreková and Jaromír Pastorek* : STRUCTURE-FUNCTION STUDY OF HYPOXIA-REGULATED CARBONIC ANHYDRASE IX USING IN VITRO MUTAGENESIS AND SPECIFIC SULFONAMIDE INHIBITORS

PO 2.11 *Jaromír Kotyza, Karin Buňatová, Miloš Pešek :* HUMORAL CHANGES IN PLEURAL SPACE ASOCIATED WITH CANCER, INFLAMMATION AND INJURY

PO 2.12 *Dana Grebeňová, Kateřina Kuželová, Michaela Pluskalová, Iuri Marinov, Petr Halada, Zbyněk Hrkal* : PROTEOME CHANGES IN CHRONIC LEUKEMIA CELLS JURL-MK1 INDUCED BY IMATINIB MESYLATE TREATMENT

PO 2.13 *Michaela Pluskalová, Dana Grebeňová, Kateřina Kuželová, Petr Halada and Zbyněk Hrkal* : HOW IMATINIB MESYLATE DOES OPERATE IN CML-DERIVED CELL LINE JURL-MK1?

PO 2.14 *Katarína Bíliková, Hans Lehrach, Jozef Šimúth :* ANTIMICROBIAL AND PHYSIOLOGICAL ACTIVITY OF SOME EXOGENOUS HONEYBEE PROTEINS AND PEPTIDES.

PO 2.15 *Ján Blahovec, Zuzana Kostecká, Alica Kočišová :*

LARVAL PROTEINASES OF HOUSEFLY *MUSCA DOMESTICA*.Endo- and exopeptidolytic activity into natural and chromogenic substrates

PO 2.16 *Zuzana Kostecká, Ján Blahovec :* PROPERTIES OF PARTIALLY PURIFIED LARVAL AMINOPEPTIDASES OF HOUSEFLY MUSCA DOMESTICA

PO 2.17 *Ivana Holková, Marián Vanko, Marek Obložinský, Andrea Balážová, Víťazoslava Blanáriková, Lýdia Bezáková :* LIPOXYGENASE ACTIVITY OF POPPY SEEDLINGS AND CELL CULTURES OF *PAPAVER SOMNIFERUM* L.

PO 2.18 *Marián Vanko, Ivana Holková, František Bilka, Andrea Bilková, Andrea Balážová, Lýdia Bezáková :* POLYPHENOL OXIDASE FROM LATEX OF COMMON CELANDINE (*Chelidonium majus*).

PO 2.19 *Veronika Doubnerová, Alena Jirásková, Karel Müller, Noemi Čeřovská, Helena Ryšlavá :* THE EFFECT OF BIOTIC STRESS ON THE ACTIVITY OF NADP-MALIC ENZYME IN PLANTS FROM *NICOTIANA* GENUS

PO 2.20 *Dana Flodrová, Eva Stratilová, Mária Dzúrová , Danica Mislovičová, Jiřina Omelková* **:** CHARACTERISATION OF EXOPOLYGALACTURONASE AND OLIGOGALACTURONATE HYDROLASE FROM PARSLEY ROOTS

PO 2.21 *Soňa Garajová, Eva Stratilová, Danica Mislovičová, Vladimír Farkaš :* TWO FORMS OF XTH IN PARSLEY ROOTS

PO 2.22 *Lenka Luhová , Marek Petřivalský, Milan Navrátil, Pavel Peč:* IMMUNOCHEMICAL ANALYSIS OF PLANT NITRIC OXIDE SYNTHASE

PO 2.23 *Marek Petřivalský, Lenka Luhová, Pavel Peč :* THE INHIBITION OF COPPER DIAMINE OXIDASE BY REACTIVE NITROGEN SPECIES

PO 2.24 *Ludmila Zajoncová, Lenka Luhová, Marek Petřivalský and Marek Šebela :* DETERMINATION OF HYDROGEN PEROXIDE IN PLANT EXTRACTS BY AMPEROMETRIC SENSOR

PO 2.25 *Hana Vodičková , Ludmila Staszková , Jan Táborský, Dana Hradecká :* THE IMPACT OF SOME PLANT HORMONES ON THE METABOLISM OF PROLINE IN WHEAT

PO 2.26 *Zuzana Brnáková, Andrej Godány, Jozef Timko :* STREPTOMYCETES EXTRACELLULAR NUCLEASES EXPRESSED IN *E. COLI*

PO 2.27 *Darina Mikulášová, Michaela Kohárýová, Jana Maderová, Miroslava Škrlantová, Marta Kollárová :* MALATE DEHYDROGENASE FROM *STREPTOMYCES AUREOFACIENS*

PO 2.28 *Elena Tichá, Polakovičová V., Obernauerová M. :*REGULATION OF THE PHOSPHATIDYLGLYCEROLPHOSPHATE SYNTHASE IN KLUYVEROMYCES LACTIS

PO 2.29 *Jana Godočíková, Marcel Zámocký, Viera Boháčová, Mária Bučková and Bystrík Polek :* SCREENING FOR CATALASE-PEROXIDASE GENES IN ENVIRONMENTAL SAMPLES OF BACTERIA.

PO 2.30 *Eva Szabová, Dana Urminská, Ivan Michalík, Eva Sendrejová :* PURIFICATION OF BACTERIAL ALKALINE PEPTIDASE BY CHROMATOGRAPHICAL METHODS

PO 2.31 *Eva Otyepková, Michal Otyepka, Pavel Banáš, Zbyněk Prokop and Jiří Damborský :* DIFFERENCE IN ENZYMATIC AND NONENZYMATIC DEHALOGENATION OF 1,2-DICHLOROETHANE AND 1,2-DIBROMOETHANE

Section 3 MEMBRANE BIOCHEMISTRY AND BIOENERGETICS

PO 3.1 *Zuzana Nováková, Stanislav Šurín, Alan Majernik, Peter Šmigáň :* DCCD RESISTANT MUTANT OF METHANOTHERMOBACTER THERMAUTOTROPHICUM : BIOCHEMICAL CHARACTERIZATION

PO 3.2 *Stanislav Šurín, Alan Majerník, Zuzana Nováková and Peter Šmigáň : BIOCHEMICAL CHARACTERIZATION OF A MUTANT*

OF THE Methanothemobacter thermautotrophicus deficient in Na^+/H^+ *ANTIPORTER*

PO 3.3 *Petra Dunajčíková, Zdeněk Verner, Petr Man, Julius Lukeš & Anton Horváth :* NADH DEHYDROGENASE OF TRYPANOSOMATIDS

PO 3.4 *Hana Rauchová, Marek Vrbacký, Zdeněk Drahota, Christian Bergamini, Romana Fato, Giorgio Lenaz :* THE ROLE OF IDEBENONE IN ACTIVATION OF GLYCEROLPHOSPHATE OXIDATION IN BROWN ADIPOSE TISSUE MITOCHONDRIA

PO 3.5 *Gabriel Bukor, František Jurský :* THE EFFECT OF GLYCINE UPTAKE ON SYNAPTIC PLASTICITY: A COMPUTATIONAL MODEL

PO 3.6 *Marek Basler, Jiří Mašín, Radim Osička and Peter Šebo :* PORE-FORMING AND ENZYMATIC ACTIVITIES OF *BORDETELLA* ADENYLATE CYCLASE TOXIN SYNERGIZE IN PROMOTING LYSIS OF MONOCYTES

PO 3.7 *Martin Šimkovič, Peter Ditte, Boris Lakatoš, Ľudovít Varečka :* ROLE OF THE ELECTROCHEMICAL H^+-GRADIENT IN TRANSMEMBRANE CA^{2+} FLUXES IN VEGETATIVE MYCELIUM *TRICHODERMA VIRIDE* ADAPTED TO DIFFERENT CA^{2+} CONCENTRATIONS.

PO 3.8 *Veronika Štofániková, František Jurský :* ROLE OF N-TERMINAL REGION IN DOPAMINE TRANSPORTER REGULATION

PO 3.9 *Zuzana Sumbalová, Jarmila Kucharská, Ria Koprdová, František Kristek :* BRAIN MITOCHONDRIA FUNCTION IN SPONTANEOUS HYPERTENSION – EFFECT OF TREATMENT WITH LOSARTAN AND PRAZOSIN

PO 3.10 *Ondřej Toman, Petr Halada, Radmila Pustějovská, Aleš Ulrych, Lenka Šemberová , Denisa Petráčková , Jaroslava Svobodová :* MEMRANE PHOSPHOPROTEOME ANALYSIS OF BACILUS SUBTILIS UNDER ETHANOL STRESS

PO 3.11 *Zuzana Tomáškova, Jana Gaburjákova, Marta Gaburjákova :* PERMEABILITY PROPERTIES OF RAT CARDIAC RYANODINE RECEPTOR

PO 3.12 *Vladimíra Tomečková, Krisztina Fodor, Jaroslav Kušnír, Pal Perjési :* THE INTERACTION OF MITOCHONDRIA WITH THE CYTOTOXIC CHALCONES INVESTIGATED BY FLUORESCENCE POLARIZATION

PO 3.13 *Martin Valachovič, Lucia Hronská, Ivan Hapala :* ROLE OF ABC-TRANSPORTERS IN STEROL HOMEOSTASIS

PO 3.14 *Vladimír Kůs, Michal Hensler, Tomáš Pražák, Pavel Flachs, Jan Kopecký :* DIFFERENCES IN PREDISPOSITION TO OBESITY BETWEEN MOUSE STRAINS C57BL/6J AND A/J: THE ROLE OF MUSCLE AND ADIPOSE TISSUE METABOLISM

Section 4 CELL REGULATIONS AND TRANSFER OF SIGNALS

PO 4.1 *Václav Brázda, Eva Brázdová Jagelská, Miroslav Fojta, Emil Paleček :* LINEAR DRIFTING OF p53 FOR ITS CONSENSUS SEQUENCE

PO 4.2 *Eva Brázdová Jagelská, Václav Brázda, Petr Pečinka, Emil Paleček:* SEQUENCE SPECIFIC DNA BINDING OF P53 CAN BE ENHANCED BY DNA SUPERCOILING

PO 4.3 *Adriana Gibadulinová, Ingrid Ovečková, Lýdia Jakubíčková, Jaromír Pastorek & Silvia Pastoreková :* TRANSCRIPTIONAL REGULATION OF S100P GENE CODING FOR THE CANCER-RELATED CALCIUM-BINDING PROTEIN INVOLVES EGF- AND HORMONE-DEPENDENT PATHWAYS

PO 4.4 *Tereza Holotňáková, Anna Ohraďanov[1], Miroslav Ferko, Silvia Pastoreková, Jaromír Pastorek, and Attila Ziegelhőffer :* HYPOXIA MIMETICS INDUCE DIFFERENTIAL RESPONSES IN RAT IMMORTALIZED RAT2TK^{-} CELLS AND RAT TRANSFORMED BP6 CELLS

PO 4.5 *Robert Ihnatko, Miroslav Kubeš, Martina Takáčová, Oľga Sedláková, Jaromír Pastorek, Juraj Kopáček and Silvia Pastoreková :*CARBONIC ANHYDRASE IX IS INDUCED BY ACIDOSIS INDEPENDENTLY OF HYPOXIA IN HUMAN GLIOBLASTOMA CELLS.

PO 4.6 *Michal Kaliňák, Katarína Majerníková, Agáta Jakubová, Ľudovít Varečka :* METABOLISM OF GABA IN FILAMENTOUS FUNGI

PO 4.7 *Ivan Kalousek, Petra Otevřelová, Barbora Brodská :* INDUCTION AND REPRESSION OF PROTEIN P21 IN LYMPHOCYTES TREATED WITH ACTINOMYCIN D AND N-BUTYRIC ACID.

PO 4.8 *Věra Klenerová, Pavel Šída, Stanislav Štípek, Sixtus Hynie :* WHAT IS THE ROLE OF LEPTIN IN ACUTE STRESS?

PO 4.9 *Eva Kmoníčková, Antonín Holý, Zdeněk Zídek :* INVOLVEMENT OF ADENOSINE RECEPTORS IN ACTIVATION OF ANTIVIRAL IMMUNE RESPONSES BY ACYCLIC NUCLEOSIDE PHOSPHONATES

PO 4.10 *Lenka Kočí, Martina Hýžďalová, Jiřina Hofmanová, Alois Kozubík:* INDUCTION OF CELL DEATH AND ADHESIVE PROPERTY CHANGES IN COLON EPITHELIAL CELLS USING NONADHERENT CULTIVATION

PO 4.11 *Ján Kormanec, Henrieta Mitická, Gary Rowley, Bronislava Rezuchová and Mark Roberts* : NEW TARGETS FOR SALMONELLA TREATMENT: RPOE REGULON OF *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM

PO 4.12 *Zuzana Koubková, Jiřina Hofmanová, Alena Vaculová, Alois Kozubík :* CHANGES OF CELL LIPIDS IN HUMAN COLONIC CELL LINES AFTER TREATMENT WITH FATTY ACIDS.

PO 4.13 *Evžen Křepela, Jan Procházka, Ilona Roušalová, Pavel Fiala, Kamila Benková :* EXPRESSION OF INHIBITOR OF APOPTOSIS PROTEINS SURVIVIN AND XIAP IN LUNG CARCINOMA CELLS AND TISSUES

PO 4.14 *Jana Křížková, Petra Melkusová, Eva Kmoníčková, Antonín Holý, Zdeněk Zídek :* ROLE OF MITOGEN-ACTIVATED PROTEIN KINASES (MAPK) IN EXPRESSION OF IMMUNOBIOLOGICA EFFECTS OF ACYCLIC NUCLEOSIDE PHOSPHONATES

PO 4.15 *Ingrid Lajdová, Dušan Chorvát Jr., Alžbeta Chorvátová :*

NON-GENOMIC INHIBITION OF HUMAN PURINERGIC $P2X_7$ RECEPTOR BY 1□,25(OH)?$_2$D3

PO 4.16 *Ingrid Lajdová, Dušan Chorvát Jr., Viera Spustová, Alžbeta Chorvátová* : RAPID EFFECTS OF 1□,25$(OH)_2$D3 IN RESTING HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS.

PO 4.17 *Eva Lincová, Karel Souček, Viktor Horváth, Jiřina Hofmanová, Alois Kozubík :* INDUCTION OF EXPRESSION OF PTGF-□/NAG-1/MIC-1/GDF-15 IN PROSTATE AND COLON EPITHELIAL CELLS USING NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

PO 4.18 *Šárka Adámková, Lenka Luhová, Marek Petřivalský, Barbora Mieslerová, Aleš Lebeda, Pavel Peč :* THE PRODUCTION OF NITRIC OXIDE AND THE ACTIVITY OF NO SYNTHASE DURING PATHOGENESIS IN TOMATO

PO 4.19 *Alena Gaudinová, Petre I. Dobrev, Jiří Malbeck, Alena Trávníčková, Zuzana Mýtinová, Naďa Wilhelmová, Daniel Haisel, Václav Motyka :* MULTIPLE RESPONSES OF RADISH AND TOBACCO LEAVES TO SALINITY AND DROUGHT STRESS

PO 4.20 *Barbora Drobcová, Marek Mentel, Ingrid Kiššová, Jordan Kolarov, Peter Polčic :* BH3-ONLY PROTEINS IN THE BCL-2 FAMILY AS STUDIED IN YEAST

PO 4.21 *Katarína Polčicová, Jana Tomášková, Martina Labudová, Jana Blaškovičová, Jaromír Pastorek, Silvia Pastoreková & Juraj Kopáček :* MODULATORY EFFECTS OF HYPOXIA ON VIRUS GENE EXPRESSION AND INFECTIOUS PROPERTIES

PO 4.22 *Oľga Sedláková, Anna Ohraďanová, Miriam Zaťovičová, Jaromír Pastorek & Silvia Pastoreková :* TRANSCRIPTION OF THE GENES ENCODING METALLOPROTEINASES AND THEIR INHIBITORS IN TUMOR CELLS EXPOSED TO HYPOXIA

PO 4.23 *Štěpánka Veselková, Martin Potocký, Olga Valentová, Jan Martinec* : EXPRESSION OF *ZEA MAYS* PHOSPHATIDYLCHOLINE - SPECIFIC PHOSPHOLIPASE C GENES UNDER STRESS CONDITIONS

PO 4.24 *Jitka Vostálová, Jitka Šantorová, Katarína Smolková, Petr Ježek, Martin Modrianský :* ROLE OF CIDEa PROTEIN IN VALINOMYCIN-INDUCED APOPTOSIS IN RAT NEONATAL CARDIOMYOCYTES

PO 4.25 *Miriam Zaťovičová, Adriana Gibadulinová, Eliška Švastová, Norbert Žilka, Alžbeta Hulíková, Jaromír Pastorek & Silvia Pastoreková :*MONOCLONAL ANTIBODY M20 BINDS TO CATALYTIC DOMAIN AND INDUCES INTERNALIZATION OF CANCER-RELATED CARBONIC ANHYDRASE IX INDEPENDENTLY OF HYPOXIA

Section 9 GLYCOBIOCHEMISTRY

PO 9.1 *Fairouz Ait Mohand and Vladimír Farkaš :* A NEW XYLOGLUCAN ENDOTRANSGLYCOSYLASE ENZYME FROM NASTURTIUM (*TROPAEOLUM MAJUS*) SEEDS

PO 9.2 *Martina Beláňová, Petronela Dianišková, Martin Kopál, Katarína Mikušová :* FURTHER STUDIES ON MYCOBACTERIAL GALACTOSYL TRANSFERASE Rv3782

PO 9.3 *Jana Cabálková, Richard Čmelík and Josef Chmelík :* COMBINATION OF TANDEM MASS SPECTROMETRY (MS/MS) TECHNIQUES WITH HPLC FOR IDENTIFICATION OF PARTICULAR CARBOHYDRATES IN COMPLEX MIXTURES

PO 9.4 *Petronela Dianišková, Zuzana Svetlíková, Jana Korduláková, Katarína Mikušová :* BIOCHEMICAL CHARACTERISATION OF MYCOBACTERIAL MANNOSYL TRANSFERASE PimA

PO 9.5 *Tomáš Dráb , Pavla Maňásková Jiří Liberda :* ROLE OF COWPER GLANDS SECRETION IN BULL

PO 9.6 *Michaela Havrlentová, Alena Gajdošová, Ján Kraic :* VARIATION IN β-D-GLUCAN CONTENT IN CEREALS AND PSEUDOCEREALS WHOLE GRAINS

PO 9.7 *Ondřej Kosík, Vladimír Farkaš :* OPTIMALIZATION OF FLUORIMETRIC ‘PAPER DOT-BLOT’ ASSAY FOR ACTIVITY ASSESSMENT OF XTHS ACTING IN XET MODE

PO 9.8 *Daniela Mikulíková, Ján Kraic* : EVALUATION OF HEALTH-PROMOTING STARCH IN SEVERAL PLANT CROPS

PO 9.9 *Jana Morová, Ladislav Bumba, Zuzana Marčeková, Jiří Mašín, Radim Osička and Peter Šebo :* INTEGRIN CD11B/CD18 – RECEPTOR FOR ADENYLATE CYCLASE TOXIN

PO 9.10 *Richard Čmelík, Josef Chmelík :* STRUCTURAL ANALYSIS OF NEUTRAL STARCH OLIGOSACCHARIDES BY MASS SPECTROMETRY

PO 9.11 *Silvia Špániková, Peter Biely :* GLUCURONOYL ESTERASE - NOVEL CARBOHYDRATE ESTERASE PRODUCED BY SCHIZOPHYLLUM COMMUNE

PO 9.12 *Marcela Fodorová, Pavol Vadovič, Katarína Slabá, Ľudovít Škultéty, Rudolf Toman :* TENTATIVE PRIMARY STRUCTURE OF LIPID A FROM *RICKETTSIA TYPHI* LIPOPOLYSACCHARIDE

PO 9.13 *Ján Štěrba, Marie Vancová, Natasha Rudenko , Maryna Golovchenko, John Kelly, Susan Logan, Roger MacKenzie, Libor Grubhoffer* **:** GLYCOSYLATION IN LYME BORRELIOSIS SPIROCHETES

PO 9.14 *Pavol Vadovič, Marcela Fodorová, Marianne Bordevik, Ľ udovít Škultéty,Katarína Slabá, Rudolf Toman :* COMPOSITION AND TENTATIVE STRUCTURE OF A LIPOPOLYSACCHARIDE FROM *PISCIRICKETTSIA SALMONIS*, THE ETIOLOGICAL AGENT OF SALMONID RICKETTSIAL SEPTICEMIA

PO 9.15 *Ondřej Vaněk, Petr Novák, Vladimír Kopecký Jr., Vladimír Křen, Jiří Brynda, Karel Bezouška :* STRUCTURAL STUDIES OF LIGAND BINDING OF NATURAL KILLER CELL RECEPTOR, PROTEIN CD69

POSTER SESSION II

Posters should be exhibited between Thursday 8:00 and Friday 14:30

Section 5 XENOBIOCHEMISTRY

PO 5.1 *Zdeněk Andrysík, Pavlína Polášková, Miroslav Machala , Alois Kozubík, Jan Vondráček* : AhR LIGANDS AFFECT BOTH POST-TRANSLATIONAL MODIFICATION OF CONNEXIN 43 AND GAP-JUNCTIONAL INTERCELLULAR COMMUNICATION

PO 5.2 *Viktor Horváth, Olga Blanářová, Lenka Švihálková-Šindlerová, Karel Souček, Jiřina Hofmanová, Petr Sova, Aleš Kroutil, Petr Fedoročko, Alois Kozubík* : PLATINUM(IV) COMPLEX WITH ADAMANTYLAMINE OVERCOMES INTRINSIC RESISTANCE TO CISPLATIN IN OVARIAN CANCER CELLS

PO 5.3 *Viera Boháčová, Miroslav Barančík, Ima Dovinová,Albert Breier:* MULTIDRUG-RESISTANCE ASSOCIATED WITH OVEREXPRESSION OF P-GLYCOPROTEIN IN L1210/VCR AND L1210/DOX CELLS.

PO 5.4 *Evžen Buchar , Eva Kmoníčková , Pavel Anzenbacher , Jana Křížková , Eva Anzenbacherová ,Petr Potměšil, Ludmila Kameníková , Antonín Holý Zdeněk Zídek :* EFFECTS OF ACYCLIC NUCLEOSIDE PHOSPHONATE TENOFOVIR DISOPROXIL FUMARATE ON CYP450 METABOLIZING SYSTEM OF PRIMARY RAT HEPATOCYTES AND LIVER MICROSOMES

PO 5.5 *Peter Dočolomanský, Viera Boháčová, Albert Breier, Miroslav Barančík* : INTERACTION OF PENTOXIFYLLINE IMMOBILIZED ON BEAD CELLULOSE WITH PROTEINS FROM SENSITIVE AND MULTIDRUG RESISTANT CELLS

PO 5.6 *Ima Dovinová, Renáta Janíková, Lýdia Jendeková, Stanislava Kojšová, Albert Breier, Oľga Pecháňová :* DETERMINATION OF ANTIOXIDANT POTENTIAL OF SOME ANTIHYPERTENSIVES

PO 5.7 *Helena Dračínská, Jana Mizerovská, Martina Svobodová, Heinz H. Schmeiser, Volker M. Arlt, David H. Phillips, Marie Stiborová:* STUDY ON METABOLISM OF CARCINOGENIC

3-NITROBENZANTHRONE AND
3-AMINOBENZANTHRONE AND THEIR POTENTIAL TO INDUCE BIOTRANSFORMATION ENZYMES

PO 5.8 *Martina Gavelová, Jana Hladíková, Romana Novotná, Miroslav Machala, Lenka Skálová :* EFFECTS OF SELECTED CYTOSTATIC DRUGS ON CARBONYL REDUCING ENZYMES IN HUMAN BREAST CARCINOMA MCF-7 CELLS

PO 5.9 *Lenka Gibalová, Ján Sedlák, Albert Breier, Miroslav Barančík, Zdenka Sulová :* EFFECT OF CISPLATIN ON L1210 CELLS

PO 5.10 *Ludmila Kameníková, Nikolina Kutinová Canová, Jindřich Martínek, Eva Kmoníčková, Zdeněk Zídek, Hassan Farghali:* NITRIC OXIDE AND HEPATOCYTE APOPTOSIS: EFFECTS OF LIPOPOLYSACCHARIDE AND D-GALACTOSAMINE IN CELL CULTURE

PO 5.11 *Věra Kotrbová, Barbora Mrázová, Marie Stiborová :* CYTOCHROME b_5 POTENTIATES PARTICIPATION OF CYP1A1 AND 1A2 IN OXIDATION OF ANTICANCER DRUG ELLIPTICINE TO PHARMACOLOGICALLY MORE EFFICIENT METABOLITES

PO 5.12 *Eliška Kondrová, Pavel Stopka, Pavel Souček and Ivan Gut :* THE INFLUENCE OF SEVERAL PHENOLIC ANTIOXIDANTS ON THE TOXIC EFFECTS OF DOXORUBICIN

PO 5.13 *Pavla Křiváková, Tomáš Roušar, Otto Kučera, Halka Lotková, Anna Lábajová, Zuzana Červinková, Zdeněk Drahota :* OXIDATIVE DAMAGE OF ISOLATED RAT HEPATOCYTES BY TERT-BUTYLHYDROPEROXIDE

PO 5.14 *Otto Kučera, Tomáš Roušar, Pavla Křiváková, Halka Lotková, Zuzana Červinková :* MODEL OF ACETAMINOPHEN-INDUCED INJURY OF RAT HEPATOCYTES IN PRIMARY CULTURE

PO 5.15 *Veronika Křížová, Michal Šavlík, Barbora Szotáková, Jiří Lamka, Lenka Skálová :* SINGLE ADMINISTRATION OF ALBENDAZOLE – EFFECT ON INTESTINAL AND HEPATIC CYP1A ACTIVITY IN MOUFLON *(OVIS MUSIMON)*

PO 5.16 *Václav Martínek, Marcela Semanská, Marie Stiborová :*

PREDICTION OF PEROXIDASE MEDIATED CARCINOGEN-RADICAL AND DNA-ADDUCTS STRUCTURE

PO 5.17 *Milan Miko, Silvia Letašiová and Milan Melník :* ARE COPPER(II) COMPLEXES WITH DIFFERENT BIOLOGICALLY ACTIVE LIGANDS MEMBRANOUS EFFECTIVE?

PO 5.18 *Markéta Mikšanová, Karel Naiman, Heinz H Schmeiser, Eva Frei, Marie Stiborová* : CHARACTERIZATION OF DEOXYGUANOSINE ADDUCT GENERATED IN DNA BY CARCINOGENIC *o*-ANISIDINE

PO 5.19 *Jana Nekvindová, Pavel Anzenbacher, Eva Anzenbacherová, Zdeněk Zídek, Damjana Rozman, Antonín Holý :* CYTOCHROMES P450: INTERACTIONS WITH ACYCLIC NUCLEOSIDE PHOSPHONATES

PO 5.20 *Romana Novotná, Vladimír Wsól :*
PURIFICATION OF NOVEL HUMAN LIVER MICROSOMAL CARBONYL REDUCTASE

PO 5.21 *Vladimír Wsól, Romana Novotná, Guangming Xiong, Edmund Maser :*
CLONING , EXPRESSION AND PURIFICATION OF AKR1C3

PO 5.22 *Slavomíra Ondková, Dana Macejová, Ján Líška, Sandra Scheiblhofer, Richard Weiss, Josef Thalhamer, Július Brtko :*
EFFECTS OF CpG AND/OR 13-*cis* RETINOIC ACID ON EXPRESSION OF VITAMIN D RECEPTOR IN MNU-INDUCED MAMMARY GLAND TUMOURS

PO 5.23 *Ingrid Pauliková, Anna Hrabovská, Otto Helia, Eva Faklová :*
INTER-TISSUE AND INTER-SPECIES VARIABILITY OF BUTYRYLCHOLINESTERASE ACTIVITY BY RAT AND RABBIT IN VITRO

PO 5.24 *Kateřina Pěnčíková, Josef Slavík, Pavlína Polášková, Jan Vondráček, Miroslav Machala :* MODULATIONS OF LIPID SIGNAL TRANSDUCTION PATHWAYS ARE INVOLVED IN INHIBITION OF GJIC BY ENVIRONMENTAL AROMATIC CONTAMINANTS

PO 5.25 *Pavlína Polášková, Kateřina Pěnčíková, Jan Vondráček, Josef Slavík , Pavel Krčmář, Zdeněk Andrysík, Miroslav Machala :* DEREGULATION OF GJIC BY NONDIOXIN-LIKE PCB 153 IN RAT LIVER WB-F344 CELLS

PO 5.26 *Mário Šereš, Jozef Orlický, Branislav Uhrík, Albert Breier, Zdenka Sulová :* RELATION BETWEEN P-GLYCOPROTEIN OVEREXPRESSION AND CALCIUM HOMEOSTASIS IN L1210/VCR CELLS

PO 5.27 *Lenka Umannová, Jiří Neča, Pavel Krčmář, Zdeněk Andrysík, Miroslav Machala, Jan Vondráček, Alois Kozubík :* INTERACTIONS OF AHR LIGANDS AND ENDOGENOUS GROWTH REGULATORS IN RAT LIVER "STEM-LIKE" CELLS

PO 5.28 *Kateřina Valentová, Isabelle de Waziers, Arlette Moncion, Jitka Ulrichová :* EFFECT OF PHENOLIC COMPOUNDS ON GLUCOSE METABOLISM IN RAT HEPATOCYTES

PO 5.29 *Jiří Vrba, Jitka Ulrichová :* CYTOTOXICITY OF ISOQUINOLINE ALKALOIDS IN HL-60 CELLS DURING DIFFERENTIATION INTO NEUTROPHILS

PO 5.30 *Jiřina Zatloukalová, Pavel Krčmář, Kateřina Chramostová, Alois Kozubík, Miroslav Machala, Jan Vondráček* : MOLECULAR MECHANISMS OF FLAVONE-INDUCED EFFECTS ON AHR IN RAT LIVER „STEM-LIKE" CELLS

PO 5.31 *Peter Ditte, Zuzana Sulová, Tatjana Vergieva :* REPRODUCTIVE FUNCTION STUDY OF THE WISTAR RATS AFTER PERINATAL TREATMENT WITH CARBENDAZIM

Section 6 FREE RADICALS IN BIOLOGY AND MEDICINE

PO 6.1 *Martina Bancířová, Marika Janovská* : ROS: CHEMILUMINESCENT DETECTON *VS*. PHOTODYNAMIC EFFECT

PO 6.2 K*atarína Bauerová, Danica Mihalová, Silvester Poništ, Ema Paulovičová :* STUDY OF SYSTEMIC INFLAMMATION IN THE MODEL OF ADJUVANT ARTHRITIS ON DETERMINING LEVELS OF IL-6 AND GAMMA-GLUTAMYL TRANSFERASE ACTIVITY

PO 6.3 *Hana Bochořáková, Hana Paulová, Marie Nováková and Eva Táborská* : DETERMINATION OF HYDROXYL RADICALS IN PERFUSED RAT HEARTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH COULOCHEMICAL DETECTION

PO 6.4 *Viera Danielisová, Miroslav Gottlieb, Miroslava Némethová, Jozef Burda :* THE CHANGES SOD AND CAT ACTIVITY AFTER POSTCONDITIONING

PO 6.5 *Monika Ďurfinová, Marta Brechtlová, Branislav Líška, Oľga Uličná, Oľga Pecháňová, Anna Liptáková, Jana Muchová :* EFFECT OF ANAESTHETIC ADMINISTRATION OF KETAMINE AND DIETHYLETHER ON LIPID PEROXIDATION IN RAT LIVER HOMOGENATES.

PO 6.6 *Dvořák M., Matejovičová M., Slaninová I., Slunská Z.,Táborská E. :* EFFECT OF SOME NATURAL COMPOUNDS ON DNA DAMAGE IN HUMAN LEUKEMIA CELL LINE HL-60

PO 6.7 *Peter Gbur, Slávka Kaščáková, Daniel Jancura, Roman Dědic, Dušan Chorvát jr., Jan Hála, and Pavol Miškovský :* PHOTOGENERATION OF SINGLET OXYGEN BY HYPERICIN IN COMPLEX WITH LOW DENSITY LIPOPROTEINS

PO 6.8 *Jarmila Kucharská, Zuzana Sumbalová, Olga Uličná, Peter Božek, Ria Koprdová, František Kristek :*RELATIONS BETWEEN REDOX STATUS AND BIOCHEMICAL PARAMETERS IN SPONTANEOUSLY HYPERTENSIVE RATS: EFFECTS OF PRAZOSIN AND LOSARTAN.

PO 6.9 *Roman Konopka, Jiří Pachernik, Antonín Lojek, Lukáš Kubala* : RETINOIC ACID-INDUCED NEURAL DIFFERENTIATION OF P19 EMBYONAL CARCINOMA CELLS IS EFFECTED BY MODULATION OF INTRACELLULAR REDOX STATE

PO 6.10 *Simona Macuchová , Ivana Márová , Veronika Turková, Andrea Mikulcová, Renata Mikulíková :* ANTIOXIDANT AND ANTIMUTAGENIC PROPERTIES OF BEER, MALT AND BARLEY

PO 6.11 *Pavla Havlová, Renata Mikulíková, Jaroslav Prýma, Jana Folkmerová :* INFLUENCE OF SELECTED CHEMICAL AND BIOLOGICAL FACTORS ON SUPEROXIDE DISMUTASE LEVELS IN BARLEY AND MALT

PO 6.12 *Jana Muchová, Katarína Sumegová, Monika Sivoňová, Peter Korytár and Zdeňka Ďuračková :* IS OUR HEALTHY POPULATION EXPOSED TO THE RISK OF OXIDATIVE DNA DAMAGE?

PO 6.13 *Anna Liptáková, Jana Muchová, Monika Sivoňová, Oľga Uličná, Jozef Čársky, Zdeňka Ďuračková :* INFLUENCE OF PYRIDOXALYLAMINOGUANIDINE ON SELECTED PARAMETERS OF OXIDATIVE STRESS IN RATS WITH DIABETES MELLITUS

PO 6.14 *Zuzana Országhová, Marta Hrnčiarová, Vladimír Jakuš, Jozef Čársky, Zdeňka Ďuračková, Marek Nocun, Cezary Watala :* PROTEIN OXIDATION PRODUCTS IN RATS WITH STREPTOZOTOCINE-INDUCED DIABETES MELLITUS

PO 6.15 *Jana Pečivová, Tatiana Mačičková, Antonin Lojek, Rado Nosál, Milan Číž, Dagmar Holomáňová :* CARVEDILOL, PROFESSIONAL PHAGOCYTES AND REACTIVE OXYGEN SPECIES GENERATION

PO 6.16 *Silvester Poništ, Katarína Bauerová, Danica Mihalová, Vladimír Šnirc :* INFLUENCE OF ANTI-RADICAL ACTIVE SUBSTANCES OF PYRIDOINDOLE TYPE ON INFLAMMATION AND OXIDATIVE STRESS IN THE MODEL OF ADJUVANT ARTHRITIS

PO 6.17 *Lucia Račková, Daniela Košťálová, Štefánia Štefaňáková, Milan Štefek :*ANTIOXIDANT AND PROOXIDANT PROPERTIES OF BOSWELLIA SERRATA EXTRACTS

PO 6.18 *Tomáš Roušar , Pavla Křiváková, Otto Kučera , Halka Lotková , Vladimíra Mužáková, Zuzana Červinková* : ANALYSIS OF

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PO 6.19 *Martina Srbová, Jiří Wilhelm* : MECHANISM OF FREE RADICAL REACTIONS IN ERYTHROCYTE MEMBRANES

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BOOK OF ABSTRACTS

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PL 1

GENOMIC AND PROTEOMIC APPROACHES TO THE ANALYSIS OF p53 ACTIVITY IN TUMOR CELLS

Šárka Pospíšilová, Boris Tichý, Jitka Malčíková, Jana Kotašková, Branislav Kusenda, Lenka Juračková, Martin Trbušek, Jiří Mayer

Center of Molecular Biology and Gene Therapy, Department of Hematooncology, University Hospital Brno, Černopolní 9, Czech Republic

Genomic and proteomic approaches have become the centerpiece of modern experimental oncology and have significantly contributed to an improved understanding of tumor progression mechanisms. Tumor suppressor p53 is a powerful transcription factor responsible for cell cycle regulation, DNA repair and many other cellular processes and can regulate several hundred genes in the human genome. Disruption of p53 function by mutation or deletion belongs to the most frequent alterations observed in human cancers. However, recent results show that there exist significant differences in the properties of specific mutants, especially in the DNA-binding ability to activate particular promoters (e.g. Pospíšilová et al., Mol. Cancer Res. 2004). Using RNA interference and subsequent microarray analysis we were able to specifically modify aberrant expression of p53 mutants and analyze their behavior within the cellular background. Using functional protein arrays bearing p53 mutants and reporter gene assays we analyzed the DNA-binding activity of particular mutants towards promoter sequences of p53-response genes. Detection of p53 mutants in oncological patients and characterization of their properties, especially their ability to induce apoptosis, are very important for tumor diagnostics. Results of such analyses are starting to play a role in anticancer therapy planning and prognosis estimation.

The work was supported with grants MŠMT ČR 1K04017, IGA MZ ČR 8448-3/2005 and Elpida-Nukleus. E-mail: sarka.pospisilova@fnbrno.cz

PL 2

ALTERNATIVE SPLICING PRODUCES TRUNCATED VARIANT OF CARBONIC ANHYDRASE IX THAT INHIBITS CA IX-MEDIATED EXTRACELLULAR ACIDIFICATION IN HYPOXIA

Monika Baráthová[1], Martina Takáčová[1], Tereza Holotňáková[1], Adriana Gibadulinová[1], Anna Ohrad'anová[1], Juraj Kopáček[1], Seppo Parkkila[2], Claudiu Supuran[3], Silvia Pastoreková[1] & Jaromír Pastorek[1]

[1]Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovak Republic, [2] Institute of Medical Technology, University of Tampere, Finland, [3]Universita degli Studi di Firenze, Sesto Fiorentino (Firenze) Italy

Alternative splicing of precursor mRNA, one of the most important sources of protein diversity in vertebrates, appears to be behind a large repertoiare of human diseases. It is also involved in the processing of *CA9* mRNA coding for the carbonic anhydrase IX (CA IX), a catalytically active transmembrane zinc metalloenzyme isoform whose expression is predominantly associated with tumors and strongly induced by hypoxia. The clinical importance of CA IX resides in its diagnostic, prognostic and therapeutic value.

The alternative splicing variants of CA9 were detected in both human and mouse mRNA populations. The human alternative isoform (hAS-CA IX) is lacking exons 8, 9 and does not contain corresponding C-terminal part of the catalytic domain as well as the transmembrane and intracytoplasmic domains. Ectopic expression of this truncated isoform clearly showed its cytoplasmic localization and secretion to the extracellular space. Overexpression of (hAS-CA IX) in the cells with hypoxia-inducible endogenous expression of the wild type CA IX led to reduced capability of these cells to acidify culture medium and compensate the changes in pH caused by hypoxia. Because hAS-CA IX expression is not so strictly hypoxia-dependent and its level is relatively constant, it can serve as a molecule that influences the activity of the wild type CA IX under specific conditions.

Supported by EUROXY (LSHC-CT-2003-502932).

PL 3

CATECHOLAMINES IN STRESS: FROM GENE EXPRESSION OF BIOSYNTHETIC ENZYMES TO METABOLIC DEGRADATION

Richard Kvetňanský

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Catecholamines (adrenalin, noradrenaline, dopamine) are derivatives of phenylethylamine, which are biologically very active. They act as neurotransmitters and regulate many physiological, neuroendocrine and metabolic processes. Activity of peripheral and brain catecholaminergic systems is many fold increased during exposure of the organism to stress.

The aim of this presentation is to show changes in catecholamine (CA) levels, biosynthesis, transport, release, uptake/reuptake, leakage, and degradation in various organs, e.g. adrenal medulla (AM), sympathetic ganglia (SG), and small nuclei of the brain in rats and mice during a single or repeated exposure to various stressors. Changes in CA biosynthetic enzymes tyrosine hydroxylase (TH), dopamine-beta-hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT) gene expression, immunoprotein levels and activities during stress exposure will be presented. Quantification of mRNA levels in the organs, regulation by specific factors involved in transcriptional activation, and pathways initiating changes in gene expression will be shown. These processes will be demonstrated also in various knockout mice (KO), e.g. c-fos KO, DBH KO, and corticotropin releasing hormone KO mice. Regulation of gene expression in rats adapted to a homotypic stressor shows exaggerated responses of mRNA levels after an exposure to heterotypic novel stressors.

Thus, presented data demonstrate biochemical and genetical mechanisms of increased activity in catecholaminergic systems during stress.

PL 4

MEMBRANE-ASSOCIATED PROTEINS TO REGULATE THE CELL CORTEX IN MOTILITY AND MORPHOGENESIS

Stefan Wacha, Tamara Golub and Pico Caroni

Friedrich Miescher Institut, Basel, Switzerland

In the nervous system, process outgrowth and synaptic rearrangements coincide with the expression of specific sets of genes in neurons, including the GAP43-like proteins GAP43, CAP23, MARCKS, and MacMARCKS. Within cells, GAP43-like proteins accumulate at cholesterol-dependent lipid microdomains, where they codistribute with $PI(4,5)P_2$. Functional studies in vivo have provided strong evidence that GAP43-like proteins are intrinsic determinants of anatomical plasticity, conferring competence for morphogenetic processes to cells that express them. Significantly, the combined expression of GAP43 and CAP23 in adult neurons is sufficient to confer competence for lesion-induced regeneration of axons in the adult.

Here we present new results addressing the mechanisms through which GAP43 and CAP23 promote and shape cell growth. We show that the two proteins exert distinct functions in morphogenesis: GAP43 promotes stimulus-induced and PKC-sensitive motility and adhesion throughout the cell edge, whereas CAP23 promotes calcium-sensitive polarized motility, and stabilizes non-motile sections of the cell surface. Stabilization of cell cortex by CAP23 is required to organize subplasmalemmal cytosol, as highlighted by its critical role in intermediate filament and mitochondria docking. We further show that these distinct activities of GAP43 and CAP23 reflect distinct distributions of the two proteins at the plasmalemma, which in turn direct corresponding local accummulations of cholesterol. Taken together, the results provide evidence that GAP43-like proteins shape the local composition of the plasmalemma through a direct stoichiometric mechanism, and that this shaping in turn controls morphogenesis.

PL 5

LEARNING AND GETTING HELP FROM BACTERIAL TOXINS: FROM THE MECHANISM OF ADENYLATE CYCLASE TOXIN ACTION TO ANTIGEN DELIVERY FOR VACCINATION AND TUMOR IMMUNOTHERAPY

Peter Šebo[1], Jiří Mašín[1], Marek Basler[1], Radovan Fišer[1,2], Jana Vodolánova[1], Jana Kamanová[1], Jana Morová[1], Marcela Šimšová[1], Ladislav Bumba[1], Radim Osička[1], Adriana Osičková[1], Ivo Konopásek[2]

[1]Laboratory of Molecular Biology of Bacterial Pathogens, Institute of Microbiology, Academy of Sciences, Prague, [2]Department of Genetics and Microbiology, Faculty of Science, Charles University, Prague, Czech Republic,

The *Bordetella* adenylate cyclase toxin (ACT) targets host phagocytes bearing the $\alpha_M\beta_2$ integrin receptor Mac-1 (also known as CR3 or CD11b/CD18), such as in particular the professional antigen presenting dendritic cells (DC, $CD11b^{high}$). ACT penetrates the cytoplasmic membrane of cells, binds cytosolic calmodulin and catalyzes conversion of ATP to cAMP. This causes phagocyte impotence and apoptotic death. Moreover, ACT permeabilizes cellular membrane by forming small (hemolytic) cation-selective channels. Using a set of specific substitutions of residues $Glu^{509,516,570 \text{ and } 581}$, dissecting the toxin action into individual steps, we show that cell killing results from synergy of cellular ATP depletion, cAMP signaling and membrane permeabilization by ACT. Toxin segments were identified that determine size, lifetime, cation selectivity and assembly (oligomerization) of ACT channels. The mechanism of toxin-induced calcium entry into cells was analyzed in detail and the results show that calcium entry into cells is tightly linked to AC domain translocation across cellular membrane, without the need for its catalytic activity.

The amazing capacity of ACT to accommodate foreign antigenic polypeptides and the capacity of ACT to promote maturation of dendritic cells allowed development of genetically detoxified ACT (dACT) into a novel carrier for delivery of antigens for processing into both the MHC class I and II-restricted presentation pathways. This enables efficient induction of prophylactic as well as therapeutic antigen-specific $CD4^+$ and $CD8^+$ T cell immune responses. I will conclude by reviewing recent applications of ACT technology for immunotherapy of certain experimental tumors and for improvement of diagnostic methods allowing detection of latent tuberculosis infection.

Wednesday September 13, 2006

Lecture room L1
10:00 – 13:00

Section 5 XENOBIOCHEMISTRY I

Chairs: Miroslav Barančík, Jiří Hudeček

L 5.1

ABC-TRANSPORTERS – PROTEINS WITH DIVERSE FUNCTIONS

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ABC transporters are members of the superfamily of membrane transporters that share a common structural feature – the ABC (ATP binding cassette) motif. They are:

- Multidomain integral membrane proteins that utilize the energy of ATP hydrolysis to translocate solutes across cellular membranes.
- Widely distributed across all taxa and are often involved in protection of cells against the toxic actions of various chemicals.
- Form one of the largest of all protein families and are central to many important biomedical phenomena, including resistance of cancers and pathogenic microbes to drugs.
- Play a key role in the function of the blood-brain, cerebrospinal fluid, and blood-testis barrier, as well as the function of epithelial cells in the intestine, airway, liver, kidney and placenta.

P-glycoprotein, the best known ABC transporter, was discovered by **Victor Ling** in 1974. It is the first discovered member of the ABC family. Overexpression of P-glycoprotein in neoplastic tissue confers the elevation of cell capacity to resist the cytotoxic attack by structurally unrelated drugs, i.e., multidrug resistance (MDR). Several others ABC transporters like multidrug resistance associated proteins (MRP1-MRP7) and breast cancer resistance protein (BCRP) were described to be involved in MDR.

In animals a very important role is played by sulfonylurea receptor (another ABC protein) that in complex with Kir6.2 forms a functional ATP sensitive potassium channel. This channel is responsible for depolarization of pancreatic β-cell plasma membrane after stimulation with elevated external glucose that finally induces insulin release.

Mutations of several ABC proteins cause many diseases. One example is the cystic fibrosis transmembrane regulator (CFTR), the mutation of which is associated with cystic fibrosis. Mutations in the ABCC6 (another ABC protein) gene cause another disease – *pseudoxanthoma elasticum* by a not elucidated mechanism. *Pseudoxanthoma elasticum* is a progressive disorder that affects connective tissue, the material supporting and holding together different structures of the body. This condition is characterized by accumulated deposits of calcium (calcification) and other minerals in elastic fibers, a component of connective tissue.

L 5.2

LY294,002, AN INHIBITOR OF PI3K/AKT KINASE PATHWAY, REVERSES THE P-GLYCOPROTEIN-MEDIATED MULTIDRUG RESISTANCE IN L1210/VCR CELLS

Miroslav Barančík[1], Vierka Boháčová[2], Jan Sedlák[3], Zdenka Sulová[2], Albert Breier[2]

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P-glycoprotein (P-gp) is transmembrane transport pump that causes the efflux of chemotherapeutic agents from cells and is responsible for multidrug resistance (MDR) of neoplastic cells. In the present study drug sensitive L1210 and multidrug resistant L1210/VCR mouse leukemic cell lines were used as an experimental model. We found that LY294,002, a specific inhibitor of PI3K/Akt kinase pathway, significantly and in concentration-dependent manner reduced the degree of vincristine (VCR) resistance in L1210/VCR cells. FACS analysis of proportion of cells in apoptosis or necrosis by Annexin-V apoptosis kit showed that LY294,002 applied together with VCR significantly increased the number of apoptotic cells in resistant cells. Transport activity of P-gp monitored in cells using calcein/AM as substrate was in resistant cells depressed by LY294,002 and that in concentration dependent manner. The development of MDR phenotype in L1210/VCR cells was associated with increased level of Bcl-2 protein and decreased activation of caspase-3 by proteolytic cleavage. On the other hand, the reversal of VCR resistance by LY294,002 was associated with marked activation of caspase-3 in L1210/VCR cells.

The results point to the possible involvement of PI3K/Akt pathway in modulation of P-gp mediated MDR in L1210/VCR cell line and show that MDR reversal effect of LY294,002 is accompanied with influence of this compound on VCR-induced apoptosis.

Supported by grants: VEGA SR 2/6080/26, APVT 51-027404

L 5.3

OVEREXPRESSION OF P-GLYCOPROTEIN IN L1210 CELLS IS ASSOCIATED WITH ALTERATION IN CELL SURFACE GLYCOSIDES

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Multidrug resistance of murine leukaemic cell line L1210/VCR (obtained by adaptation of parental drug sensitive L1210 cells to vincristine) is associated with overexpression of P glycoprotein (Pgp) – the ATP-dependent drug efflux pump. 31P-NMR spectra of L1210 (Pgp negative cells) and L1210/VCR cells (the latter in the presence of vincristine) revealed besides the decrease of ATP level a considerable lower level of UDP-saccharides in L1210/VCR cells. Recently we have assumed that biosynthesis of oligo and polysaccharides were considerably depressed [1]. Histochemical staining of negatively charged cell surface binding sites (mostly sialic acid) by ruthenium red (RR) revealed a compact layer of RR bound to the external coat of sensitive cells (S). In resistant cells cultivated in the absence (R) or presence of vincristine (V) the RR layer is either reduced or absent. Consistently with this, we observed differences in interaction of S and R cells with ConA lectin and tomato lectin (*lycopersicum esculentum* agglutinin).

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Acknowledgements. Acknowledgement: This work was supported from grant APVT-51/027404 and Slovak Grant Agency for Science VEGA grant No. 2/4155, 2/6080

L 5.4

DIFFERENCES IN CELL CYCLE REGULATION AFTER PLATINUM DERIVATIVES TREATMENT IN SENSITIVE AND CISPLATIN RESISTANT OVARIAN CANCER CELL LINES

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Cisplatin is one of the most potent antitumour agents which display high efficiency in the treatment of ovarian cancer. However, cisplatin is active in a limited range of cancer types, and its effects are often decreased due to intrinsic or acquired resistance. LA-12 (currently under the 1st phase of clinical evaluation) is a novel octahedral platinum(IV) complex containing a bulky hydrophobic ligand – adamantylamine. In our previous studies [1,2] we showed that LA-12 was able to overcome both the acquired and the intrinsic cisplatin resistance. In addition, we investigated the effects of LA-12 on cytokinetic parameters of ovarian cancer cell lines with different sensitivity to cisplatin - A2780 (ciplatin sensitive), A2780cis (with acquired cisplatin resistance) and SK-OV-3 (with intrinsic resistance to cisplatin), and compared them with the effects caused by cisplatin. Interestingly, we found the differences in cell cycle perturbation after 24 h cisplatin or LA-12 sustained treatment. Activation of cell cycle checkpoints is a general cellular response after exposure to cytotoxic agents. Therefore we characterize in more detail several signal transduction pathways that are activated in response to exposure to the DNA damage-inducing agents such are studied platinum-based compounds.

Exposure to LA-12 resulted in accumulation of A2780 and A2780cis cells in S phase, while cisplatin caused G_2/M arrest in sensitive and S phase arrest in resistant cells after 24 h of treatment. Detailed simultaneous flow cytometric analysis of cell cycle distribution and DNA synthesis revealed that cisplatin in A2780 cells primarily caused transient S-phase arrest as LA-12, but this arrest was shifted to G_2/M at later time points (24 h). Western blot analysis indicated a concentration-dependent accumulation of p53 protein which is implicated modulation of cell cycle, as the result of cellular response to DNA damage. Equitoxic LA-12 (IC_{50}) and cisplatin (IC_{50}) concentrations increased p53 protein levels already after 6 h of treatment followed by increasing of $p21^{waf1}$ levels at 12-24 h time points in studied cell lines, both significantly higher after cisplatin treatment. In the present study, we expanded our investigations to examine the effects of equitoxic concentrations of LA-12 or cisplatin in A2780 and A2780cis cells on the protein expression of either p53-targeted genes that have been shown to be important in the cellular response to DNA damage including Bax, Gadd45, Mdm2 or cell cycle regulatory genes such as Cyclin A, Cyclin B1 or cdk-2.

Supported by the IGA grant No. 1QS500040507 of the Academy of Sciences of the Czech Republic and by the Ministry of Industry and Trade of the Czech Republic, Contract No. PZ-Z2/29, "New Medicines for Cancer Therapy"

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L 5.5

THE ROLE OF CARBONYL REDUCING ENZYMES IN BIOTRANSFORMATION OF XENOBIOTICS

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NADPH-dependent enzymes, e.g. carbonyl reductases, aldo-keto reductases, mediate carbonyl reduction of many endogenous compounds such as prostaglandins, biogenic amines and steroids as well as the pharmacologically active xenobiotic carbonyl compounds (aromatic and aliphatic aldehydes and ketones) to the corresponding alcohols prior to their further metabolism and/or elimination.

The modulation of biotransformation enzymes activities may have an important impact on pharmacological or toxicological properties of drugs and other xenobiotics. Induction of biotransformation enzymes may reduce plasmatic levels of drugs resulting in failure of pharmacotherapy. In case of anthelmintics, the risk of development of parasite resistance can be increased.

Carbonyl-reducing enzymes metabolize some anticancer drugs as well. In case of anthracycline antibiotics reduction of carbonyl group lead to lowering of antitumour effect of these drugs. A major problem in cytostatic treatment of malignant tumours is the development of resistance to multiple anticancer drugs. Non-classical multidrug resistance is also associated with the overexpression of drug-detoxifying enzymes, including conjugation enzymes and carbonyl-reducing enzymes.

With respect to the mentioned facts, the modulation of biotransformation enzymes activities by drugs should be carefully tested.

This project is supported by Grant Agency of Charles University (108/2005/C).

L 5.6

IRON CHELATORS IN ANTHRACYCLINE-INDUCED CARDIOTOXICITY

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To date iron chelation remains the only approach that have been successfully used to mitigate the cardiotoxicity of anthracyclines such as doxorubicin (DOX). Clinical requirements for ideal chelator are: oral administration, good tissue penetration and access to iron pools, low costs, low toxicity, specificity for iron and easy mobilization of the iron-chelator complex. For use in the combination anthracycline therapy it is also necessary that the chelator does not interfere with the anticancer activity of the cytostatic drug. Currently, dexrazoxane (DXZ) is a single chelator clinically used as a protector against DOX toxicity. In our study with the A549 cells we compared the effects of five structurally different chelators on DOX toxicity. For comparison we also employed other oxidative stress-inducing agents such as bleomycin (BLM) and hydrogen peroxide (H_2O_2) to elucidate the role of free radicals in DOX-induced toxicity. Interestingly, we have found that the chelators that efficiently diminished DOX-induced oxidative stress and membrane damage failed to protect the cells against H_2O_2 and vice versa. Moreover, these chelators slightly compromised both the antiproliferative effects of DOX and the DOX-induced apoptosis in the A549 cells. We therefore conclude that the Fenton-derived oxidative stress is not crucial in the pathogenesis of DOX cardiotoxicity and the ability of a compound to chelate iron is not the sole determinant of a suitable cardioprotector.

This work was supported by GAUK 97/2005 and GAČR 305/05/P156

Wednesday September 13, 2006

Lecture room L1
15:30 – 18:30

Section 5 XENOBIOCHEMISTRY II

Chairs: Miroslav Machala, Albert Breier

L 5.7

RETINOIC ACIDS-INDUCIBLE TRANSCRIPTION FACTORS AND THEIR ROLE IN THERAPY OF SELECTED MALIGNANT DISEASES

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Retinoids are natural and synthetic compounds related to retinoic acid that act through interaction with two basic types of nuclear receptors: retinoic acid receptors (RAR α, RARβ and RARγ) and retinoid X receptors (RXRα, RXRβ and RXRγ) as retinoid-inducible transcription factors. Thus, the retinoid receptors are considered to be ligand-activated, DNA-binding, trans-acting, transcription-modulating proteins involved in a general molecular mechanism responsible for transcriptional responses in target genes. They exert both beneficial and detrimental activity; they have tumor-suppressive activity but on the other hand they are teratogenic. Retinoids inhibit carcinogenesis, suppress premalignant epithelial lesions and tumor growth and invasion in a variety of tissues. Natural and synthetic retinoids have therapeutical effects due to their antiproliferative and apoptosis-inducing effects. They are known to cause redifferentiation or to prevent further dedifferentiation of various neoplastic tissues. A number of novel chemical compounds, receptor selective retinoids and rexinoids, have been synthesized up to now and tested both in vitro and in vivo, using animal models against different cancer cells. In spite of that progress, there is still an urgent call for novel synthetic retinoids and rexinoids with greater retinoid receptor selectivity, reasonable chemotherapeutic or chemopreventive effects and reduced toxicity and side effects.

This work was supported partly by the VEGA grant No. 2/5017/5.

L 5.8

EFFECTS OF MICROTUBULES INTERFERING AGENTS AND/OR ALL-*TRANS* RETINOIC ACID ON EXPRESSION OF NUCLEAR RECEPTORS IN PRIMARY RAT HEPATOCYTES

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Retinoids and thyroid hormones regulate many biological processes, *in vitro* as well as *in vivo* [1,2]. These effects are mediated through their nuclear receptors which are ligand-inducible transcription factors. Cellular signalling by nuclear receptors is influenced by variety of endogenous factors as well as exogenous stimuli such as inflammation, infection, stress, drugs, environmental pollutants, UV-light, etc. Perturbation of cytoskeleton can be considered as pathophysiological factor affecting the function of several nuclear receptors with consequences in endogenous metabolism as well as in drug metabolism [3]. For instance, microtubules interfering agents (MIAs) colchicine and nocodazole down-regulated expression of important biotransformation enzymes in primary human hepatocytes via inhibition of glucocorticoid receptor (GR) nuclear import and suppression of its transcriptional activity [3,4]. Retinoic acid receptors (RARs) are typical nuclear receptors that are involved in essential endogenous processes and they are often targets in human pharmacotherapy [5]. Since both MIAs and retinoids are used in human pharmacotherapy, possible drug interactions may occur. The aims of present study were to examine the effects of MIAs (colchicine, nocodazole, taxol) and/or all-*trans* retinoic acid (ATRA) on expression of nuclear receptors and their coregulators in primary rat hepatocytes as model no-proliferating cells.

This work was supported by grant MSM 6198959216 from the Ministry of Education, Youth and Sports of the Czech Republic grant VEGA 2/5017/5.

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L 5.9

EXPRESSION OF ENZYMES OF METABOLIC ACTIVATION OF PAHs IN RAT LIVER CELLULAR MODELS

Miroslav Machala[1], Pavel Krčmář[1], Lenka Skálová[2], Barbora Szotáková[2], Martina Plíšková[2], Martin Bunček[3], Šárka Holasová[3], Lenka Dostálová[1], Jiřina Zatloukalová[4], Jan Vondráček[1,4]

[1]Veterinary Research Institute, Brno; [2]Pharmaceutical Faculty of Charles University, Hradec Králové; [3]Generi Biotech, Hradec Králové; [4]Institute of Biophysics ASCR, Brno

The poorly differentiated liver oval progenitor cells are considered to be important target of hepatocarcinogens, however it is generally believed that these cells, contrary to hepatocytes, contain none or only negligible levels of drug- and steroid hormone-metabolizing enzymes. We report here that in rat liver epithelial 'stem-like' WB-F344 cells, there are relatively high levels of mRNA expression of enzymes involved in metabolic activation of promutagens including polycyclic aromatic hydrocarbons (PAHs), and/or in antioxidant defense systems. These include CYP1A1, CYP1B1, AKR1A1 (aldehyde reductase), AKR1C9 (3alpha-hydroxysteroid dehydrogenase), NQO-1 and HO-1 mRNA. We found also CYP1A2 mRNA to be present in the cells. CYP1A1, CYP1B1, AKR1C and NQO-1 were significantly induced after exposure of the cells to model AhR agonists. The presence and inducibility of CYP1, AKR1 and NQO-1 were confirmed by the measurement of specific enzymatic activities. Based on our findings in hepatoma cells with or without AhR, and in WB-F344 cells stably transfected with shRNA targeting AhR, it seems that AhR/DRE pathway and not antioxidant responsive elements play a principal role in gene expression of CYP1, AKR1C9 and NQO-1. Therefore, activation of AhR may directly lead to oxidative metabolism of PAHs, formation of PAH-DNA adducts and induction of oxidative stress, resulting in genotoxicity, tumor promoting effects and modulations of steroid hormone metabolism. [Supported by the Czech Ministry of Agriculture, grant No. 00002716201.]

L 5.10

ROLE OF MICROTUBULES IN CELLULAR SIGNALING BY ARYL HYDROCARBON AND GLUCOCORTICOID RECEPTOR – CONSEQUENCES IN REGULATION OF DRUG METABOLIZING ENZYMES

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Expression of drug metabolizing cytochromes P450s (CYP) is controlled mainly by arylhydrocarbon receptor (AhR), pregnane X receptor (PXR), constitutive androstane receptor (CAR), and glucocorticoid receptor (GR). In this work, we have investigated the role of cytoskeleton in cellular signaling by AhR and GR (1-3). We observed:
(i) Microtubules interfering agents (MIAs) inhibit basal and inducible expression of CYPs 1A1, 1A2, 2B6, 2C8, 2C9, 2C19, 3A4 in primary human hepatocytes (4-6). Similar effects were observed in HepG2 cells and primary rat hepatocytes (4). MIAs down-regulated PXR and CAR but not GR mRNAs (5).
(ii) MIAs inhibits transcriptional activity of GR in human hepatocytes and HeLa cells. GR targed genes (TAT, CYPs, PXR, CAR) were down-regulated but binding of corticoids to GR was not affected by MIAs. MIAs restricted cytosol to nucleus translocation of GR in human hepatocytes and HEK293 cells (5). MIAs activate c-Jun-N-terminal kinase which in turn phosphorylates GR and negatively modulates its functions (2,7).
(iii) Microtubules disruption in HeLa cells affects the stability of GR protein. We observed ultra-rapid, time- and dose-dependent, proteasome-ubiquitine mediated degradation of GR by MIAs (8,9).
(iv) MIAs inhibits transcriptional activity of AhR in rat hepatocytes and HepG2 cells. Cytosol to nucleaus translocation of AhR was restricted by MIAs in HepG2 cells (4).

Our experiments show that intact cytoskeleton is indispensable for correct function of GR-CAR/PXR-CYP and AhR-CYPs signaling/metabolic cascades. Microtubules disruption induced changes in these cascades and results in negative regulation of important genes including drug metabolizing enzymes.

This research was supported by grant MSM 151100003 from the Ministry of Education, Youth and Sports of the Czech Republic, and by grant from Grant Agency of the Czech Republic GACR 303/04/P074.

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L 5.11

BIOTRANSFORMATION ENZYMES OF PARASITES AND THEIR ROLE IN DRUG-RESISTANCE DEVELOPMENT

Lenka Skálová, Martina Gavelová, Viktor Cvilink

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Biotransformation enzymes can, to a certain extent, protect the parasitic worms against the toxic effects of anthelmintics, and an increased expression of genes coding certain biotransformation enzymes (the so-called induction) can represent one of mechanisms of helmintoresistance.

The biotransformation mechanisms of parasitic worms have been relatively little investigated so far. In worms, the key role in the biotransformation of xenobiotics is ascribed to the reduction, hydrolytic and conjugation enzymes. The existence of oxidation metabolism of xenobiotics was being rejected for a long time and only in recent years several studies have brought unequivocal evidence in favour of the oxidation of drugs (albendazole and triclabendazole) by means of the enzymatic apparatus in some species of worms. The parasitic helmints possess a number of hydrolytic enzymes. Among the reduction enzymes of helmints it was possible to find reductases of aldehydes and ketones, azoreductases and nitroreductases. In conjugation of xenobiotics, the dominant role belongs to glutathion-*S*-transferases.

After exposing the *Echinococcus granulosus* and *Schistoma mansoni* parasites to xenobiotics, induction of glutathion-*S*-transferases activity was observed. A significantly higher oxidation (deactivation) of triclabendazole was found in triclabendazole-resistant strains of *Fasciola hepatica* than was that in the sensitive strains. Hence, it is obvious that also parasitic helmints can struggle against chemical stress by induction of activity (expression) of biotransformation enzymes.

This project is supported by Grant Agency of Czech Republic (524/06/1345)

L 5.12

BERBERINE – POSSIBLE POTENTIAL ANTICANCER COMPOUND AND ITS MECHANISM OF ACTION

Silvia Letašiová, Soňa Jantová, Milan Miko

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Berberine, an isoquinoline plant alkaloid widely used in traditional Chinese and Ayurvedic medicine, has a broad range of pharmacological and biochemical effects. In our study, we evaluated cytotoxic/antiproliferative activity and mechanism of action of berberine using B16, L1210, NIH-3T3, EAC, HeLa and U937 cells. Apoptosis was detected in berberine-treated suspension-growing EAC, L1210 and U937 cells. The caspase 3 activity was found in L1210 and U937 cells measured by the amount of released paranitroaniline. A time-dependent increase in caspase 3 like activity of L1210 and U937 cells was observed up to 14 h (L1210 cells) and 6 h (U937 cells), then activity decreased very slightly. On the other hand, in berberine-treated adherent-growing B16, HeLa and NIH-3T3 cells we observed cell lysis/necrosis as the result of the cytoplasmic membrane integrity damage. By the study of the effect on biosynthesis of macromolecules (DNA, RNA, proteins) in berberine-treated EAC cells was found out that the biosynthesis of proteins was inhibited in a greater extent than the biosynthesis of nucleic acids. Moreover, berberine inhibited the biosynthesis of DNA more than the biosynthesis of RNA. Next we monitored the possible interaction of berberine with dsDNA on electrode surface. Berberine induced intercalation of berberine with DNA and formation of dsDNA breaks. Direct DNA strand breaks in EAC and NIH-3T3 cells were confirmed by comet assay.

This study was supported by the Slovak State Committee for Scientific Research VEGA 1/1173/04 and APVT project 20-007304.

L 5.13

IMPACT OF DNA BINDING INTERCALATORS ON ALTERNATIVE STRUCTURAL MOTIFS IN DNA

Viktor Viglaský and Patrik Danko

Department of Biochemistry, Institute of Chemistry, Faculty of Sciences, P.J. Šafárik University, Košice, Slovakia

Structural perturbations of DNA can be manifested by the interactions of small molecules with nucleic acids and may result in alterations to the informational content that may be nonlethal (i.e. mutagenesis) or lethal leading to cell lethality. The interactions of small molecules with nucleic acids have provoked considerable interest in the field of anticancer drug design over the past three decades. Here, we are focused on the impact of DNA intercalating drugs on the DNA cruciform.

Generally, any DNA-drug interactions are accompanied by a local DNA distortion; intercalators usually locally unwind the DNA thereby decrease negative DNA supercoiling. However, the local change of DNA helical parameter has a great impact on DNA supercoiling and negative DNA supercoiling is known to facilitate cruciform extrusion from appropriate palindromic sequences [4]. Therefore, intercalating drugs should have a great influence on the cruciform extrusion in supercoiled DNA. Despite the existence of a wealth of structural and theoretical data relating to palindromic sequences in genomes, the mechanism of cruciform extrusion in miscellaneous biological processes at the presence of intercalating agents are still poorly understood.

Acknowledgements

This study was supported by grants from the Slovak Grant Agency (1/1274/04 and 1/3254/06), and the Science and Technology Assistance Agency (APVT-20-006604).

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Wednesday September 13, 2006

Lecture room L2
10:00 – 12:30

Section 3 MEMBRANE BIOCHEMISTRY AND BIOENERGETICS I

Chairs: Hana Rauchová, Miloslav Greksák

L 3.1

LIFE WITHOUT OXYGEN: ADAPTATION OF YEAST LIPID METABOLISM TO ANAEROBIOSIS.

Ivan Hapala, Lucia Hronská, Martin Valachovič, Zuzana Mrózová

Institute of Animal Biochemistry and Genetics SAS, Ivanka pri Dunaji

Yeast *Saccharomyces cerevisiae* is a able to grow in the absence of oxygen. This ability is important for its survival in natural environment and is utilized in several industrial processes (e.g. brewing). To adapt to anaerobiosis, yeast cell has to undergo complex restructuring of its metabolism. Changes in lipid biogenesis represent one of dominant adaptations to hypoxia reflecting primarily the requirement of molecular oxygen for fatty acid desaturation and ergosterol synthesis. Moreover, heme (synthesized itself in oxygen-dependent manner) is involved in ergosterol synthesis as well as in regulation of expression of several genes participating in lipid synthesis. Growth of anaerobic or heme-deficient yeast *S. cerevisiae* is dependent on external supply of ergosterol and unsaturated fatty acids. Adaptation of lipid biogenesis to lack of oxygen includes changes in internal lipid synthesis and activation of systems for uptake of external lipid molecules (sterols and fatty acids). We will discuss basic principles in the regulation of these processes and present experimental data showing that changes in lipid biogenesis induced by anaerobiosis can be considered as an adaptive response to a complex environmental stress.

Acknowledgements: This work was supported by grants APVT-51-029504, APVT-51-024904 and VEGA 2/4130/24.

L 3.2

VOLTAGE GATED L-TYPE CALCIUM CHANNELS AND THEIR ROLE IN ORGANISM

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L-type calcium channels form most ubiquitously expressed group of voltage activated calcium channels. $Ca_v1.2$ channels have three isoforms: cardiac $Ca_v1.2a$, smooth muscle-specific $Ca_v1.2b$ and neuronal $Ca_v1.2c$. Because of their functional importance they are target for various commonly used drugs.

Sequence difference between $Ca_v1.2a$ and $Ca_v1.2b$ is responsible for differential sensitivity of vascular and cardiac tissue to dihydropyridines.

L-type calcium channels may form unintentional target mediating severe side effects of various drugs. Haloperidol, drug used as a treatment for delusional agitation in critically ill patients, causes inhibition of I_{Ca} characterised by two IC_{50}s of 2 nmol/l and 2.4 μmol/l as well as moderate shortening of action potential duration.

In hippocampal pyramidal cells, which participate in forming of a long-term memory $Ca_v1.2$ channels take part in regulation of excitability. Mice with the $Ca_v1.2$ gene inactivated in the forebrain including the hippocampus had significantly enhanced threshold for generating a series of action potentials and firing frequency was lower in CA1 pyramidal cells from $Ca_v1.2$ mutants.

$Ca_v1.3$ subtype of L-type voltage gated calcium channels is responsible for calcium current in inner hair cells (IHC) and is necessary for normal hearing. I_{Ca} of IHCs are inhibited by various phenylalkylamines and benzothiazepines. For verapamil, IC_{50} was 199 μM, for diltiazem IC_{50} was 334 μM and for gallopamil 467 μM.

L 3.3

DNA REPLICATION INITIATION IN ARCHAEA – IS THERE A SPACE FOR FUNCTIONAL COUPLING TO CYTOPLASMIC MEMBRANE?

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[2]Department of Biology University of York York UK

Genomic sequencing of Archaea has revealed that a number of genes involved in information processing, such as DNA replication, repair and transcription are more closely related to eukaryotic ORFs. In particular, protein homologues to eukaryotic DNA initiator proteins such as CDC6, Orc1 or MCM have been found in Archaea. In recent years, several studies confirmed that CDC6/Orc1 and MCM proteins are *de facto* archaeal DNA replication initiators. We have performed physiological characterization of DNA initiation in the *Methanothermobacter thermautotrophicus* and characterized nucleotide sequences of Origin Recognition Boxes (ORBs) responsible for CDC6/Orc1 binding. Surprisingly, this protein shares significant structural resemblance to the bacterial initiator DnaA. Moreover, CDC6/Orc1 binds cooperatively to ORBs in a similar way as DnaA. Another novel feature of DnaA and bacterial initiation processes is a interaction to a cytoplasmic membrane. Membrane is not only a site of an initiation but it seems to have a critical regulation role for re-initiation processes in bacteria. Elucidation of possible advantages of functional coupling of the archaeal initiation of DNA replication to cytoplasmic membrane will be discussed.

L 3.4

MEMBRANE TRANSLOCATION OF *Bordetella ADENYLATE CYCLASE TOXIN PROMOTES CALCIUM ENTRY INTO* CD11b$^+$ J774A.1 MACROPHAGE CELLS

Jiří Mašín[2] Radovan Fišer[1,2], Marek Basler[2], Jan Krůšek[3], Veronika Špuláková[1], Ivo Konopásek[1,2], Peter Šebo[2]

[1]Department of Genetics and Microbiology, Charles University, Prague [2]Laboratory of Molecular Biology of Bacterial Pathogens, Institute of Microbiology, Prague, [3]Department of Cellular Neurophysiology, Institute of Physiology, Prague

The *Bordetella* adenylate cyclase toxin-hemolysin (CyaA) targets phagocytes expressing the CD11b/CD18 integrin, permeabilizes their membranes by forming small cation-selective pores and delivers into cells a calmodulin-activated adenylate cyclase enzyme that dissipates cytosolic ATP into cAMP. We describe here a third cytotoxic activity of CyaA that causes elevation of cytosolic calcium concentration ($[Ca^{2+}]_i$) in target cells. CyaA-mediated $[Ca^{2+}]_i$ increase in CD11b$^+$ J774A.1 monocytes was inhibited by extracellular La^{3+} ions but not by nifedipine, SK&F 96365, flunarizine, 2-APB, or thapsigargin, suggesting that influx of Ca^{2+} into cells was not due to opening of conventional calcium channels by cAMP or receptor signaling. Compared to intact CyaA, a CyaA-AC$^-$ toxoid unable to generate cAMP promoted a faster, albeit transient elevation of $[Ca^{2+}]_i$. This was not due to cell permeabilization by the CyaA hemolysin pores, since a mutant exhibiting a strongly enhanced pore-forming activity (CyaA-E509K+E516K) but unable to deliver the AC domain into cells, was also unable to elicit $[Ca^{2+}]_i$ increase. Further mutations interfering with AC translocation into cells, such as proline substitutions of glutamate residues 509 or 570, or deletion of the AC domain as such, reduced or ablated the $[Ca^{2+}]_i$-elevating capacity of CyaA. Moreover, structural alterations within AC domain, due to insertion of various oligopeptides, differently modulated the kinetics and extent of Ca^{2+} influx elicited by the respective AC$^-$ toxoids. The translocating AC polypeptide appears, hence, to participate in formation of a novel type of membrane conduit for calcium ions, contributing to cytotoxicity of CyaA in an unexpected manner.

L 3.5

EFFECT OF METHYLMERCURY ON NEURONAL T-TYPE CALCIUM CHANNEL

Bohumila Tarabová, Zdenka Sulová, Ľubica Lacinová

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Methylmercury (MeHg) is environmental intoxicant, which affects different organs including central nervous system during acute and chronic exposure. It was shown that exposure to mercury may disrupt function of voltage gated calcium channels in the cells. Possible interaction of MeHg with neuronal T-type calcium channel remains unclear. We have investigated effects of MeHg on the current the through the $Ca_V3.1$ calcium channel stably expressed in HEK 293 cell line. MeHg in concentrations above 1 nM inhibited the current through the $Ca_V3.1$ calcium channel with an IC_{50} 13.0 ± 5.0 μM and a Hill coefficient of 0.47 ± 0.09. MeHg in concentrations between 10 pM and 1 nM had both positive and negative effects on the calcium current. Furthermore, micromolar MeHg concentrations enhanced time constant of current activation and decreased time constants of current inactivation and deactivation. Current voltage relation was not altered in the presence of MeHg.

Chronic effects of MeHg on current through the $Ca_V3.1$ calcium channel expressed in HEK 293 cells were also investigated. Up to 72 hours exposure of 10 nM MeHg had no significant effect on current amplitude but 1 nM MeHg caused significant increase in average current density. Viability of HEK 293 cells during acute treatment with MeHg was tested using MTT assay and FACS flow cytometry. 4 hours long exposure to 10 μM MeHg did not affect the viability of the cells.

In conclusion, the interaction of MeHg with $Ca_V3.1$ calcium channel may contribute to neuronal symptoms during acute or chronic mercury poisoning.

Wednesday September 13, 2006

Lecture room L2
15:30 – 17:00

Section 3 MEMBRANE BIOCHEMISTRY AND BIOENERGETICS Ii

Chairs: Eva Kutejová, Ivan Hapala

L 3.6

NA^+ CYCLE AND ITS FUNCTION IN BIOENERGETICS OF METHANOARCHAEA

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Institute of Animal Biochemistry and Genetics,Slovak Academy of Sciences

Methanoarchaea are a group of strictly anaerobic organism that posses the unique feature of producing methane by the process of methanogenesis. In this process, redox reactions of methanogenesis drive the formation of two primary electrochemical gradient – proton electrochemical gradient and sodium ions electrochemical gradient across a very specialized archaeal membrane. The presence of primary generated electrochemical gradients $\Delta\mu_{H}^{+}$ and $\Delta\mu_{Na}^{+}$ that can be directly coupled to ATP synthesis is unusual among energy conserving systems. In spite of many studies of this bioenergetic machinery over the past decades these process has not been satisfactorily elucidated .

A systematic genetic approach to the problem of energy conservation in yeast and eubacteria has proved to by very valuable. In methanoarchae, a genetic approach to the solution of bioenergetic problems was started in our laboratory in 1997.The purpose of this work was to isolate a series of methanoarchaea mutants with modified energy conservation systems which could give further information on structure, function and organization of the energy conserving system . This paper deals with the use of mutants of *M.therautotrophicum* in the study of sodium ions bioenergetic system

Hopefully, the genetic and molecular biology approach, combined with biochemical and new physical techniques will extend the study of Na^+-dependent bioenergetic problems of Archaea to the molecular level.

Acknowledgement:
This investigation was supported in part from the Science and Technology Assistance Agency (Slovak Republic) APVT-51-024904 and by a Research Grant VEGA 2/6025/26 from the Slovak Academy of Sciences

L 3.7

RESPIRATORY CHAIN OF TRYPANOSOMATIDS

Anton Horváth[1], Petra Dunajčíková[1], Vladislava Benkovičová[1], Zdeněk Verner[2] and Julius Lukeš[2]

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Trypanosomatids are parasitic protozoans, among which several species cause serious diseases in humans such as sleeping sickness *(Trypanosoma brucei)*, Chagas disease *(T. cruzi*) and leishmaniasis *(Leishmania* spp.). Study of bioenergetism and particularly study of complexes of respiratory chain of these interesting flagellates brings not only news to basic science but could be useful in applied medicinal research as well. Respiratory chain is localized in inner mitochondrial membrane. It finalizes oxidation processes and energy of transferred electrons is used form electrochemical potential on inner mitochondrial membrane. Besides this standard respiratory pathway mitochondria from various organisms (included trypanosomatids) contain alternative route for electrons from NADH to oxygen. Two single peptides do the same job as whole respiratory chain, but they do not contribute to the generation of transmembrane potential. In order to study the respiration of *T. brucei*, by RNA interference (RNAi) we have generated knock-downs for three nuclear-encoded subunits of mitochondrial complexes III and IV. mRNA of the silenced genes was virtually completely degraded within 24 hrs upon RNAi induction. Western analysis of mitochondrial lysates of RNAi interfered cells using specific polyclonal antibodies revealed almost complete elimination of the targeted proteins at days 4 to 6. We have showed that down regulation of either of the targeted proteins lead to the disruption of respective complexes and subsequent switch to alternative oxidase presented in *T. brucei*. Depletion of one subunit leads to elimination of another investigated subunit within complex III but not in complex IV. No connection was observed between stability complexes III, IV and I.

L 3.8

MITOCHONDRIAL PROTEASES AND THEIR SUBSTRATES

Gabriela Ondrovičová[1], Natalya Parkhomenko[2], Jiří Janata[2], Eva Kutejová[1]

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Several serious diseases are connected with the dysfunction of the mitochondrial biogenesis. Proteases in mitochondria are essential for the homeostasis of these organelles. Processing peptidases are responsible for the removing of presequences after transport of the proteins into the mitochondria either in the mitochondrial matrix or in the inter membrane space and unable them to fold properly and/or assemble into the mitochondrial complexes. ATP- dependent proteases are localized in mitochondrial matrix and also in the inner mitochondrial membrane. There are important for the removing of non assembled complex subunits, play a crucial role for the stabilization of mtDNA and in stress response.

Our work is focused on Lon protease that is located in mitochondrial matrix. Lon protease plays an important role in two different functions in mitochondria. Degradation of certain regulatory proteins, misfolded and unassembled polypeptides, represents the quality control of protein biogenesis. Stabilization of the mitochondrial DNA is important for mitochondrial coded protein subunits formation. Human Lon protease recognizes and cleaves folded endogenous substrates. Substrate determinants are on the surface of the proteins and represents specific adjacent hydrophobic residues surrounded by the highly charged amino acids.

Acknowledgement

This study was supported by grants VEGA No. 2/5118/25 and the Science and Technology Assistance Agency No. APVT-51-029604.

Wednesday September 13, 2006

Lecture room L3
10:00 – 13:10

Section 9 GLYCOBIOCHEMISTRY I

Chairs: Peter Biely, Karel Bezouška

L 9.1

BACTERIAL LECTINS AND RECOGNITION OF HOST GLYCANS: STRATEGIES FOR HIGH AFFINITY BINDING

Michaela Wimmerová[1,2], Martina Pokorná[1], Charles Sabin[3], Jan Adam[1], Stephanie Perret[3], Edward P. Mitchell[4], Anne Imberty[3]

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Carbohydrate-mediated recognition plays an important role in the ability of pathogenic bacteria to adhere to the surface of the host cell in the first step of their invasion and infectivity. Lectin-carbohydrate interactions are usually characterised by a low affinity for monovalent ligands that is balanced by multivalency resulting in high avidity for complex glycans or cell surfaces. Bacterial lectins involved in pathogenesis display much higher affinity than observed for plant or animal lectins [1].

Contribution is focused on a bacterial lectin from *Pseudomonas aeruginosa* and its homologues from other pathogens displaying sub-micromolar range affinity towards their carbohydrate ligands. The combination of protein mutagenesis, X-ray crystallography and isothermal titration microcalorimetry approaches is used to decipher the structural and thermodynamical basis for high affinity binding of these lectins to host carbohydrates. Discovery of a three amino acid motif of the „ligand binding loop" that is responsible for lectin specificity toward different monosaccharides will be discussed.

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This work has been supported by Ministry of Education of Czech Republic (MSM 00216224138, MSMT LC06030), by Mizutani Foundation for Glycosciences, by French Association Vaincre la Mucoviscidose and by Czech Grant Agency (303/06/0570).

L 9.17
MIMETICS OF THE CARBOHYDRATE LIGANDS FOR NK CELL RECEPTORS ARE EFFECTIVE IN EXPERIMENTAL TUMOR THERAPIES

Karel Bezouška*[1,2], Jan Sklenář[1,2], Ondřej Plíhal[1,2], Petr Pompach[1,2], Petr Man[2], Petr Novák[2], Vladimír Havlíček[2], Anna Fišerová[2], Luca Vannucci[2], Miloslav Pospíšil[2], Thisbe K. Linhorst[3], Joachim Thiem[4], Pavel Krist[2], Vladimir Křen[2]

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Dendrimeric carbohydrates are becoming interesting and efficient tools of today's molecular medicine, especially in the area of infectious diseases and tumor therapies [1]. In our laboratory we have identified carbohydrate ligands for important activation antigens of natural killer lymphocytes, NKR-P1 and CD69 [2,3]. Although *N*-acetylhexosamines are the most important monosaccharide ligands, these receptors display a remarkable specificity for the linear and branched oligosaccharide sequences, respectively. Although the search for the optimal oligosaccharides interacting with these receptors has been continuing in our laboratories [4], we have found that *N*-acetylhexosamines can *per se* become very efficient ligands provided they are appropriately clustered through binding to dendrimeric backbones [5]. *N*-acetyl-D-glucosamine-coated dendrimers are high-affinity ligands for NKR-P1A, but not NKR-P1B, the corresponding D-mannose-coated dendrimers are inactive. These dendrimers represent one of the most specific functional marker of active NK cells [6]. The ability of these compounds to activate NK cells through NKR-P1 and receptors associated in the activation membrane microdomain [7] can be use to activate natural killing *in vitro* [8] as well as *in vivo* [9]. The relation between the structure and antitumor activities of carbohydrate dendrimers will be discussed from the point of view of (a) active oligosaccharide sequences; (b) linker structures; and (c) the degree of clustering.

This work has been supported by Ministry of Education of Czech Republic (MSM 21620808 and 1M0505), and by the Institutional Research Concept (AVOZ50200510)

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L 9.3

PREPARATION OF ANTENNARY OLIGOSACCHARIDE LIGANDS FROM OVOMUCOID AND THEIR BINDING TO CD69 RECEPTOR

Michal Navrátil[1], Petr Pompach[2], Karel Bezouška[1,2]

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[2]Institute of Microbiology, Academy of Sciences of the Czech Republic

It is well known that saccharide structures play an important role in immune system, from direct recognition of pathogenic saccharide structures to protein glycosylation, which can have also modulatory or self-protective function. One group of very interesting oligosaccharide compounds are branched antennary oligosaccharides which are present at the surface of tumour cells and are recognized by C-type lectin-like receptors of NK cells.

Chemical synthesis of antennary oligosaccharide structures is quite difficult. In contrast, there are glycoproteins that provide defined oligosaccharide structures in sufficient yields. Thus, the isolation of native structures from natural sources seems to be an appropriate way as well. In order to research for physiological ligands for C-type lectin-like receptors of NK cells we developed a relatively fast method for preparation of pure and well defined antennary oligosaccharides from hen egg white protein ovomucoid. The purity and identity was verified by MALDI-TOF mass spectrometry. Biochemical binding and inhibition experiments with CD69 receptor revealed the highest affinity for the pentaantennary oligosaccharide. Oligosaccharides were used for structural studies by means of cocrystallisation with CD69, and examination of CD69-saccharide complexes by MS and NMR titration.

L 9.4

PLANT XYLOGLUCAN ENDOTRANSGLYCOSYLASE (XET) – PROPERTIES AND FUNCTION

Vladimír Farkaš

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Polysaccharide xyloglucan (XG) is a major hemicellulose in growing plant cell walls. Owing to its high sorption affinity to cellulose, the XG molecules function as tethers between individual cellulose microfibrils in primary plant cell walls thus strengthening the wall structure.

Xyloglucan endotransglycosylases (XETs) are group of enzymes catalyzing breakdown and re-formation of xyloglucan chains thereby enabling continuous expansion of plant cell walls during growth. Analytical methods for assessment of XET activity include viscometric, radiometric, colorimetric and fluorescent methods. Fluorescent technique proved extremely valuable in making zymograms detecting XET isoenzymes after their separation by native PAGE and/or PAG-IEF [1]. Extracts from nasturtium (*Tropaeolum majus*) contain at least five isoenzymes of XET differing in their substrate specificity and mode of action on xyloglucan [2, 3]. Some isoenzymes of XET catalyze hetero-transfer between different types of polysaccharides. In model experiments where a polysaccharide served as the glycosyl donor and an oligosaccharide fluorescently labeled with sulphorhodamine (SR) as the acceptor, the following transglycosylation donor:acceptor pairs were found: XG: XGOS-SR; XG:CEOS-SR; XG:LAOS-SR; HEC:XGO-SR; CMC:XGO-SR [3]. The ability to catalyze the hetero-transfer indicates that XETs may be involved in cross-linking of diverse polysaccharides in plant cell walls.

Abbreviations: CEOS, cellooligosaccharides; LAOS, laminarioligo-saccharides; HEC, hydroxyethyl cellulose; CMC, carboxymethyl cellulose

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L 9.5

MODE OF ACTION OF ENDOXYLANASES OF GLYCOSIDE HYDROLASE FAMILY 10, 11 AND 5 ON GLUCURONOXYLAN AND ACIDIC XYLOOLIGOSACCHARIDES

Mária Vršanská, Katarína Kolenová, Peter Biely

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Action of endo-β-1,4-xylanases (EXs) of three glycoside hydrolase families, GH-10, GH-11 and GH-5, was examined on glucuronoxylan and acidic xylooligosaccharides. The structure of products of hydrolysis was determined by combination of NMR and enzymatic treatment by β-xylosidase and α-glucuronidase. EXs of GH-10 liberate from glucuronoxylan aldotetraouronic acid $MeGlcAXyl_3$ and EXs of GH-11 aldopentaouronic acid $Xyl(MeGlcA)Xyl_3$, as the shortest acidic fragments [1]. EX of GH-5 of *Erwinia chrysanthemi* catalyzed the depolymerization of glucuronoxylan to longer aldouronic acids [2].

Aldotetraouronic acid $MeGlcAXyl_3$ and aldopentaouronic acid $Xyl(MeGlcA)Xyl_3$ were resistant to the action of EXs of GH-10 and GH-11. EXs of GH-5 attacked aldotetraouronic and aldopentaouronic acids at the first glycosidic linkage from the reducing end [3, 4].

The greatest differences in the mode of action of EXs of the above three families were observed with aldohexaouronic acid $Xyl_2(MeGlcA)Xyl_3$. The hydrolysis of this substrate by EXs of GH-10 afforded Xyl_2 and $MeGlcAXyl_3$. The degradation of aldohexaouronic acid by EXs of GH-11 did not correspond to simply hydrolysis and involved glycosyl transfer reaction. EX of GH-5 attacked the substrate at the reducing end to give Xyl plus $Xyl_2(MeGlcA)Xyl_2$.

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Wednesday September 13, 2006

Lecture room L3
15:30 – 18:40

Section 9 GLYCOBIOCHEMISTRY II

Chairs: Jozef Čársky, Vladimír Farkaš

L 9.6

UNIQUE TRANS-α-GALACTOSYLATION TO INTERNAL SUGAR RESIDUES OF OLIGOSACCHARIDES CATALYZED BY *ASPERGILLUS FUMIGATUS* IMI 385708 α-GALACTOSIDASE

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In agreement with its retaining character and glycoside hydrolase family 27 classification, a purified extracellular α-galactosidase of a thermotolerant fungus *Aspergillus fumigatus* IMI 385708 was found to catalyze efficiently transgalactosylation reactions using 4-nitrophenyl α-D-galactopyranoside as glycosyl donor. Self-transfer reactions with this substrate afforded in low yields several 4-nitrophenyl α-galactobiosides. Monosaccharides also served as poor glycosyl acceptors. Disaccharides and particularly higher oligosaccharides of α-1,4-gluco- (maltooligosaccharides), β-1,4-gluco- (cellooligosaccharides) and β-1,4-manno-series were efficiently α-galactosylated, the latter being the best acceptors that were also doubly galactosylated. With mannooligosaccharides product yields increased with polymerization degree of acceptors reaching 50 % at DP of 4-6. Longer oligosaccharide acceptors were galactosylated at internal sugar residues. All galactosyl residues were transferred exclusively to the primary hydroxyl group at *C*-6 position of oligosaccharide acceptors. This is in accordance with the inability of the enzyme to transfer galactose to β-1,4-linked xylooligosaccharides.

Glycosyl transfer reaction to internal sugar residues of oligosaccharides catalyzed by *A. fumigatus* α-galactosidase is a unique property not only among α-galactosidases but also among exo-acting glycosidases in general. In structural terms, the high affinity to oligosaccharide acceptors may be explained on the basis of a homologous 3D model that predicts a pair of catalytic aspartates located in a pocket within a shallow binding cleft accommodating the oligosaccharide acceptors. All these findings indicate that the enzyme has a great potential for galactosylation of polysaccharides, an important way to modulate their physico-chemical and biological properties.

The work has been financially supported by Centre of Excellence of the Slovak Academy of Sciences (GLYCOBIOS) and Slovak Scientific Grant Agency VEGA (No. 2/6130/26).

L 9.7

FORMATION OF THE SPERM RESERVOIR IN REPRODUCTIVE TRACT

Jiří Liberda [1] ***Ivana Tichá*** [1]***, Tomáš Dráb*** [1]***, Lucie Prelovská*** [1]***, Marie Tichá*** [1]***, Pavla Maňásková*** [2]

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Several mechanisms have been established during evolution to prolongate sperm life, to test and select good ones, and to elongate sufficient sperm concentration in the female urogenital tract. Basic principles are similar in wide range of species: sperm binding to the epithelium, selection and releasing according to their quality and at proper time for fertilisation event. Sperm reservoirs are formed for this purpose.

Two of them are studied in detail in our laboratory. First one is the semen itself as is stored at the place of ejaculation. The role of bulbourethral glands have been studied in two species, bull and boar. Second one is the oviductal reservoir formed in the isthmic part of the oviduct in cattle and swine. Mechanisms involved in establishing of both types of sperm reservoirs are based on the same principles : saccharide – protein interactions. On the sperm we identified the key proteins, which are well known. Surprisingly same proteins participate on both types of sperm reservoir formation: boar seminal plasma proteins AQN, AWN, DQH and PSP I+II; bull seminal plasma PDC 109 protein. We characterized female urogenital tract components due to their saccharide structure by lectin saccharide binding study and inhibition study. Saccharide structures exposing Mannose, Galactose and N-acetyl-hexosamine epitopes, which are involved in sperm binding to the isthmic part of the oviduct.

This work was supported by grant No. 303/06/0895 of the Grant Agency of the Czech Republic.

L 9.8

MYCOBACTERIAL GLYCOSYL TRANSFERASES – CHALLENGE AND EXPECTATIONS

Katarína Mikušová, Martina Beláňová, Petronela Dianišková, Zuzana Svetlíková, Jana Korduláková

Department of Biochemistry, Comenius University in Bratislava, Faculty of Natural Sciences

Mycobacterial cell wall is an attractive target for rational drug design against tuberculosis. Not only it forms a protective, almost impermeable barrier on the surface of mycobacteria, but also it contains important structures responsible for effective establishment of the infection. Covalently linked complex of mycolyl-arabinogalactan-peptidoglycan (mAGP) forms the backbone of mycobacterial cell wall; intercalating mAGP complex is lipoarabinomannan (LAM) – one of the dominant lipoglycans and crucial immunomodulator in the course of tuberculosis infection. Despite the fact that structures of both key polysaccharides of mycobacterial cell wall, AG and LAM, are known, information on their biosynthesis is rather limited. Our aim is identification of glycosyl transferases involved in biosynthesis of these two components of mycobacterial cell wall that could be exploitable for TB drug development.

In the presentation we will report on our latest data towards understanding initial steps of mycobacterial galactan build-up. We will also present our progress in the development of spectrophotometric assay for high throughput screening of inhibitors against essential mycobacterial enzyme – mannosyl transferase PimA.

Acknowledgements: This work is supported by EC 6th Framework Programme, Contract No. LSHP-CT-2005-018923; NIH, Fogarty International Center; R03 TW 006487; Slovenská grantová agentúra VEGA, grant 1/2324/05.

L9.9

ANTIOXIDANT, ANTIGENOTOXIC, AND IMMUNOMODULATING PROPERTIES OF YEAST CELL WALL POLYSACCHARIDES

Grigorij Kogan[1], Eva Miadoková[2], Darina Slameňová[3], Viera Vlčková[2], Melánia Babincová[4], Peter Rauko[3]

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Polysaccharides constitute the major part of the dry weight of the yeast cell wall and build the skeletal carcass defining cell wall stability and cell morphology (β-D-glucans) or compose amorphous matrix and cell surface fibrous material (mannans and mannoproteins). Recently it has been discovered that yeast cell wall β-D-glucans reveal immunomodulating properties, which allows for their application in antiinfective and antitumor therapy. We have prepared two water-soluble derivatives of β-D-glucan isolated from the cell wall of baker's yeast *Saccharomyces serevisiae*, and isolated glucomannan from the industrial yeast *Candida utilis*. The derivatives of β-D-glucan demonstrated potent inhibitory effect on lipid peroxidation comparable to that of the known antioxidants and exerted DNA protection from oxidative damage. Antimutagenic and antigenotoxic activity of the yeast polysaccharides was demonstrated using yeast, bacterial, and algal models. The presented results indicate significant protective antioxidant, antimutagenic, and antigenotoxic activities of the yeast polysaccharides and imply their potential application in anticancer prevention/therapy.

Financial support from the grants VEGA 2/4143/04, 1/2337/05/, 2/4056/04, APVT-20-002604, as well as of Center of Excellence of Slovak Academy of Sciences, CEDEBIPO is gratefully acknowledged.

L 9.10

CYCLIC OLIGOSACCHARIDES – INTERMEDIATES OF STARCH UTILIZATION IN SOME BACTERIA

***Peter Biely*[1], *Gregory L. Côté*[2]**

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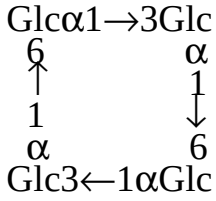
Utilization of starch that involves hydrolysis by amylolytic enzymes is wide-spread among microorganisms and mammals. An alternative way of starch degradation occurrs in some bacteria, which, in a competitive environment convert starch to non-reducing cyclic structures that are not degraded by other microorganisms. The best known are α-1,4-linked cyclic glucoligosaccharides composed of 6 to 8 glucose residues, so called cyclodextrines, which found a remarkable application in pharmacy and cosmetics. In 1994 we discovered the smallest cyclic oligosaccharide occurring in nature [1]. It is composed of four glucopyranosyl residues linked alternatively by α-1,3 a α-1,6-glycosidic linkages, and is called cycloalternan [2]. The formation of this oligosaccharide was observed for the first time with a *Bacillus* sp. from the bacterial polysaccharide alternan in which the glucopyranosyl residues are linked by alternating α-1,3 a α-1,6-glycosidic linkages. Japanese scientists recently showed that the natural pathway leading to cycloalternan starts similarly as with cyclodextrines from starch.



The formation of the cyclic tetramer involves two enzymes, one of which is identical with the enzyme catalyzing the formation of cyclic tetramer from alternan [3]. Interesting that bacteria producing cycloalternan or cyclodextrines are capable of translocating of these large molecules to the cell interior by cytoplasmic membrane systems operating against concentration gradients [4]. Cycloalternan finds its application as aroma stalilizer in food and cosmetic products [5] as well as a factor delaying cataract formation in lenses [6].

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[3] Kim Y.K. et al. Carbohydr. Res. 338 (2003) 2213-2220.
[4] Kim Y.K. et al. Carbohydr. Res. 339 (2004) 1179-1184.
[5] Kazuyuki O., et al. US 2005/0013914 A1.
[6] Toshiko M. Cell Pres. Technol. 3 (2005) 238-243.

L 9.11

ELECTROCHEMICAL AFFINITY SENSORS FOR GLYCATED HEMOGLOBIN

Jan Halámek, Ulla Wollenberger, Walter Stöcklein, Frieder W. Scheller

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The glycated hemoglobin in blood is an important marker of the glycaemia level. With a normal turn-over number of erythrocytes, the glycated hemoglobin provides an accurate index of the average glucose concentration over the preceeding 2 to 3 months. Due to their higher average glucose level diabetes patients, whose glucose level is not well adjusted, show a significantly elevated fraction of glycated hemoglobin.

Different methods such as ion-exchange chromatography, boronate-based chromatography or various immunoassays are used for the detection of glycated hemoglobin at clinical diagnostics.

Here we describe a piezoelectric sensor coupled with an electrochemical detection step for simultaneous determination of total and glycated hemoglobin. In the first step total hemoglobin content is measured by binding to a surfactant-modified piezosensor followed by the specific recognition of the glycated fraction. Different surface modifications were examined, as well as various formats to read-out the content of glycated hemoglobin.

Wednesday September 13, 2006

Lecture room L4
10:00 – 13:30

Section 1 GENOMICS

Chairs: Jaromír Pastorek, Ján Turňa

L 1.1

BACTERIOPHAGE BFK20 GENOME ANOTATION

Luboš Kľučár[1], Gabriela Bukovská[1]

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The entire genome of bacteriophage BFK20, a lytic phage of the Brevibacterium flavum CCM 251, industrial producer of L-lysine, was sequenced and analyzed. Bacteriophage BFK20 morphologically belongs to a taxonomical group of unclassified Siphoviridae. The phage particle is composed of 50 nm polyhedral head and 200 x 10 nm noncontractible tail. The genome of BFK20 contains a linear double-stranded DNA molecule with 3' cohesive ends and a G+C content of 56.2 % (EMBL, accession no. AJ278322). The BFK20 genome consists of 42,968 bp and it is the first corynephage to be completely sequenced. Fifty-five potential open reading frames were identified and annotated using various bioinformatics tools. Clusters of functionally related putative genes were defined (structural, lytic, replication and regulatory). To verify the annotation of structural proteins, they were resolved by 2D gel electrophoresis and were submitted to N-terminal amino acid sequencing. Structural proteins identified included the portal and major and minor tail proteins. Based on the overall genome sequence comparison, similarities with other known bacteriophage genomes include primarily bacteriophages from Mycobacterium spp. and some regions of Corynebacterium spp. genomes - possible prophages. Our results support the theory that phage genomes are mosaics with respect to each other.

L 1.2

SEGMENTAL TRISOMY OF CHROMOSOME 17: A MOUSE MODEL OF HUMAN ANEUPLOIDY SYNDROMES

Tomáš Vacík[1], Michael Ort[2,3], Soňa Gregorová[1], Petr Strnad[1], Radek Blatný[1], Nathalie Conte[4], Allan Bradley[4], Jan Bureš[2], and Jiří Forejt[1]

Institutes of [1]Molecular Genetics and [2]Physiology, Academy of Sciences of the Czech Republic, 14220 Prague, Czech Republic; [3]Department of Psychiatry, First School of Medicine, Charles University, 11636 Prague, Czech Republic; and [4]The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, United Kingdom

Triplication of whole autosomes or large autosomal segments is detrimental to the development of a mammalian embryo. The trisomy of human chromosome (Chr) 21, known as Down's syndrome, is regularly associated with mental retardation and a variable set of other developmental anomalies. Several mouse models of Down's syndrome, triplicating 33-104 genes of Chr16, were designed in an attempt to analyze the contribution of specific orthologous genes to particular developmental features. However, a recent study challenged the concept of dosage-sensitive genes as a primary cause of an abnormal phenotype. To distinguish between the specific effects of dosage-sensitive genes and nonspecific effects of a large number of arbitrary genes, we revisited the mouse Ts43H/Ph segmental trisomy. It encompasses >310 known genes triplicated within the proximal 30 megabases (Mb) of Chr17. We refined the distal border of the trisomic segment to the interval bounded by bacterial artificial chromosomes RP23-277B13 (location 29.0 Mb) and Cbs gene (location 30.2 Mb). The Ts43H mice, viable on a mixed genetic background, exhibited spatial learning deficits analogous to those observed in Ts65Dn mice with unrelated trisomy. Quantitative analysis of the brain expression of 20 genes inside the trisomic interval and 12 genes lying outside on Chr17 revealed 1.2-fold average increase of mRNA steady-state levels of triplicated genes and 0.9-fold average down-regulation of genes beyond the border of trisomy. We propose that systemic comparisons of unrelated segmental trisomies, such as Ts65Dn and Ts43H, will elucidate the pathways leading from the triplicated sequences to the complex developmental traits.

L 1.3

EXPRESSION OF CYTOCHROMES P450 mRNA IN HUMAN CARCINOMA BREAST PATIENTS

Radka Václavíková[1], Miluše Hubáčková[1] Jana Stříbrná[1], Roman Kodet[2], Marcela Mrhalová[2], Ivan Gut[1] and Pavel Souček[1]

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Background: Cytochromes P450 (CYP) belong to crucial enzymes metabolizing a variety of endogenous and exogenous compounds including drugs. Changes in expression of CYPs in tissue affected by tumorigenesis may have an important role in progression of cancer and in metabolism of anticancer drugs. The aim of this study was to characterize the expression levels of four important CYPs (*CYP1B1, 2C9, 2E1* and *3A4*) by measuring mRNA content. Methods: The mRNA levels were monitored in 40 human carcinomas of the mammary gland, breast tissue of the same patients without morphological evidence of presence of tumor cells and peripheral blood lymphocytes. mRNA expression was determined by real-time PCR with absolute quantification and normalization to the control gene cyclophilin A. Results and conclusions: The expression of *CYP1B1* was significantly higher than that of *CYP2E1* in majority of samples. Expression levels of *CYP2C9* and *CYP3A4* in all kinds of samples were mostly under limit of quantification. In peripheral blood lymphocytes *CYP2E1* and *CYP1B1* were unambiguously expressed, but the level of their expression was lower than that in breast tissue and there was lack of correlation. The high expression of *CYP1B1* in breast tissue unaffected with carcinoma may evoke formation of some carcinogens, e.g. metabolic activation of polycyclic aromatic hydrocarbons or the formation of 4-hydroxyestradiol during estradiol metabolism. Our results suggest that the expression of *CYP1B1* should be followed in research of mammary gland carcinogenesis.

This study was supported by grants IGA 1A/8248-3, and COST B20.001.

L 1.4

FUNCTIONAL ANALYSIS OF AN UPSTREAM REGULATORY REGION OF THE GENE CODING FOR TUMOR ENDOTHELIAL MARKER (TEM1)/ENDOSIALIN

Anna Ohraďanová, Monika Baráthová, Miriam Zaťovičová, Juraj Kopáček, Silvia Pastoreková and Jaromír Pastorek

Centre of Molecular Medicine, Institute of Virology,
Slovak Academy of Sciences, Bratislava

The search for novel molecular markers that distinguish neoplastic from normal vasculature is producing many attractive therapeutic targets. One of them is endosialin, which is a member of a glycoprotein family involved in cell-cell interactions. It has been identified as one of the most differentially expressed markers of cells lining tumor blood vessels and adjacent fibroblast-like stromal cells of tumors vs normal tissue (hence tumor endothelial marker 1, TEM1), and its role is believed to be in vascular reorganization to form new capillaries in oxygen and nutrition deprived regions of actively growing tumors.

So far, a density-dependent expression pattern of endosialin has been observed *in vitro*, but transcription factors that regulate TEM1 expression have not been identified. High cell density could modulate gene expression by establishment of direct contacts between neighboring cells, by depletion of serum growth factors, or by decreased oxygen supply, i.e. hypoxia. In order to shed some light on endosialin transcription regulation, we performed *in silico* analysis of endosialin 5´ flanking region and its study by dual luciferase reporter system. According to our results, when cells transfected by cloned -1091/+43 TEM1 genomic fragment in pGL3 vector were treated with hypoxia-mimicking agent DFO, several fold increase of the luciferase activity was observed. We found certain putative HREs (hypoxia response element) within TEM1 promoter and confirmed one of them by serial deletion analysis of cloned TEM1 promoter as well as by ChIP assay.

Supported by VEGA 2(6112/26)

L 1.5

OBSERVATION OF SINGLE STRANDED DNA AND EXONUCLEASE III ACTIVITY BY ATOMIC FORCE MICROSCOPY

Jozef Adamčík[1,2], Guillaume Witz[1], Dmitry V. Klinov[3], Sergey K. Sekatskii[1] and Giovanni Dietler[1]

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Linear single stranded DNA (ssDNA) molecules adsorbed on different substrates were studied by Atomic Force Microscopy (AFM). ssDNA molecules deposited on mica from a solution containing magnesium ions or 3-aminopropyltriethoxysilane modified mica formed very compact structures with lumps, loops and intramolecular base-pairings. It was not possible to obtain native ssDNA without secondary structures, while ssDNA molecules adsorbed on modified highly oriented pyrolytic graphite (HOPG) were without secondary structures. We have found a simple method for immobilization of single stranded DNA to eliminate intramolecular base-pairing. The modified HOPG can be used for AFM studying of processes involving ssDNA, like DNA replication and transcription, the mechanism of enzyme action (digestion of DNA by Exonuclease III) or DNA hybridization.

L 1.6

QUANTIFICATION OF SPECIFIC ABC TRANSPORTER GENES EXPRESSION BY NOVEL MULTIPLEX ANALYSIS ON LUMINEX PLATFORM

Jana Jakubíková, Ján Sedlák

Laboratory of Tumor Immunology, Cancer Research Institute, Slovak Academy of Sciences, Vlárska 7, Bratislava, Slovak Republic

Abstract

Several different approaches have been used for quantification of mRNA expression profile but most of them require RNA isolation, reverse transcription and also target amplification. To avoid these steps, novel technology was designed that adapts a multiplex branched DNA (bDNA) technology to the Luminex fluorescent bead-based platform by coupling xMAP fluorescent beads with a set of oligonucleotide capture probes. This new mRNA quantification method measures the expression levels of multiple mRNA transcripts quantitatively in crude cell lysates. The bDNA is a sandwich nucleic acid hybridization assay that affords a unique approach for mRNA quantification by amplifying the reporter signal rather than target sequences. At the core of the xMAP technology are set of 100 microspheres, each with a unique spectral signature embedded with specific quantities of two fluorescent dyes that are conjugated with a capture reactant specific to a particular bioassay. In our study we have used a 7-plex assay for human ABC transporters mRNA quantification. In addition to permanent cell lines with defined type of multidrug resistance, the peripheral blood mononuclear cell panel of AML patients was analyzed. Our experience suggests that this assay might be useful tool for high-throughput parallel quantitative gene expression analysis in basic research of drug resistance but also in clinical applications using biological samples.

Wednesday September 13, 2006

Lecture room L4
15:30 – 17:45

Section 6 FREE RADICALS IN BIOLOGY AND MEDICINE I

Chairs: Zdeňka Ďuračková, Ján Lehotský

L 6.1

OXIDATIVE STRESS IN PATHOGENESIS OF DISEASES AND ITS MONITORING

Zdeňka Ďuračková

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Oxidative stress (OS) is a phenomenon characterizing a disequillibrium between reactive oxygen/nitrogen species (ROS) formation and defence systems in the organism resulted into damage of biomolecules, organs or the whole organism. Oxidative stress is related to the pathology of many diseases.

ROS play two roles in the organism. They play a positive role in some physiological processes (phagocytosis, reproduction, systems of cytochrome P_{450}). Some of them have important signal function in regulation of physiological or pathological processes in the organism. They have a negative function if their formation gets out of control, if they in high amount and at a wrong place are formed. In such cases ROS are very toxic and harmful to lipids, proteins and nucleic acids.

Monitoring of ROS formation in the organism is very difficult, because most of them persist for only a very short period of time *in vivo* and cannot be measured directly. For this reason indirect methods are used. For the determination of total antioxidant capacity *in vivo*, several methods are used (TRAP, ORAC, TAS, TEAC, FRAP).

For the determination of oxidative damage to lipids (lipoperoxidation) wide range of products in variable amounts is formed. Validity and specificity of some methods will be discussed.

DNA exposed to attack by hydroxyl radical generates a lot of purine, pyrimidine and deoxyribose modification products which are measured by HPLC, GC-MS, LC-MS and antibody-based techniques. Also enzymaticly modified (using Fpg or endonuclease III) single cell gel electrophoresis (comet assay) is used.

Oxidatively modified proteins determination is important *in vivo*, because the function of enzymes, receptors, transport proteins can be changed primarily and because it can contribute to secondary damage to other biomolecules (inactivation of DNA repair enzymes and DNA polymerases). The most frequently used biomarker of protein damage are the protein carbonyl groups. Carbonyls can be formed after protein glycation by saccharides, by the binding of aldehydes (formed for example during lipid peroxidation) to proteins and by the direct oxidation of side chains of amino acids residues.

In conclusion - there is not one single biomarker of oxidative stress. Monitoring of oxidative stress is not easy, but at defined conditions it is possible.

L 6.2

COMPARISON OF ANTIOXIDANT ACTIVITIES OF NATURAL EXTRACTS DISSOLVED IN DIFFERENT SOLUTIONS

Zuzana Chovanová[1], Monika Dvořáková[1], Petra Högger[2], Zdeňka Ďuračková[1]

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At present, a great attention has been paid to the study of effects of different procyanidines administered as a food supplement. Mentioned substances have a wide range of biochemical and pharmacological properties. These compounds are often administered in solution form, in which fructose is present. However, during the metabolism of fructose, ATP is formed and subsequently it degrades to uric acid, which also contributes to antioxidant ability. If fructose increased antioxidant ability of serum through raising uric acid level, obtained positive results could contribute to their misunderstanding. Hence, the aim of our study is to approve or disprove this theory and to avoid the possible incorrect result evaluation.

Acknowledgements

This study was supported by Horphag Research Ltd. Switzerland, by VEGA grant 1/1157/04 and by civil association Mind and Health, Slovakia.

L 6.3

INFLUENCE OF SEVERAL TYPES OF COMPLEX ANTIOXIDANT PREPARATIVES ON METABOLIC AND ANTIOXIDANT STATUS: A COMPARATIVE STUDY

Ivana Márová [1], Simona Macuchová [1], Renata Mikulíková[2], Rostislav Kotrla[3]

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[2]Research Institute of Brewing and Malting, Brno, Czech Republic ,
[3]Department of Clinical Biochemistry, Regional Hospital Kyjov, Czech Republic

In this work results of five 8-month intervention studies were compared. In these studies relation between certain complex antioxidant micronutrients intake and some parameters characterizing antioxidant and metabolic status in non-insulin dependent diabetics, patients with hyperlipidaemia and healthy subjects was evaluated. As complex antioxidant preparative various mixtures of vitamins C, E, carotenoids, bioflavonoids and PUFA were used as well as natural food supplement *Chlorella pyrenoidosa*. Levels of antioxidants and Total antioxidant status in the control groups in all studies exhibited typical seasonal differences. After 90-day of antioxidant treatment statistically significant changes in some parameters of saccharide as well as lipid metabolism (HbA1c, LDL cholesterol and TAG decrease) were observed in group of patients with diabetes and hyperlipidaemia. Complex preparative containing more types of antioxidants (e.g. combined preparatives with tocopherol, carotenoids and flavonoids) exhibited better effect than simple antioxidant trio intake. Effect of *Chlorella pyrenoidosa* was substantially lower than in preparatives containing mixtures of antioxidants. According to our findings, an optimum supplement should contain a complex mixture of naturally occurring antioxidants. The most active component that positively influences lipid and saccharide metabolism in Czech population is probably alpha-tocopherol.

L 6.4

OXIDATIVE INJURY OF SARCOPLASMIC RETICULUM (SR) FROM RABBIT SKELETAL MUSCLE AND EFFECTS OF ANTIOXIDANTS

Ľubica Horáková[1], Miriam Štrosová[1], Janka Karlovská[2], Tilman Grune[3], Pavol Balgavý[2]

[1]Institute of Experimental Pharmacology SAS, Bratislava, [2]Department of Physical Chemistry of Drugs, Faculty of Pharmacy, Comenius University, Bratislava, [3]Research Institute for Environmental Medicine, Heinrich Heine University, Düsseldorf

We studied the effects of hypochlorous acid (HOCl) and Fenton system on sarcoplasmic reticulum (SR) or pure Ca^{2+}-ATPase. HOCl induced oxidation of SR may be associated mainly with oxidation of SH groups, no protein fragmentation was observed. HOCl altered the kinetic parameters of pure enzyme with respect to free Ca^{2+} ions and ATP by decreasing V_{max} and also by modification of the regulatory binding site for ATP. The Fenton system induced fragmentation of SR Ca^{2+}-ATPase (SERCA). According to our kinetic studies, Fe decreased the affinity to both ATP binding sites.

Trolox, stobadine, EGb 761 and Pycnogenol® exerted preventive effects against oxidation of SR induced by HOCl and Fenton system. Trolox and EGb 761 had in addition protective effects on the activity of SERCA, including mainly the increase of V_{max} with respect to the concentration of free Ca^{2+} ions. Stobadine was without any effect in spite of its scavenging against HOCl. Pycnogenol® had a significant inhibitory effect on the activity of Ca^{2+}-ATPase even in the absence of oxidants. The antioxidants studied had no protective effects on the activity of Ca^{2+}-ATPase inhibited by the Fenton system. Modification of the activity of the enzyme may be caused by its structural changes associated with binding of antioxidants or their oxidative products to the enzyme.

This work was supported by APVV 51017905, VEGA 2/5012/26 and COST B35.

L 6.5

OXIDATIVE CHANGES IN RAT BRAIN AND HEART DURING AGEING

Eva Babušíková[1], Miloš Jeseňák[1,2], Jozef Hatok[1], Peter Račay[1], Zuzana Tatarková[1], Anna Drgová[1], Ján Lehotský[1], Dušan Dobrota[1], Peter Kaplán[1]

[1]Dept. of Med. Biochem., [2]Dept. of Paed., Jessenius Faculty of Medicine, Comenius University, Martin

Ageing is characterized by a progressive deterioration of physiological functions and metabolic processes. In recent years it was demonstrated that raising oxidative stress may contribute to cumulative damage to biomolecules during ageing. Little is known about mechanisms responsible for the deleterious changes seen during both normal ageing and neurodegenerative and cardiovascular diseases.

In the present study, we investigated the effect of ageing on oxidative protein damage and lipid modification in the brain homogenate, mitochondria and synaptosomes and in the heart homogenate, sarcoplasmic reticulum and mitochondria of adult, old, and senescent *Wistar* rats. The levels of dityrosine, lysine conjugates and levels of TBARS increased in brain homogenate while the levels of free SH groups were decreased. We observed increase in levels of dityrosine and lysine conjugates in synaptosomes. In the heart homogenate we observed significant changes in fluorescence of tryptophan, dityrosine, lysine conjugates and conjugated dienes. Similarly, significant oxidative modification of proteins and lipids were observed in cardiac sarcoplasmic reticulum of aged rats. These results suggest different mechanisms of oxidative modification exist in different organs and cell compartments during ageing.

Acknowledgements: Supported by project VEGA1/2263/05 and APVT-51-027404

Thursday, September 14, 2006

Lecture room L1
10:00 – 13:00

Section 5 XENOBIOCHEMISTRY III

Chairs: Pavel Anzenbacher, Július Brtko

L 5.14

CYTOCHROMES P450: FROM HISTORY TO STRUCTURE

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Cytochromes P450 (abbrev. CYP) were discovered as pigment(s) absorbing the visible light at 450 nm in the reduced state and in the presence of carbon monoxide. During years, it has been shown these enzymes form a superfamily of proteins with unique ability to bind and simultaneously activate molecular oxygen. Their newly described functions as well as the number of known substrates increase steadily for more than forty years. Although CYP enzymes possess similar properties, they differ in the properties of their active sites. The crystallographic data and the data on flexibility and dynamics of CYP enzymes seem to allow for an approximate division of known CYP enzymes according to their flexibility into two groups.

The first comprises of relatively flexible CYPs including e.g. CYP3A4, CYP2B4, CYP2E1; the second – less flexible – are represented by CYP1A2, CYP2A6. Interestingly, water molecules play an important role in the active site. As it looks now the water molecules may be relatively mobile and less ordered – for example, recent estimates give a figure of total 24 molecules present in the active site of CYP3A4. When external pressure is applied, flexible proteins with active sites full with water molecules may be more susceptible to the denaturation as according to the Pascal´s law the pressure is more effective (hydrostatic pressure is transmitted throughout the fluid). In fact, this is the case as flexible CYP enzymes with more accessible active sites (as CYP3A4) denature quite easily under pressure.

Acknowledgments. The authors acknowledge financial support from the COST B30 (OC133) and MSM 6198959216 grants from the Czech Ministry of Education and from the Grant Agency of the Czech Republic (305/06/P139).

L 5.15

CYTOCHROME P450: FROM STRUCTURE TO FUNCTION

Jiří Hudeček, Petr Hodek, Marie Stiborová

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Elucidation of the structure-function relationships is one of the main goals of the biochemical research. In the "P450 field", the functional approach dominated for long years. Due to the considerable hydrophobicity of P450 enzymes, their primary structures remained mostly unknown till the onset of cDNA sequencing in 1980`s. Also, for the most studied and important mammalian P450s, the spatial structures were unaccessible for long time and had to be supplemented by theoretical models or indirect information.

Therefore, it comes as no surprise that the appearance of the first X-ray structures of mammalian P450 enzymes during the last five years attracted so much attention within the P450 community. In addition to the "new dimension" of knowledge and insight, established by these milestone data, there is also an undeniable "aesthetic appeal" in the pictures. Definitely, the solved structures are very valuable and their existence lifted the P450 biophysics and molecular biology to a much higher level. On the other hand, there is a tendency to overestimate the "direct" structural information when confronted with other experimental data ("one picture is worth of thousands of spectra").

There are several caveats, which should not be overlooked in discussions of structure-function relationships of P450 enzymes:
(i) Crystal conditions, (ii) truncated molecules, (iii) possible postranslational modifications, (iv) effectors and flexibility, (v) interaction with the membrane, and (vi) interaction with redox partners and other membrane proteins.

The possible influence and importance of these factors will be discussed.

This work was supported by the Grant Agency of the Czech Republic (GAČR grant No. 303/06/0928)

L 5.16

MECHANISM OF CYTOCHROME P450 1A1 AND 1A2 INDUCTION BY THE ANTICANCER DRUG ELLIPTICINE

Dagmar Aimová[1], Tereza Somolová[1], Helena Dračínská[1], Radka Václavíková[2], Marie Stiborová[1]

[1]Department of Biochemistry, Faculty of Science, Charles University, Prague;
[2]National Institute of Public Health, Center of Occupational Diseases, Prague

Ellipticines are potent antineoplastic agents used in the therapy of breast cancer and leukemia. Among their cellular targets, the inhibition of topoisomerase II after intercalation into DNA was hitherto considered the most important property for its toxicity. Recently, we found that ellipticine also forms covalent DNA adducts *in vitro* and *in vivo* and that the formation of the major adduct is dependent on the activation of ellipticine by cytochromes P450 (CYP) of a 1A and 3A subfamily and peroxidases. Expression levels of these enzymes are, therefore, crucial for ellipticine pharmacological efficiencies. Here, we evaluate the potential of ellipticine to influence the expression of CYP1A1 and 1A2 in rats, the animals found to be suitable to mimic the fate of ellipticine in humans. The expression levels of hepatic CYP1A1/2 in treated rats of both sexes are more than one order of magnitude higher than those in control animals. An increase in CYP1A1/2 protein expression correlates with an increase in EROD activity, a marker for CYP1A1/2 and with the oxidation of Sudan I, a marker for the CYP1A1 activity. The increase in expression of CYP1A1 protein and its enzymatic activity caused by ellipticine also corresponds to elevated CYP1A1 mRNA levels. However, levels of CYP1A2 mRNA are unaffected. The CYP1A/2 protein induction is strongly dependent on the dose of ellipticine administrated to the rats. The results indicate that a long treatment of humans with ellipticine might stimulate its pharmacological efficiency against cancer diseases, if the CYP induction also occurs in the target organs of therapy, e.g. the breast.

Supported by the GACR (grant 303/06/0928) and Ministry of Education of the Czech Republic (grant 1M4635608802).

L 5.17

CONTROVERSIES IN THE ROLE OF p53 PROTEIN IN CELLULAR RESPONSE TO POLYCYCLIC AROMATIC HYDROCARBONS

Jan Vondráček[1,2], Soňa Marvanová[2], Eva Hrubá[2], Pavel Krčmář[2], Jiřina Zatloukalová[1], Zdeněk Andrysík[1], Alois Kozubík[1], Miroslav Machala[2]

[1]Institute of Biophysics ASCR, Brno; [2]Veterinary Research Institute, Brno

The tumor suppressor p53 is a crucial protein involved in maintenance of genome integrity, which is by a wide array of genotoxic stresses, as well as by hypoxia or oncogenic stimuli. P53 activation and its functions strongly depend upon the nature of stress signal. Polycyclic aromatic hydrocarbons (PAHs) are a group of environmental pollutants that often induce DNA damage, and many of them are able to cause p53 stabilization and accumulation. Nevertheless, the role of PAHs in p53 regulation is controversial, as they often fail to induce some of crucial p53-dependent responses, such as G1 or G2/M cell cycle arrest. Based on literary evidence and our experimental studies using various in vitro cellular models, we characterized the role of PAHs with different toxic modes of action in p53 accumulation and activation. Only a limited number of PAHs with the highest mutagenic potencies were able to induce a significant accumulation of p53 and its phosphorylation at Ser15. We therefore propose that this parameter has only a limited use as a biomarker of PAH-induced DNA damage. Only highly genotoxic PAHs also caused significant apoptosis and accumulation of cells in S-phase of cell cycle, the latter effect being significantly dependent on the type of cell line. Using cell lines with different p53 status, we present here preliminary data on the role of p53 protein in regulation of expression of various p53-regulated genes involved in DNA repair, apoptosis and cell cycle arrest, induced by PAHs. [Supported by grant No. B6004407 from the Grant Agency of the Academy of Sciences of the Czech Republic.]

L 5.18

ANTICANCER DRUG ELLIPTICINE IS CYTOTOXIC TO HUMAN LEUKEMIA AND NEUROBLASTOMA CELL LINES

Jitka Poljaková, Jan Hraběta, Tomáš Eckschlager, Eva Frei, Marie Stiborová

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Ellipticine is an alkaloid exhibiting potent antineoplastic activities. The inhibition of topoisomerase II after intercalation into DNA, was considered the most important property for its cytotoxicity. Recently, we found that ellipticine also acts as arylation agent, covalently binding to DNA *in vitro* and *in vivo*. The formation of the major DNA adduct is dependent on the activation of ellipticine by cytochrome P450 and peroxidases. Here we show that ellipticine is an agent strongly cytotoxic against human leukemia HL-60 and CCRF-CEM cells and human neuroblastoma cell lines in culture. We also demonstrate that the enzymatic systems present in the tumor cells mediate the activation of the antineoplastic agents to form two ellipticine-derived deoxyguanosine adducts in DNA. Moreover, ellipticine cytotoxicity against human leukemia HL-60 and CCRF-CEM cells correlates with formation of these DNA adducts. Because MPO and/or COX-2 are expressed in many human leukemia cells, we have studied their potential to activate ellipticine. MPO, LPO and COX-1 and -2 oxidize ellipticine to a dimer, in which two ellipticine residues are connected *via* N(6) and C(9) atoms, and ellipticine N(2)-oxide. During the ellipticine oxidation by peroxidases DNA adducts are formed. The mechanism of formation of two major adducts is proposed. Activation of ellipticine to a DNA binding species by peroxidases is an interesting finding in view of the compound's activity against human leukemia and other tumors like neuroblastoma cancer. The results obtained in this study might, moreover, be employed for development of new drugs utilizing in gene therapy and for tumor targeting.

Supported by the GACR (grant 203/06/0329) and Ministry of Education of the Czech Republic (grant 1M4635608813).

L 5.19

INOSITOL HEXAPHOSPHATE – A NOVEL ANTI-CANCER AGENT

Ladislava Schrőterová[1], Zuzana Filarová[2], Pavlína Hašková[2] Emil Rudolf[1], Miroslav Červinka[1]

[1] Faculty of Medicine in Hradec Kralove, [2]Faculty of Pharmacy
Charles University in Prague, Hradec Kralove, Czech Republic

Use of adjuvant therapy might improve to the benefit of patients suffering from cancer. Inositol hexaphosphate (IP6), also known as a phytic acid, has recently been studied for its anti-cancer properties in variety of tumor types. This natural substance can be found in legumes, corn, wheat bran etc.. The mechanisms of its action are not completely understood; however, evidence accumulates that IP6 may show anti-oxidant properties. On the other hand, some studies have shown cell cycle arrest as well as enhanced cells differentiation and apoptosis in tumor cells treated with IP6.

Due to high incidence and mortality in colorectal carcinoma in the Czech Republic we chose three colorectal cell lines with different malignant potential. We tried to find the concentration of IP6 with maximum anti-cancer effect and compare the responses of the individual used lines. Proliferation and apoptosis after IP6 treatment was assessed biochemically, using WST, BrdU, BB assays together with activity of caspase-3. Our results demonstrate the ability of IP6 to reduce the proliferation rate and increase apoptotic activity.

This work was supported by Grant Agency of Czech Republic Research Project 301/06/P047 and Ministry of Education of the Czech Republic Research Project MSM0021620820

Thursday, September 14, 2006

Lecture room L2
10:00 – 11:55

Section 7 PATHOBIOCHEMISTRY I

Chairs: Oľga Križanová, Marie Nováková

L 7.1

ISCHEMIC-REPERFUSION INJURY OF MYOCARDIUM: FROM CALCIUM PARADOX TO FREE RADICALS

Marie Nováková[1], Hana Bochořáková[2], Hana Paulová[2], and Eva Táborská[2]

[1]Department of Physiology, [2]Department of Biochemistry, Faculty of Medicine, Masaryk University in Brno, Czech Republic

Cardiac ischemia has been extensively studied for last several decades. This adverse state of myocardium is very important clinically as numerous patients in developed countries suffer from ischemic heart disease with all its consequences (e.g. myocardial infarction). Later, the crucial role of reperfusion – the period following the successful blood flow recovery, often caused by therapeutic interventions – has been widely investigated. Still, two mainstreams can be traced in the literature concerning ischemic-reperfusion injury of myocardium. One group of researchers focuses on the calcium overload phenomenon, the others study the role of reactive oxygen species (ROS) during ischemia and reperfusion. More recently, the terms preconditioning and postconditioning have been introduced and these events are intensely studied.

The methods used for studying both electrical and mechanical events on the heart are of various origin and elaborateness. In our laboratory, the registration of monophasic action potential by means of voltage-sensitive dye di-4-ANEPPS is employed. However, very little is known about the direct effect of this compound on the heart muscle. Thus, the changes in electrogram, coronary flow and production of ROS measured by HPLC were followed. These parameters were studied in two animal models – the isolated heart of guinea pig and of rabbit. We may conclude that rabbit heart is a suitable model for studying ischemia-reperfusion injury at the organ level by voltage-sensitive dyes.

Supported by the project MSM 0021622402 of the Ministry of Education of the Czech Republic.

L 7.2

IP3 RECEPTORS AND THEIR MODULATION

Ol'ga Križanová

Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia

Inositol 1,4,5-trisphosphate (IP3) receptors are intracellular calcium channels able to release calcium from intracellular stores. They are activated upon binding of IP3 and calcium. Up to now, three types of IP3 receptors were identified, differing in their localization, sequence and probably also function. Type 1 IP3 receptor predominates in neuronal cells, where occurs in two alternatively spliced variants – longer neuronal and shorter non-neuronal form. Type 2 IP3 receptor is quite abundant in cardiomyocytes and the type 3 IP3 receptor in kidney. Depending on the tissue, IP3 receptors form homotetramers and heterotetrames. Modulation of the gene expression of IP3 receptors is affected by variety of transcription factors through the corresponding responsive elements localized in the promoter regions of genes coding these channels. Upregulation of the gene expression of IP3 receptors occurs in pathophysiological state of organism (e.g. stress) or during development of some diseases (cardiovascular, neurodegenerative, etc.). In this process, some distinct regulators (e.g. glucocorticoids) are involved.

Physiological relevance of IP3 receptors, especially individual types of IP3 receptors is not fully elucidated yet. Generally, it was proposed that IP3 receptors might be involved in the process of apoptosis, cell proliferation, etc. Precise understanding of the role of IP3 receptors during development of pathological state of organism and mechanism of this regulation can help to develop proper treatment and also to prevent detrimental consequences of pathological state.

This work was supported by grants VEGA 2/6078, APVT 51-027-404 and Genomika SP 5151/0280800/0280802.

L 7.3

HYPOXIA MODULATES GENE EXPRESSION OF THE IP_3 RECEPTORS IN MOUSE CEREBELLUM

Dana Jurkovičová[1], Juraj Kopáček[2], Peter Štefánik[1], Lucia Kubovčáková[3], Silvia Pastoreková[2], Ol'ga Križanová[1]

[1]Institute of Molecular Physiology and Genetics, [2]Institute of Virology and [3]Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia

Neurons are generally considered as one of the most hypoxia sensitive cells. The most tolerant neurons are able to recover fully, when oxygen becomes available. The fate of hypoxic or ischemic neurons depends upon both physiological and molecular events, with pro-survival and apoptotic pathways competing at the transcriptional and posttranscriptional level.

Hypoxic brain cell injury is a complex process that results from a series of intracellular events. Since hypoxia is known to induce an increase in calcium concentration, in this work we tested, whether severe hypoxia for 6 hours can affect gene expression and protein levels of intracellular calcium channels - ryanodine receptors (RyR) and inositol 1,4,5-trisphosphate receptors (IP_3R) in cerebellum of mouse. Gene expression and protein level of the type 1 and 2 IP3 receptors was significantly increased after the exposure of mice to hypoxic stimulus. Ryanodine receptors of type 1 and 2 and also SERCA 2 were not affected by hypoxia. Our results suggest the involvement of IP3 receptors in hypoxic injury. Our preliminary results show that 48-hour hypoxia resulted in delayed and slower outgrowth of the axons in cerebellar primary cultures. Deeper understanding of physiological consequences and mechanisms of regulation of intracellular calcium could point to the way towards the development of new therapeutic approaches to reduce or suppress the pathological effects of cellular hypoxia, such as those seen in stroke or ischemia or neuronal disorders.

This work was supported by grants VEGA 2/6078 and APVT 51-027-404.

L 7.4

DIVERSE FORMS OF INTRACELLULAR CALCIUM STORES: IMPLICATION FOR THE DISEASED CELLULAR STATES

Ján Lehotský, Peter Kaplán, Martina Pavlíková, Peter Urban, Radovan Murín, Eva Babušíková, Zuzana Tatarková, Dušan Dobrota

Jessenius Fac. Med., Comenius Univ., Dpt. Med. Biochem., Martin, Slovakia

Neural cells are endowed with an ordered intracellular membrane organelles which are characterized by a high luminal Ca^{2+} concentration. The view that both the endoplasmic reticulum (ER) and the Golgi apparatus (GA) act as Ca^{2+} signalling compartments triggering cytosolic processes upon release, and Ca^{2+} is also cofactor of luminal maturation processes is rather new. On the tissue level, the complex responses have evolved in response to disturbances of ER function which have a great impact to intimate cross-talk between ER/GA/mitochondria and finally to neural viability. Due to the high susceptibility to free radicals, damage to the ER is one of the facets of ER disturbances and are thought to be involved in neuronal damage including ischemia/hypoxia. Perturbation of ER homeostasis affects protein folding, dysregulates Ca^{2+} balance and causes ER stress. Despite of the vital importance of the ER for cellular functions, surprisingly fragmented knowledge exists about its luminal state and ER redox balance. We describe here an ischemia/reperfusion (IRI) (4 vessel occlusion /reperfusion in rats) induced modulation of ER facing membrane and lumenal proteins and lipids. The IRI induced both the changes in the early gene expression and the post-translational protein modification. Novel role of Golgi Ca^{2+} pump might be included in intracellular calcium signalling and cellular resistance to ischemic stress. Alterations between intracellular calcium stores function might be an alternative pathway which control neuronal loss after injury.

Supported by : VEGA 3380/06 , COST B30, MVTS 39 and Grant UK 250/06, 256/06.

L 7.5

EXPRESSION OF GOLGI SPCA1 CA2+ PUMP IN RAT NEURONAL TISSUE: EFFECT OF ISCHEMIC/REPERFUSION INSULT

Martina Pavlíková, Peter Urban, Radovan Murín, Dušan Dobrota, Ján Lehotský

Jessenius Fac. Med., Comenius Univ., Dpt. Med. Biochem., Martin, Slovakia

The neural cells of brain have important secretory functions. They secrete many neurotransmitters and secretory proteins necessary for growth and reorganization of neuronal circuits. The Golgi apparatus as a newly recognized Ca^{2+} store regulates this secretion by secretory Ca^{2+}/Mn^{2+} ATPases (SPCA1/SPCA2). While the presence of SPCA1 in the brain has already been shown, the cell-type specific expression pattern has not been established. Forebrain ischemia/reperfusion initiates cellular catastrophic cascade in which many subcellular organelles play important role. We investigated the presence and distribution of the SPCA1 pump protein in both the rat brain and the cell cultures of neurons. Experiments show that SPCA1 pump protein in neural cells is localized to structures distinct from endoplasmic reticulum. In addition, we have analyzed level of mRNA expression in *in vivo* condition after ischemia/reperfusion insult by RT-PCR in cortical brain area of rat. Forebrain ischemia was initiated by four vessel occlusion, 15 min and reperfusion for 1h, 3h and 24 h (IRI). We present data on expression mRNA pattern of SPCA1 gene after IRI. RT-PCR analysis clearly detected expression of SPCA1 gene in injured area after ischemic/reperfusion insult. In addition, mRNA expression pattern follows time dependent manner in reperfusion period. Since the pump plays major role in the refilling of Ca^{2+} stores, we disscuss here it possible contribution to altered intracellular Ca^{2+} signaling in neural cells in ischemia/reperfusion event.

Supported by : VEGA 3380/06 , COST B30, MVTS 39 AND Grant UK 250/06, 256/06.

Thursday, September 14, 2006

Lecture room L3
10:00 – 12:30

Section 4 CELL REGULATIONS AND TRANSFER OF SIGNALS I

Chairs: Ján Kormanec, Radim Černý

L 4.1

HYPOXIA SIGNALING IN HEALTH AND DISEASE – BOTH SIDES OF STORY

Silvia Pastoreková, Juraj Kopáček, & Jaromír Pastorek

Institute of Virology, Slovak Academy of Sciences, Bratislava

Decreased oxygen supply (hypoxia) induces whole spectrum of molecular responses that significantly influence cell behavior and viability in different physiological and pathological situations including embryonic development, ischemia, arthritis and cancer. Hypoxia signaling is principally mediated by hypoxia-induced factors (HIFs), which transcriptionally activate several dozens of genes involved in adaptive processes and life/death decisions. In addition, hypoxia modulates translation and functional capabilities of relevant proteins. The outcome of this signaling can be either beneficial or damaging for the organism depending on the particular changes in the transcriptome, proteome and metabolome of hypoxic cells. Deep understanding of molecular mechanisms that regulate and execute hypoxia signaling thus offers new opportunities for disease-tailored therapeutic interventions.

L 4.2

ECTOPIC EXPRESSION OF ENAMEL MATRIX PROTEINS ?

Radana Vrzáková, Radim Černý

Department of Biochemistry, Charles University, Faculty of Medicine in Plzeň

There are two principle types of mineralized tissues – bone and dentin on one side and enamel on the other side. Protein matrix of bone and dentin is based on collagen while enamel protein matrix is formed by different specialized proteins, namely amelogenin, ameloblastin, enamelin, etc. This is obviously connected with the origin of individual tissues. Bone and dentin are produced by mesenchyme-derived cells while enamel matrix is formed by ameloblasts – cells derived from oral and, therefore, it is of ectodermal origin. Tooth root is also built from dentin but it is covered with cementum, poorly understood bone-like hard tissue. The roots are also partially covered by Hertwig's epithelial root sheaths which are the remnants of the same oral epithelium that differentiated into ameloblasts.

There were several attempts to detect the expression of enamel specific proteins outside the ameloblasts. The last conclusion based on immunodetection in electron microscopy of rat models is that amelogenin but not ameloblastin was produced in epithelial root sheath.

We have analyzed 20 extracted human teeth, all fully developed, by means of RT-PCR for the presence of different mRNA related to the production of both, enamel-specific and mesenchymal proteins. We detect both, amelogenin and ameloblastin mRNAs in total RNA isolated from the extracted teeth. The specificity of the obtained PCR products was verified by DNA sequencing. We do not have the evidence concerning the cellular origin of the amelogenin and ameloblastin mRNA, however, the epithelial root sheaths are the main candidates. The number of additional mRNAs specific for hard tissue was also detected.

L 4.3

SYSTEMIC RESISTANCE OF PLANTS, THEORY VS. PRACTICE

Vladimír Mikeš, Tomáš Kašparovský, Ján Lochman

Department of Biochemistry, Faculty of Science, Masaryk University, Brno, Czech Republic

Plants react to pathogen attack through complex adaptive responses. Some of the responses are constitutive and pathogen non-specific, but a majority of them are induced after recognition of some feature of the pathogen. Several alterations in cellular metabolism occur as an outcome of pathogen interaction. The defense responses are expressed through accumulation of downstream signaling molecules which in turn regulate expression of defense through cell wall strengthening, lignification, phytoalexin synthesis and expression of localized cell death. In addition, this set of events leads to the expression of induced resistance. Based on differences in signaling pathways and spectra of effectiveness, different types of induced resistance have been defined. The classic form of induced resistance is referred as systemic acquired resistance (SAR) and takes place in distal plant parts upon localized infection by a pathogen. Accumulation of pathogenesis-related proteins occurs directly upon induction of SAR.

Many studies have focused on the mechanisms of positive regulation regarding the expression of defense genes in plants. Thus, the simplest strategy includes the application of elicitors (alginates, carrageenans, fucans, laminarin, chitosan, etc.) or signaling molecules in agriculture, which can provide an alternative strategy in crop protection to reduce or replace the overwhelming application of chemical pesticides. This paper aims to compare the advantage of these methods and their practical impacts.

L 4.4

SECRETION OF CYTOKINES IN BREAST CANCER –MOLECULAR MECHANISM OF PROCATHEPSIN D MITOGENICITY

Martin Fusek[1], Jana Větvičková[2], Václav Větvička[2]

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Procathepsin D (pCD) is one of major secreted proteins in estrogen receptor positive breast cancer cell lines. The secreted pCD has mitogenic effects on many breast, lung and prostate cancer derived cell lines. It was also shown that the proliferative effect of pCD involves both autocrine and paracrine modes of action. Recent studies have suggested that pCD could act as a key paracrine communicator between cancer cells and stromal cells. The proliferative activity of pCD resides within the sequence of the activation peptide of pCD. In our present study we have focused on the mechanism by which procathepsin D influences the proliferation of cancer cells. We have used protein array profiling of secreted cytokines following the addition of pCD to breast cancer cells. Our results clearly show that pCD initiates secretion of cytokines IL-4, IL-8, IL-10, IL-13 and MIP-1β from cell lines ZR-75-1 and MCF7. Secreted cytokines take part in the proliferation of the cancer cells as proved by selective inhibition using antibodies. Analogous pattern was observed also for fibroblasts, cells, which are *in vivo* in close contact with the tumor tissue. Co-culture experiments are in agreement with this observation. Together with previous *in vivo* data these results point to pCD as to one of key candidates for therapeutic attack in breast cancer.

L 4.5

THE REGULATION OF PRODUCTION OF POLYKETIDE ANTIBIOTIC AURICIN IN *S. AUREOFACIENS*.

***Renata Nováková*, Jana Bistaková, Ľubomíra Fecková, Ján Kormanec**

Institute of Molecular Biology, Centre of Excellence for Molecular Medicine, Slovak Academy of Sciences, Bratislava

Streptomyces are Gram-positive bacteria, which have been extensively used in the pharmaceutical industry for the past decades as a source of bioactive natural products. Many of them are clinically important products belonging to the aromatic polyketides. These secondary metabolites are usually synthetized by type II polyketide synthase (PKS). We have identified a gene cluster encoding an aromatic type II PKS in *Streptomyces aureofaciens* CCM3239. Based on sequence comparison with known polyketide clusters such that for jadomycin, urdamycin and landomycin, we supposed that this gene cluster, named *aur1*, contained biosynthetic genes for the angucycline-like polyketide, auricin. The auricin has been detected in the strain grown on solid Bennet medium, but its purification by HPLC for structural analysis has been hampered by very low yields as for other homologous angucycline clusters. It seems that these PKS clusters are very tightly regulated and investigation of the regulatory mechanisms may help to increase production of secondary metabolites. In *Streptomyces*, antibiotic gene clusters are usually regulated by pathway-specific transcriptional activators that are located in these clusters. We have identified several regulatory genes, acting negatively or positively on the production of auricin.

Acknowledgement. This work was supported by the VEGA grant 2/6010/26 from Slovak Academy of Sciences.

Thursday, September 14, 2006

Lecture room L4
10:00 – 12:35

Section 6 FREE RADICALS IN BIOLOGY AND MEDICINE II

Chairs: Ľubica Horáková, Attila Ziegelhőffer

L 6.6

FUNCTIONAL REMODELING OF THE DIABETIC MYOCARDIUM: THE ROLE OF FREE RADICALS

Attila Ziegelhöffer[1], Miroslav Ferko[1], Iveta Waczulíková[2], Jana Mujkošová[1], Terézia Holotňáková[3], Jaromír Pastorek[3], Silvia Pastoreková[3], Jozef Čársky[4]

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Background: Administration of streptozotocin (STZ) to rats induces parallelly to diabetes (DIA) also endogenous mechanisms of protection (EMP) which yield in functional remodeling (FURM) of subcellular structures and finally in certain degree of adaptation of the myocardium (MYO) to DIA. **Aims:** i) Study the effect of free radicals (FR) in FURM of MYO sarcolemma (SL) and mitochondria (MIT); ii) Elucidate the role that may play FURM in keeping the DIA MYO functioning; iii) Reveal the possible role of FR in modulation of the hypoxic genes and their involvement in adaptation of DIA MYO working in conditions of pseudo hypoxia (PHY). **Materials and Methods:** Adult male wistar rats, STZ 65 mg/kg b. wt., i. p., 8 days. Investigated: the metabolism, function of SL and MIT, cell signaling, regulation of hypoxic genes expression. **Results & Discussion:** In SL, FURM is represented by decreased transduction of sympathetic signals and cations, due to decreased membrane fluidity (MF, all $p<0.05$-0.01) caused by non-enzymatic glycation of proteins and FR. In MIT, FURM is represented by decrease in O_2 consumption and in capacitity of oxidative phosphorylation – ascribed to FR and PHY. However, this damage is not extended to FR-induced oxidation of MIT membrane lipids (MF is increased, $p<0.01$) and it is compensated considerably by enhanced transport of energy through the MIT membrane. Moreover, FR seem to be involved in regulation of hypoxic genes, particularly that of CA 9 expression. **Conclusion:** FR-induced changes in acute DIA MYO may not be considered as destructive unambiguously. Grants: VEGA 2/5110/25, APVT 51-027404, 51-017902, SP51/0280901 and SP51/0280902.

L 6.7

MYELOPEROXIDASE BINDING TO EXTRACELLULAR MATRIX

Lukáš Kubala[1], Stephan Baldus[2], Antonín Lojek[1] and Jason P. Eiserich[3]

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Myeloperoxidase (MPO) is a highly cationic hemoprotein abundantly expressed by phagocytes that plays important roles in tissue injury associated with acute and chronic inflammation. Following secretion from activated leukocytes, MPO binds to endothelial cells and undergoes transcytotic migration to the underlying extracellular matrix (ECM) where it catalyzes various oxidative reactions and compromises vascular nitric oxide (NO) signaling. However, the molecular mechanisms governing the binding of MPO to ECM proteins, and the effect this has on its enzymatic functions remains poorly characterized. Herein, we demonstrate that MPO avidly binds to cell-derived and purified ECM proteins. ECM derived from various cell types facilitates binding of MPO, which was enhanced by preincubation of isolated ECM proteins with heparin, but not chondroitin sulfate. In contrast, excess heparin or chondroitin sulfate present in the exposure medium inhibits binding of MPO to cell-derived ECM proteins. Studies utilizing ECM derived from wild-type and mutant Chinese hamster ovary cells revealed that MPO preferentially binds to heparan- and chondroitin-proteoglycans. When incubated with fibronectin or collagen, the oxidizing potential of MPO is enhanced, but its capacity to consume NO remained unchanged. Collectively, our data reveal that MPO is sequestered within ECM proteins in glycosaminoglycan-dependent manner, and that the enzymatic activity of MPO is increased when associated with ECM proteins.

L 6.8

URIC ACID STIMULATES SUPEROXIDE PRODUCTION BY NAD(P)H OXIDASE OF VASCULAR SMOOTH MUSCLE CELLS

Martin Vejražka, Marek Grega, Stanislav Štípek

Institute of Medical Biochemistry, 1st Faculty of Medicine,
Charles University in Prague

Hyperuricaemia is associated with cardiovascular disease and arterial hypertension. The exact pathogenesis remains, however, unclear. Increased concentration of uric acid (UA) decreases nitric oxide NO, an important vasodilatating agent. Possible mechanism could be overproduction of superoxide $O_2\cdot^-$ in vascular wall: $O_2\cdot^-$ rapidly reacts with NO inactivating its vasodilatating function. NAD(P)H oxidases are considered the most important source of $O_2\cdot^-$ in vessel wall. We examined the effect of UA on NAD(P)H oxidase activity in vascular smooth muscle cells (VSMC).

Cultured VSMC were originally isolated from aortas of normotensive (Wistar) and spontaneously hypertensive (SHR) rat. Activity of NAD(P)H oxidase was measured in isolated plasma membranes using water soluble tetrazolium WST-1 in presence of NADH or NADPH. In normotensive VSMC, UA (1 mM) stimulated both NADH and NADPH oxidase compared to controls (190%, $p<0.01$ and 140%, $p<0.05$, respectively). In SHR the pattern differed: basal NADH oxidase activity was similar to normotensive VSMC; however, it has not increased after UA treatment. On the other hand, basal NADPH oxidase activity of SHR was the same as in UA-stimulated normotensive VSMC and was not further increased by UA. These findings strongly suggest that UA-caused overproduction of $O_2\cdot^-$ by VSMC could play a role in pathogenesis of arterial hypertension and cardiovascular disease. Regulation of NAD(P)H oxidase activity is altered in SHR. *Supported by MSM 002162080.*

L 6.9
CELLULAR INTERNALIZATION MECHANISMS AND INTRACELLULAR LOCALIZATION OF HYPERICIN IN GLIOMA CELL LINE U-87 MG

Slávka Kaščáková[1,2], Anton Mateašik[3], Matthieu Refregiers[2,4], Daniel Jancura[1], Jean-Claude Maurizot[2] and Pavol Miškovský[1,3]

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Using methods of fluorescence spectroscopy and microscopy we have investigated uptake and the internalization mechanisms of hypericin (Hyp), a natural photosensitizing agent occurring in plants of the genus *Hypericum*, in glioma cell line U-87 MG as the function of the presence of different transport serum proteins in cultivation medium. The results show that the accumulation of Hyp by U-87 MG in the presence of LDL is proportional to the Hyp/LDL molar ratio. This indicates that the amount and aggregation state of Hyp inside LDL do not influence the ability of LDL to be internalized into cells.

We have shown that activation of LDL receptors leads to substantial increase of Hyp uptake by U-87 MG in the presence of LDL. It means that LDL particles seem to be effective transporters of hydrophobic Hyp to cells through LDL receptor pathway. Moreover, the colocalization experiments revealed the lysosomal-targeting mechanism of the uptake of Hyp-LDL complex.

Our results also suggest that the internalisation of Hyp into U-87 MG cells seems to be the result of different mechanisms depending on the composition of the cultivation medium. We detected differences in the intracellular concentration as well as in the time dependence of the uptake of Hyp in the presence of different serum proteins in the medium.

ACKNOWLEDGEMENT

This work was supported by the Scientific Grant Agency of the Ministry of Education of Slovak Republic under the grant VEGA No. 2265 and by the Slovak Science and Technology Assistance Agency under the contract APVT-20-036104.

L 6.10

THE DIVERSE MECHANISMS OF PLANT POLYPHENOL EFFECTS IN THE PREVENTION OF VASCULAR DISEASES.

***Alexey Kondrashov*[1]**

[1]First Faculty of Medicine, Charles University, Prague

A growing number of studies clearly establish the efficacy of diet rich in phytochemicals in reducing the risk of cardiovascular disorders, high cholesterol and cancer. Plant polyphenols exert multiple beneficial effects toward vascular disorders. The antioxidant properties of plant polyphenols are related to both their scavenging effect, e.g. protection of LDL and VLDL from oxidation and their capacity to suppress inflammation. The molecular bases of anti-inflammatory effects of polyphenols have been linked to inhibition of nuclear factor κB (NF-κB), which governs the regulation of cytokine release and response. Polyphenols inhibit the production of pro-inflammatory cytokines interleukin -1 (IL-1), and IL-6 by human blood mononuclear cells as well as cytokines from helper T-cells, such as IL-2 and interferon gamma (INFγ) by Th1 cells and IL-4, IL-5 by Th2 cells.

Some polyphenols (phytoestrogens) have estrogen-like effects and/or inhibit tyrosine kinases. Phytoestrogens bind to steroid receptors and supposed to have the direct inhibitory effect on activity of human 17β-hydroxysteroid dehydrogenase type 5 (17β-HSD 5) a key enzyme in the metabolism of estrogens and androgenes. This effect contributes to the cancer prevention. Many phytoestrogens possess antiproliferative properties due to inhibition of protein kinases, a key enzymes for the stimulation of cell growth.

Recent in vitro and in vivo studies have confirmed the action of soy isoflavones on nuclear receptors known as peroxisome proliferator activated receptors (PPARs) similarly to antidiabetic drugs fibrates and glitazones.Polyphenols are thought to contribute to lipolytic process by possible action of isoflavones similar to the nonestrogenic ligands that bind the estrogen-related receptors (ERRs). Isoflavones could also activate thyroid receptors that provide additional clues explaining their metabolic action. Plant polyphenols can inhibit platelet aggregation and adhesion, inhibit enzymes involved in lipid metabolism and induce endothelium-dependent vasodilatation. These are thought to be the mechanisms whereby plant polyphenols significantly improved endothelial function.

Various plant polyphenols appear to increase the activity of phase II detoxification enzymes, such as quinone reductase and glutathione S-transferase a candidates for cancer prevention. Plant polyphenols also have hypolipemic activity. These compounds have the ability to lower cholesterol in hypercholesterolemic animal models and in human hepatoma cell line by the direct reduction of overall apolipoprotein B (apoB) secretion, the structural protein of LDL. This suggests that polyphenols found in food sources exert cardioprotective effects by several mechanisms described above.

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Thursday, September 14, 2006

Lecture room L5
10:00 – 13:00

Section 8 BIOTECHNOLOGY

Chairs: Jozef Timko, Jaroslav Spížek

L 8.1

BIOTECHNOLOGY FOR PRODUCTION OF SECONDARY METABOLITES

Jaroslav Spížek[1], Jiří Janata[1], Markéta Jelínková[1], Jan Kopecký[1], Jitka Novotná[1]

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It has been more than half a century since natural low molecular weight compounds were first introduced into clinical use for the treatment of infectious diseases followed by dramatic reductions in mortality and morbidity of infectious diseases. Unfortunately, there has been a corresponding and dramatic increase in antibiotic resistance during the same period and, thus, it appears that many common infections are becoming, once again, untreatable. The appearance of resistant strains was originally not expected and has been the result of a series of microbial genetic events which are still not well understood.

Several approaches are presently used to find new antimicrobials. They include search for new targets in pathogenic bacteria based on genome sequencing, production of new protective vaccines, the use of probiotics to increase immunological response, intensive search for new producers of biologically active compounds including non-cultured microbial species, production of new hybrid compounds, mainly against resistant bacteria, and chemical transformation and biotransformation using intact cells or isolated enzymes. The knowledge of gene clusters specifying biosynthesis of antibiotics and of their protein products makes it possible to design new antibiotics or new derivatives of known antibiotics with better biological activity or improved pharmacological properties. Many tools of modern biotechnology derive largely from studies of antibiotic resistance mechanisms. The challenge of drug resistance may well be met by the application of this knowledge to our advantage.

L 8.2

PRODUCTION OF HUMAN RECOMBINANT ANTIMICROBIAL PEPTIDES

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Antimicrobial peptides play a significant role in innate and adaptive immunity, and are particularly important in the first line of defense against microbial invaders. These mostly cationic peptides have a broad-spectrum antimicrobial activity, kill invaders rapidly by interaction with the membrane, they are synergistic with endogenous antimicrobial proteins and classical antibiotics, neutralize endotoxins, and have multiple roles as immunomodulators.

We present the production and isolation of an active 48 amino acid recombinant dermcidin variant and 43 amino acid recombinant human beta-defensin-1 using a specific pLMM1-rhBD-1 expression system in *Escherichia coli.*

L 8.3

A KEY ROLE OF HIGHPRODUCING STRAINS OF MICROORGANISMS IN BIOTECHNOLOGY

***Gabriela Borošová*[1]**

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Production strains of microorganisms play a key role in the biotechnological method of the target metabolites production which is non-replaceable. The screening and selection of production strains are on the early beginning of the potential success how to achieve the biologically active substance. The development of high producing strains for the production of desired metabolite is only route to succeed in the technical realization of the project as well as the economical profitability of it.

Several potential approaches are presently used to reach these objectives. They include the classical methods of the selection and further testing of mono-colony isolates, their treatment with chemical and physical mutagens, furthermore methods of the transformation of production strains with the utilization of molecular biology tools and methods, as well as the combination of aforesaid procedures. The significant advance in the strain development process enables the most exact knowledge of the biosynthetic route of given metabolite including the identification of genes responsible for the synthesis of key enzymes.

L 8.4

PHYTOREMEDIATION OF NITROESTERS.

Radka Podlipná, ***Zuzana Vavříková, Tomáš Vaněk***

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ABSTRACT

Nitroglycerin is a highly toxic substance used as an energetic plasticizer for both gun and rocket propellants. In the past, destruction of theses materials has been accomplished by open air burning or detonation and more recently by incineration. Phytoremediation is the use of vegetation for *in-situ* treatment of hazardous wastes. It is a form of ecological engineering that has proven effective and relatively inexpensive at several pilot and full-scale sites. Plants have shown the ability to withstand relatively high concentrations of organic pollutants, and they can uptake certain chemicals quickly and convert them to less toxic metabolites. In addition, they stimulate the degradation of organic chemicals in the rhizosphere by the release of root exudates, enzymes, and the build-up of organic carbon in the soil. In the cooperation with some explosive factory we are studying the possibility of cleaning up the wastewater in constructed wetland. The small models of wetlands were planted with selected helophytes. We will present the results collected during last two years in comparison with the experiments in the laboratory scale.

Acknowledgements : this work was supported by projects 1P05OC042 and 1P0ME730

L 8.5

MOLECULAR METHODS FOR DETECTION OF DNA VARAIBILITY IN CLOSELY RELATIVE SALMONELLA STRAINS

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Infections caused by *Salmonella enterica* constitute a major part of food-born diseases and evoke serious problems in human as well as veterinary medicine. Although the genus *Salmonella* includes more than 2 thousand different serovars, only a limited number of serovars, including Enteritidis and Typhimurium, has been frequently identified in infections. For epidemiological studies and for evaluation of the strain virulence, it is necessary to discriminate between relative and non-relative strains belonging to the same serovar.

The aim of the work was to study genotype differences in *Salmonella* Typhimurium strains using several molecular methods. The strain relatedness at the whole genome level was monitored by AFLP, which combines the restriction endonuclease cleavage of chromosomal DNA with amplification of resulting fragments by PCR at stringent conditions. The multilocus variable number tandem repeat analysis (MLVA) was used for typing of strains belonging to same phage type. Based on the allelic profiles of five loci, high discrimination was obtained in MLVA. Lysogenic bacteriophages integrated into bacterial chromosome are source of strain to strain variability and can participate on horizontal gene transfer between salmonella strains. In our study, the presence of selected genes from lysogenic bacteriophages (P22, ST104, Gifsy-1, Gifsy-2, Fels-1 and SopEfi) was monitored in salmonella strains. The high variability in gene occurrence was observed.

In concussion, similar grouping of salmonella strains was observed in case of all the employed DNA techniques and results corresponded well with the phage type and antimicrobial resistance of strains. The highest discriminating power was achieved with use of the MLVA, yet the AFLP, and PCR-phage typing also proved to be valuable in typing the *S.* Typhimurium strains.

Friday, September 15, 2006

Lecture room L1
10:00 – 12:55

Section 2 PROTEOMICS AND ENZYMOLOGY I

Chairs: Lenka Hernychová, Ľudovít Škultéty

L 2.1

CHARACTERIZATION OF SELECTED BIOLOGICAL WARFARE AGENT PROTEOMES USING MASS SPECTROMETRY

Lenka Hernychová[1] and Ľudovít Škultéty[2]

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The threat of bioterrorism, long ignored and denied, has heightened over the past few years and it represents a major security problem to deal with. National and international organizations have created rules concerned the public health emergencies. Thus the unambiguous detection and identification of biological warfare (BW) agents became extremely important. Proteomic methods based on mass spectrometry (MS) are promising tools for obtaining data in this respect.

We have analyzed different bacterial organisms belonging to the list of BW agents. Our investigations have focused on the both reliable protein identification of whole bacterial lysates as resolved by two-dimensional electrophoresis (2-DE) or liquid chromatography and fishing for specific bacterial biomarkers. The proteins separated by 2-DE were either subjected to identification based on peptide mass fingerprinting (MALDI-TOF instrument) or the MS/MS approach (Q-TOF and/or MALDI-TOF/TOF instruments). Furthermore, in order to find unique peaks of protein biomarkers, we have prepared acetonitrile extracts from these bacteria and subjected them to MALDI-TOF/MS and/or SELDI MS analysis.

These results will serve for screening of immunodominant and/or immunoprotective bacterial proteins that might be used in diagnosis or vaccine development. In addition they might be also utilized for both rapid detection, identification and typing of the microbial taxons.

Supported by OBVLAJEP20031, OBVLAJEP20033, APVT-51-032804 and VEGA5030/25.

L 2.2

CHANGES IN THE URINARY PROTEOMES IN PATIENTS WITH NEPHROTIC SYNDROM

Lucie Vojtová[1], Tomáš Zima[1], Vladimír Tesař[2], Markéta Kazderová[2]

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Heavy proteinuria is caused by increased glomerulal basement membrane permeability and membrane or podocytes damage. There isn´t known an accurate composition of modified or degraded urine proteins in proteinuria. However, it is assumed possible proteins´ toxic effect on tubular cells and disease progression. In this study, forty urine samples of patients with nephrotic syndrome and other nephropathy (amyloidose AL, Wegener-granulomatose, membranous nephropathy) were analyzed by 2D electrophoresis method. We have studied the albumin´s separation effect on samples analyze. The study was also focused on comparison of the urinary proteomes and on finding the specific diagnosis proteins.

In the first step, the proteins were devided by isoelectric focusing method using polyacrylamide strips (pH 3-10 linear). The second dimensional SDS electrophoresis was performed in 12 % polyacrylamide gel, which separate proteins according to their molecular weight. The proteins were visualized by silver method. The gels were evaluated by Phoretix 2D expression software.

Albumin´s separation caused the higher lucidity of the urinary proteomes and higher proteins resolution in area with molecular weight > 30 kDa. The urinary maps comparison brought out that there are some proteins´ changes, which are typical for specific disease.

The 2D electrophoresis for patients´samples with nephrotic syndrom was optimized. We found out that albumin´s separation by ammonium sulfate caused the higher lucidity of proteins spectrum and that some proteins could be diagnostic important. *This study is supported by MZ ČR VFN č. 64165 and GA UK č. 203434/54.*

L 2.3

ANTIBODIES SPECIFIC FOR INFLUENZA A VIRUS PROTEIN PB1-F2 ARE PRESENT IN HUMAN CONVALESCENT SERA

Hana Pančuchárová[1], Ingrid Krejnusová[1], Magdaléna Bystrická[1], Hana Blaškovičová[2], Gustáv Russ[1]

[1]Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovak Republic, [2]Public Health Authority of the Slovak Republic, National Influenza Centre, Bratislava, Slovak Republic

The PB1-F2 protein of influenza A virus (IAV) is encoded by an alternative ORF (+1) in the highly conserved PB1 gene. We hypothesize that cells infected with IAV may release PB1-F2 that kills uninfected cells, mostly immune cells recruited to the site of infection. Accordingly, anti-PB1-F2 antibodies are induced and these antibodies might in turn neutralize PB1-F2 cytotoxicity. To investigate whether antibodies specific for PB1-F2 are induced following influenza A virus infection we analyzed acute and convalescent serum samples from patients with clinical influenza during winter season of 2003/2004. All convalescent sera exhibited a significant rise in anti-IAV antibody titers as assayed by ELISA. The reactivity of these sera was further tested with synthetic full length PB1–F2 protein and peptide fragments representing different regions of PB1–F2. Altogether, 20 paired samples were tested and a rise in PB1-F2 antibodies was confirmed in 40% of infected inviduals. The antibodies present in human sera reacted well with all synthetic peptides; nevertheless the binding to peptide corresponding to N-terminus (1-16) was significantly reduced. Importantly, the specificity of PB1-F2 antibodies was also confirmed by immunoprecipitation and by indirect immunofluorescence.

So far our experiments did not indicate any biological significance / role for the PB1-F2 antibodies present in human sera.

L 2.4

MASS SPECTROMETRY ANALYSIS OF THE GLYCOSPHINGOLIPID-ENRICHED MICRODOMAINS OF RAT NATURAL KILLER CELLS

***Petr Man*[1,2], *Petr Novák*[1,2], *Marek Cebecauer*[3], *Ondřej Horváth*[1], *Anna Fišerová*[1], *Vladimír Havlíček*[1], *Karel Bezouška*[1,2]**

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Natural killer (NK) cells are cytotoxic effector lymphocytes which do not express antigen-specific surface receptors. NK receptors that mediate activation signals leading to the initiation of natural cytotoxicity may be associated with membrane microdomains (MM). Objective of the study was to characterize proteins in MM of rat NK cells. Methods were based on a shotgun strategy for full mapping and characterization of MM from the rat NK cell leukemia RNK-16. Plasmatic membranes prepared by hypotonic procedure were lysed in detergents and extracts subjected to sucrose gradient centrifugation under various conditions. Each fraction was analyzed by μLC-MS/MS and Western blotting. Results indicate that we were successful in identifying unambiguously up to 250 proteins in each density gradient fraction separated from MM of rat NK cells. The first study of the proteome of NK cell MM brought several surprising findings including identification of molecules not expected to be expressed in rat NK cells (e.g. NAP-22), or associated with MM (e.g. NKR-P1, CD2, CD45). It also provided clear data consolidating controversial views on the occurence of MHC glycoproteins and RT6.1/CD38/CD73 complex in NK cells. A large number of new candidate receptors has also been identified. In conclusion, shotgun proteomic strategy has proved successful for identification of proteins associated with membrane microdomains of NK cells.

This work was supported by Ministry of Education of Czech Republic (MSM 21620808), by Institutional Research Concept (AVOZ5020903), by Czech Grant Agency (204/06/0771), and by Grant Agency of Academy of Sciences (A5020403).

Reference*:* Man P. et al. (2005) *Proteomics* 5, 113-122.

L 2.5

OPTICAL SPECTROSCOPY, MASS SPECTROMETRY AND NMR ARE ESSENTIAL TOOLS IN THE PRODUCTION OF SOLUBLE RECEPTORS OF NATURAL KILLER CELLS

Ľuboslav Mihók[1,2], Daniel Kavan[1,2], Ondřej Vaněk[1], Petr Pompach[1,2], Jan Bílý[1], Petr Novák[2], Kateřina Hofbauerová[2,3], Vladimír Kopecký, Jr.[3], Monika Nálezková[4], Lukáš Žídek[4], Vladimír Sklenář[4], Karel Bezouška[1,2]

[1]Department of Biochemistry, Faculty of Science, Charles University in Prague, Prague 2; [2]Institute of Microbiology, AS CR, Prague 4; [3]Institute of Physics, Faculty of Mathematics and Physics, Charles University in Prague, Prague 2; [4]National Centre for Biomolecular Research, Faculty of Science, Masaryk University in Brno, Brno;E-mail: mihok@biomed.cas.cz

Natural killer (NK) cells represent an important component of the early immune response capable of elimination of transformed, virally infected and otherwise stressed cells. Target cell recognition is controlled by a variety of activation and inhibitory surface receptors. In our laboratory we have been producing soluble recombinant forms of two important activating receptors of NK cells, CD69 and NKR-P1, physiological ligands of which still remain unknown. The globular extracellular domain of the above receptors is produced in bacterial expression system, refolded *in vitro* and purified. We use a range of methodologies for verification of the stability and proper folding of our recombinant proteins. The initial assessment of the refolding efficiency is performed by combination of the UV, FT-IR and Raman spectroscopy. For the final verification of the folding status we use ion Fourier transform-ion cyclotron resonance mass spectrometry to look at the identity of the entire protein and the number of closed disulfide bonds. Measurement of the 1H – 15N HSQC spectra in a 600 MHz NMR spectrometer using the uniformly ^{15}N labeled protein provides the definitive evidence for the proper folding and long-term stability of the protein preparations. The refolded proteins are very stable up to 80 °C (CD 69) and 55 °C (NKR-P1) under nonreducing conditions. The use of NMR as well as protein crystallography and other methods for the ligand identification and verification experiments is under progress.

L 2.6

INVESTIGATION OF POSTTRANSLATIONALLY MODIFIED (MALTED) PROTEINS BY COMBINATION OF IEF AND MALDI-TOF/TOF MS

Karel Mazanec*, *Karel Šlais and Josef Chmelík

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Protein amount and composition are radically changed in consequence of malting of barley (*Hordeum Vulgare*) grains. New formed or modified proteins play important role in starch degradation and some of them go through whole brewing process and may be identified also in beer. Protein Z is one of abounding proteins in beer and it contributes to foam stability and/or haze formation. Protein Z contains 22 lysine units in chain allowing glycation during malting.

Our work is concentrated on study of posttranslational modifications namely of protein Z in malt extract. Complex water extracts of barley and malt were simplified using size exclusion LC or Nanosep centrifugation filters with proper pore diameters. Fractions enriched with protein Z were then separated using gel IEF with 3/7 pH gradient. The color low-molecular mass pI markers were used for calibration of pH gradient of IEF gels and were identified by MS. The use of color pI markers allowed us to avoid a time consuming protein staining. For comparison, the gels were also stained with Coomassie Brilliant Blue R 250. Proteins were in gel digested with trypsin and peptides masses were measured by MALDI-TOF/TOF MS. The protein Z was identified in different positions in pH gradient proving posttranslational modifications.

Acknowledgement: *This work was supported by the Ministry of Education, Youth and Sports, Czech Republic (1M0570) and Grant Agency of the Academy of Sciences of the Czech Republic (No. A4031302).*

Friday, September 15, 2006

Lecture room L1
15:00 – 18:05

Section 2 PROTEOMICS AND ENZYMOLOGY II

Chairs: Štefan Janeček, Ľudovít Škultéty

L 2.7

AMYLOLYTIC ENZYME FAMILIES IN THE POST-GENOME ERA

Štefan Janeček

Institute of Molecular Biology, Slovak Academy of Sciences, Bratislava

Proteins form their evolutionarily related families. Glycosidases have been classified into the families of glycoside hydrolases (GHs) based on the similarities in their primary structures, reaction mechanism, catalytic machinery and fold. This means the evolution is reflected rather than the EC numbers. At present there are more than one hundred GH families. The amylolytic and related enzymes are found in the families 13, 14, 15, 31, 57, 70 and 77.

The main α-amylase family is the family GH13 that, together with families GH70 and GH77, forms the clan GH-H. β-Amylase and glucoamylase should seemingly be closely related to the α-amylase, however, these two amylases are different from each other as well as from the α-amylase. They thus form their own GH families, GH14 and GH15, respectively. The family GH31 is of special interest because it shares with the main α-amylase family GH13 several characteristics (the catalytic nucleophile). Since the general acid/base catalyst is not conserved within these two families, the family GH31 cannot be classified with GH13 as a member of clan GH-H. The family GH57 was for a long time considered to be a remote homologue of the clan GH-H, but solving the GH57 structures has shown differences in both catalytic fold and machinery.

Of the seven GH families mentioned above, the members of GHs 13, 14, 15, 31 and 77 contain a module outside their catalytic domains that is usually responsible for raw starch binding and degradation. Starch-binding domain was originally known of microbial-amylase origin only. Homologues of the starch-binding domains have recently been found also in various regulatory proteins involved in the metabolism of starch in plants and glycogen in mammals.

L 2.8

STUDY OF PROTEINASE INHIBITORS IN BOAR EPIDIDYMAL FLUID

Nina Davidová[1,2], Pavla Maňásková[2], Věra Jonáková[2]

[1]Department of Biochemistry, Faculty of Science, Charles University, Prague, Czech Republic [2]Department of Biochemistry of Reproduction, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Proteinase inhibitors are present in all tissues and body fluids. They interfere with the activity of the proteinases and thus maintain the homeostasis. Role of proteinase inhibitors in reproductive tract is an inactivation of prematurely released acrosin from damaged spermatozoa and protection against proteolytic degradation.

Epididymal fluid was separated by size exclusion chromatography. Serine proteinase inhibiting activity was measured by the colorimetric method. Further separation of these fractions was carried out by RP HPLC. In several HPLC-separated fractions proteinase-inhibiting activity was found by the reverse substrate zymographic method after SDS-electrophoresis. Moreover, we immunodetected the seminal plasma inhibitor of acrosin in some HPLC-separated epididymal fluid fractions.

In the epididymis, spermatozoa undergo their maturation and are stored there. Epididymal fluid contains various enzymes that affect the sperm surface. We suggest that acrosin inhibitors present in epididymal fluid might be important for preservation of natural balance in this tissue.

This work was supported by the Grant Agency of the Czech Republic, grants Nos. 303/04/P070 and 303/06/0895; by the Ministry of Education, Youth and Sports of the Czech Republic grant No. 1M06011, and in part by project AVOZ50520514 awarded by the Academy of Sciences of the Czech Republic.

L 2.9

STUDY OF HYALURONIDASE IN BOAR REPRODUCTIVE TRACT

Eva Cibulková[1,2], Pavla Maňásková[1], Věra Jonáková[1], Marie Tichá[2]

[1]Institute of Molecular Genetics, AS CR, Prague 6, [2]Department of Biochemistry, Faculty of Science, Charles University, Prague 2, Czech Republic

Two forms of hyaluronidase are present in the male reproductive tract of mammalian species: (i) the sperm mebrane bound enzyme, that degrades hyaluronic acid in extracellular matrix of the oocyte (PH-20, Spam1) and (ii) a soluble form of the enzyme whose role is not fully understood.

In the present study, we have investigated different forms of hyaluronidase in boar seminal plasma, in spermatozoa and in fluids isolated from boar reproductive organs. Two forms of soluble hyaluronidase were detected in boar seminal plasma (rel. mol. masses 50 000 and 65 000). They differ in their affinity to heparin and in the pH optimum of their enzyme activity. The form with rel. mol. mass 50 000 was active both at acidic (pH 3.7) and the neutral pH (pH 7.4) and was detected in epididymal fluid and in extract of boar spermatozoa. The other form (rel. mol. mass 65 000) was active only at acidic pH and was found in boar seminal vesicle fluid.

Further studies are necessary to find out the relationship between the soluble forms of boar hyaluronidase and the membrane-bound form and to elucidate the role of hyaluronidase forms in the reproduction process in mammals.

Acknowledgement

This work was supported by the Grant Agency of the Czech Republic, grants Nos. 303/04/P070 and 303/06/0895; by the Ministry of Education, Youth and Sports of the Czech Republic, grant No. 1M06011; and by the Academy of Sciences of the Czech Republic project AVOZ50520514.

L 2.10

ENZYMES OF SULFUR METABOLISM IN *ACIDITHIOBACILLUS FERROOXIDANS*

Oldřich Janiczek, Blanka Pokorná, Martin Mandl

Department of Biochemistry, Faculty of Science, Masaryk University, Brno

This study describes the distribution, characterization, and possible significance of the sulfur-oxidizing enzymes in *A. ferrooxidans*. *A. ferrooxidans* is a Gram-negative, acidophilic, chemolithotrophic bacterium able to derive energy for growth from the oxidation of ferrous to ferric iron and elemental sulfur or reduced inorganic sulfur compounds to sulfate using oxygen as electron acceptor. The first step of the sulfur compound oxidation is enzymatic oxidation of thiosulfate to tetrathionate. The enzyme, catalyzing this reaction is **thiosulfate dehydrogenase**, a periplasmic enzyme with pH optimum 3.0. **Tetrathionate hydrolase** is a periplasmatic enzyme with pH optimum 2.5. The rate of tetrathionate oxidation was strongly stimulated by the presence of sulfate ions. After formation of sulfur in the periplasm, sulfur was actively transported across the cell membrane. Further oxidation via sulfite to sulfate occurs in the cytoplasm. **Sulfuroxygenase** is a cytoplasmatic enzyme with pH optimum 7.4. Enzyme required GSH for its activity. The final step in the oxidation of reduced sulfur compounds; oxidation of sulfite to sulfate, is catalyzed by **sulfite dehydrogenase**, a cytoplasmatic enzyme with pH optimum 8.0.

This work was supported by grant No. 525/04/1309 from the Czech Science Foundation.

L 2.11

STRUCTURAL-FUNCTIONAL DIFFERENCES IN THREE CLASSES OF PECTIC ESTERASES

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There were studied differences in three classes of pectic esterases, enzymes which catalyse the hydrolysis of esters present in pectin backbone: (a) pectin methylesterases (EC 3.1.1.11) that hydrolyze methylesters from O6 of galacturonic acid in the homogalacturonan part; (b) pectin acetylesterases (EC 3.1.1.6) that remove acetyl groups from O2 and/or O3 in the homogalacturonic part; (c) rhamnogalacturonan acetylesterases (3.1.1.-) that remove acetyl groups in the rhamnogalacturonan part[1].

The pectin methylesterases belong to family CE8 of the carbohydrate esterase classification and represent a new type of esterases with β-helical structure and with aspartic acid as nucleotide[2].

The plant pectin acetylesterases belong to the family CE13, while the microbial acetylesterases belong to the family CE10 and some of them together with rhamnogalacturonan acetylesterases to the family CE12.

In contrast to pectin methylesterases which are Asp-esterases, the pectin acetyl esterases and rhamnogalacturonan acetylesterases are serine esterases with conserved catalytic triad Ser-Asp-His[3].

The sequences of all these three pectic esterase classes were aligned and the evolutionary trees based on the alignments were calculated.

References

[1]Benen, J. A. E. *et al.* In *Handbook of Food Enzymology;* Whitaker, J. R. *et al.* Eds. M. Decker: New York, 2003; pp.849-856.
[2]Markovič, O. & Janeček, Š. Carbohydr. Res. **339,** 2281-2295 (2004).
[3]Mølgaard, A. *et al.* Structure **8,** 373-383 (2000).

L 2.12

REGULATION OF THE PHOSPHOENOLPYRUVATE CARBOXYLASE UNDER STRESS CONDITIONS

Helena Ryšlavá[1], Karel Muller[1], Noemi Čeřovská[2]

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Phosphoenolpyruvate carboxylase (PEPC; EC 4.1.1.31) catalyzes the carboxylation of PEP to yield oxaloacetate and inorganic phosphate. It is one of the CO_2-fixing enzymes; bicarbonate (HCO_3^-) act as substrate and presence of divalent ion is required. PEPC plays a key photosynthetic role in primary CO_2 fixation in C4 and CAM plant leaves. In addition, PEPC produces oxalacetate and malate that replenish the citric acid cycle and provides carbon skeletons for amino acids. PEPC is also involved in pH regulation in the cell. PEPC is an allosteric enzyme, the activity of which is affected by metabolites such as glucose-6-phosphate (activator) and malate (inhibitor). In addition, PEPC is regulated by reversible phosphorylation at the conserved serine residue located near the N terminus. This phosphorylation is catalyzed by PEPC kinase.

In tobacco plants under biotic stress caused by viral infection was three times higher activity of PEPC than in control plants. The amount of PEPC mRNA, amount of PEPC protein and the extension of PEPC phosphorylation in infected and healthy plants were compared. Detailed kinetic studies concerning PEPC regulation were carried out on PEPC isolated from tobacco leaves. The possible role and regulation of PEPC under stress condition will be discussed.

Acknowledgement: *This work was supported by grant: Grant Agency of the Charles University 428/2004*

L 2.13

THE IMPORTANCE OF THE OXYANION HOLE IN ESTER HYDROLYSIS OF ENZYMATIC DEHALOGENATION CATALYZED BY HALOALKANE DEHALOGENASE REVEALED BY QM/MM CALCULATIONS

Michal Otyepka[1], Pavel Banáš[1], Jiří Damborský[2], Alessandra Magistrato[3], and Paolo Carloni[3]

[1]Department of Physical Chemistry, UP Olomouc, CZ; otyepka@prfnw.upol.cz, [2]Loschmidt Laboratories, MU Brno, CZ, [3]Department of Biophysics, ISAS, Trieste, Italy

Described QM/MM calculation reveals mechanism and energy profile of the second reaction step of dehalogenation catalyzed by haloalkane dehalogenase LinB. The catalytic triad Asp-His-Asp/Glu is common to all enzymes in alpha/beta-hydrolase family and shares some similarities with the catalytic triad of serine proteases. The reaction step studied comprises a general base catalyzed nucleophile ester hydrolysis. The catalytic base, His272, accepts proton from the catalytic water molecule attacking the ester intermediate and transfers it to newly formed alcoholate ion. The catalytic base works as a proton carrier. The Glu132 polarizes His272 to become more basic and to accept proton of the catalytic water molecule easily. The reaction proceeds through tetrahedral intermediate, which appears to be metastable at 300 K. The hydrolyzed ester forms enzyme's protonated aspartic acid (Asp108) and a primary alcohol. The protonated aspartic acid shows easy reconformation to the relaxed state found in the free enzyme. The enzyme lowers the energy barrier by about 3 kcal·mol−1 in comparison with model reaction. Such energy lowering is caused by strong stabilization of the reaction intermediate and transition state by oxyanion-hole.

Friday, September 15, 2006

Lecture room L2
10:00 – 12:25

Section 7 PATHOBIOCHEMISTRY II

Chairs: Nadežda Lukáčová, Peter Račay

L 7.6

MITOCHONDRIAL DYSFUNCTION INDUCED BY BRAIN ISCHEMIA

Peter Račay, Zuzana Tatarková, Peter Kaplán, Dušan Dobrota

Ústav lekárskej biochémie JLF UK, Martin

Dysfunction of mitochondria is considered to be key event triggering neuronal cell death after brain ischemia. The exact mechanism of ischemia-induced dysfunction of mitochondria and the link between mitochondrial dysfunction and induction of apoptosis is still elusive.

Here we report the effect of brain ischemia-reperfusion on functions of mitochondria isolated from the most vulnerable region of brain, hippocampus. By performing standard 4-vessel occlusion model of global brain ischemia, we have observed that 15 minutes of ischemia, followed by reperfusion in duration of 1, 3 and 24 hours led to the partial inhibition of both complex I activity and complex IV in all investigated intervals. Since core subunits of both complexes are coded by mitochondrial DNA, we have determined effect of ischemia-reperfusion on the rate of mitochondrial proteosynthesis. Ischemia and consequent reperfusion led to the inhibition of mitochondrial proteosynthesis. Depressed proteosynthesis was not a result of diminished mitochondrial transcription or increased proteolysis of mitochondrial proteins. Finally, an effect of ischemia-reperfusion on mitochondrial calcium transport was investigated. Although succinate-driven mitochondrial calcium uptake was not affected by ischemia-reperfusion, capacity of mitochondria to accumulate calcium was significantly decreased after ischemia but not reperfusion when mitochondria were energized by mixture pyruvate/malate.

Our results indicate that brain ischemia-reperfusion affects several mitochondrial functions, however, with distinct outcome.

This work was supported by grants VEGA 1/1192/04 and MVTS 39.

L 7.7

GENE EXPRESSION OF ADRENERGIC RECEPTORS IN FAILING HEART OBTAINED BY TRANSPLANTATION

Andrej Tillinger[1], Viliam Fischer[2], Ivan Gabauer[2], Barbara Grantnerová[3] Oľga Križanová[4], Richard Kvetňanský[1]

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Catecholamines (noradrenaline and adrenaline) are involved in many physiological and pathophysiological processes (including control of cardiovascular functions) and they act via adrenergic receptors. These receptors exist in multiple subtypes. Based on pharmacological properties and molecular structure, adrenergic receptors (ARs) are divided into three subtypes: α_1-, α_2- and β-ARs. In human heart mainly β- and α_1-ARs are present. The increased activity of the sympathetic nervous system with elevated plasma levels of noradrenaline is a well-known feature in patients with chronic heart failure. These changes are certainly closely related to alterations in ARs gene and protein expression.

In our studies we investigated gene expression of β_1, β_2, β_3 and α_{1A}, α_{1B} -ARs in cardiac ventricles and atria of patients that were subjected to the heart transplantation (mainly due the dilated cardiomyopathy) and compared to healthy hearts of people died at accidents. We found significant increases in mRNA levels of β- and α_1-ARs in the left ventricle, however, no changes were found in the other cardiac areas studied, when compared to healthy controls. It seems that β- and α_1-ARs in the left ventricle are activated in spite of the increased sympathetic activity, when compared to the other parts of failing heart and thus could affect the increase in heart rate and contractility leading to heart failure. Based on our results we can assume that cardiac gene expression of β- and α_1-ARs is involved in processes leading to development of the pathophysiological state of the heart.

This work was supported by: SP 51/028 08 00/028 08 02, Slovak Grant Agency VEGA 2/5125 and 2/6078 and APVT 51-027-404.

L 7.8

MODULATION OF OXIDATIVE STRESS AND GLYCATION BY PLANT ANTIOXIDANTS IN EXPERIMENTALLY INDUCED DIABETES

Oľga Uličná[1], Oľga Vančová[1], Peter Božek[2], Jozef Čársky[3], Katarína Šebeková[4], Peter Boor[4], Miloslav Greksák[5]

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The purpose of our study was to investigate effects of aqueous (RT) and alkaline extracts (AERT) of rooibos tea (*Aspalathus linearis*), as a natural source of antioxidants, on prevention and treatment of oxidative stress and glycation in experimental diabetes. N-acetyl-L-cysteine (NAC) for the comparison was used. Male Wistar rats (290-340g) were divided into five groups: 1. Control. Four other groups were treated with streptozotocin (45 $mg.kg^{-1}$) (STZ), 2. STZ. 3. STZ+RT (5 $ml.kg^{-1}$), 4. STZ+AERT (300 $mg.kg^{-1}$), 5. STZ+NAC (150 $mg.kg^{-1}$). The rats in the third group had free access to RT instead of tap water. Eight weeks after STZ administration, blood samples and liver, kidney, lens were removed and subjected to analysis.

Significant changes of biochemical parameters characteristic for experimental diabetic state in plasma and tissue were found. Glucose, glycated Hb, fructosamine (FRUCA), advanced glycation end-products (AGEs), advanced oxidation protein products (AOPPs) in plasma and malondialdehyde (MDA) levels in liver, kidney and lens were significantly ($p<0.001$) higher. Administration of RT, AERT and NAC to diabetic rats did not affect markers of diabetic status (glucose, glycated Hb, FRUCA), while AGEs were significantly and AOPPs slightly lowered. All these three antioxidants significantly decreased MDA in plasma and lens. MDA in liver decreased only in the case of RT administration and in kidney in the case of NAC administration.

We can conclude that the wide scale of compounds with a potent antioxidant properties present in RT, partially prevents oxidative stress induced by hyperglycemic conditions and that it is effective in both, hydrophilic and hydrophobic biological systems. Therefore, roobois tea can be recommended as an excellent adjuvant support for the prevention and therapy of the diabetes-induced oxidative stress complications.

This research was supported by Science and Technology Assistance Agency SR No. APVT-51-024904 and VEGA No. 1/3442/06

L 7.9

THE PARTICIPATION OF NITRIC OXIDE IN ANTEROGRADE SIGNALING

Nadežda Lukáčová, Jozef Maršala

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In recent years, nitric oxide has been identified as a peculiar neurotransmitter in the CNS, being involved in signaling and in trophic and neuroprotective functions. The use of biochemical, immunohistochemical and histochemical approaches has revealed heterogeneous distribution of nitric oxide synthase pools in the segments and regions of the spinal cord along its rostrocaudal axis, providing a basis for identifying the participation of nitric oxide in the modulation of the spinal microcircuits after various experimental interventions. Our experiments have revealed the presence of nitric oxide synthase containing axons in four long, nitric oxide synthase-immunoreactive pathways, i.e. the sensitive dorsal column pathway connecting medium- and large-sized dorsal root ganglion neurons with somata of the gracile nucleus in the medulla; the bulbospinal respiratory descending pathway forming a direct connection between the bulbar respiratory centers and the motoneurons of the phrenic nucleus located in the ventral horn of C3-C5 segments; the premotor propriospinal pathway underlying the connection between the neurons in the lumbosacral enlargement and the ventral motor nucleus at the cervicothoracic level, and finaly, in the monosynaptic Ia-motoneuron pathway forming the afferent limb of the stretch H-reflex. These findings indicate that 1) nitrergic terminal arborizations innervate relevant motoneurons, and that 2) nitric oxide may act as an anterograde neuromodulator influencing the postsynaptic neurons.

Supported by the VEGA Grant No. 2/5134/25 from the SAS and PAS/SAS project 17/24-26.

Friday, September 15, 2006

Lecture room L2
15:00 – 16:35

Section 7 PATHOBIOCHEMISTRY III

Chairs: Juraj Kopáček, Michal Svoboda

L 7.10

ROLE OF TUMOR MICROENVIRONMENT IN THE MODULATION OF CARBONIC ANHYDRASE IX EXPRESSION

Juraj Kopáček , Jaromír Pastorek & Silvia Pastoreková

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Tumor development is a multi-stage process that results from accumulation of genetic alterations, epigenetic changes and abnormal physiological processes in the tumor tissue.

Signaling of the extracellular conditions in tumor microenvironment as well as of the molecular changes inside the tumor cells is executed via regulatory molecules that act in particular signal transduction pathways. One of the molecules whose expression is influenced by tumor microenvironment is carbonic anhydrase IX (CA IX), which belongs to the most strongly inducible proteins in response to hypoxia. Moreover, the enzyme activity of CA IX is increased by hypoxia what enables tumor cells to regulate pH and better adapt to acidosis.

Based on the available data, CA IX expression is subjected to positive regulation by hypoxia via transcriptional complex HIF-1, by transcription factors AP-1 and SP-1 and by phophatidylinositol-3- kinase and MAPK kinase pathways. It is also negatively regulated by the tumor suppressor protein pVHL and AhR mediated dioxin signaling pathway. Thus CA IX expression may be influenced by several different pathways which may compete or cooperate for the crucial transcriptional factors and cofactors. Crosstalk between hypoxia, dioxin signaling and acidosis modulates CA IX expression and thereby can directly influence tumor progression.

Supported by APVV -51-024905

L 7.11

COMPARISON OF ABC TRANSPORTERS EXPRESSION WITH CLINICAL OUTCOME IN ACUTE LEUKAEMIA

[1]Jozef Hatok, [1]Tatiana Matáková, [2]Mária Franeková, [1]Dušan Dobrota, [1]Peter Račay

[1]Department of Medical Biochemistry, JMF CU Martin, [2]Department of Biology, JMF CU Martin

Drug resistance can be caused by ATP-binding-cassette (ABC)-transporters which function as outward pumps for chemotherapeutic drugs. In presented study, expression P-glycoprotein (P-gp), multidrug resistance-associated protein (MRP) and breast cancer resistance protein (BCRP), have been studied by RT-PCR in leukaemic cells of patients with acute leukaemia. In addition, our study focuses on the clinical relevance and prognostic value of these efflux pumps. P-gp exhibited strong variation in transcription level among different leukaemia patients; however, it was significantly higher in relapsed than in de novo patients. The expression of MRP was more consistent and no significant differences between de novo and relapsed patients were observed. The expression level of BRCP was very low, however, significantly higher in patients with unfavourable clinical outcome. High P-gp expression prior to treatment has been associated, whereas expression of MRP does not seem to play a major role. Higher expression of BCRP prior to treatment was moderately associated with poor clinical outcome, but larger studies are needed to confirm these results. In addition, other factors (e.g. expression of apoptotic and anti-apoptotic molecules) might affect significantly the clinical outcome of acute leukaemia treatment.

Supported by grant aAV/1106/2004 from Ministry of Education of Slovak Republic and grant of Comenius University UK/265/2006.

L 7.12

METALOTHIONEIN AS A POTENTIAL TUMOR DISEASE MARKER

Michal Svoboda[1], Ondřej Blaštík[1], Pavlína Šobrová[1], Richard Průša[2], Jiří Kukačka[2], Hana Binková[3], Zuzana Horaková[3], Vojtěch Adam[1], René Kizek[1]

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According to World Health Organisation (WHO), more than 11 millions people are diagnosed with tumor disease every year and it is supposed that this number will be dramatically increased in following years. On this account Czech Republic has been beginning an intensive program to fight with tumor diseases containing declaration of National Oncological Program of the Czech Republic. One of aims of this program is also to improve early diagnosis including searching for new potential markers of tumor diseases. According to recently published papers, metallothionein (MT) could serve as a new potential tumour disease marker. MT belongs to group of intracellular, low molecular and cysteine-rich proteins with molecular weight from 6 to 10 kDa. Recently published papers showed a possibility that link between amount of MT and rate of tumor disease progression really exists.

Our aim has been to study a relation of MT and cancer diseases. For this purposes we have chosen Brdicka reaction (electrochemical method), which was high sensitive and also allowing quick routine analysis of bigger blood and/or blood serum samples amount. We analyzed blood serum of patients with different tumor diseases. Our results show that MT content at all studied cancer diseases increased in comparison with control samples.

This work was supported by grant GACR 525/04/P132 a FRVS 699/F4a.

References. 1. Kizek, R., L. Trnkova, et al. Anal. Chem. **73**(20): 4801-4807 (2001). 2. Prusa, R., O. Blastik, et al. Clin. Chem. **51**(Suppl. 6): A56-A56 (2005). 3. Prusa, R., D. Potesil, et al. Mol. Biol. Cell **15**(1376 Suppl. S): 249A-249A (2004).

L 7.13

MATURATION OF ATP YIELDING IN NEONATAL RAT BRAIN IN RELATION TO ITS SENSITIVITY TO HYPOXIA – AN *IN VIVO* ^{31}P-MRS STUDY

Ivo Juránek, Ladislav Bačiak[1], Eduard Ujházy, Svatava Kašparová[1]

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Perinatal asphyxia involves serious hypoxia-ischaemia mediated brain injury of newborns. Our previous findings demonstrated that survival of newborn rats exposed to acute hypoxia was inversely proportional to their age and duration of hypoxic insult. Rat pups 1-2 day old did survive up to 20-25 min lasting anoxia (100% N_2) without any detectable consequences in psychomotor development. Moreover, using conventional neurobehavioral tests, rats exposed neonatally to hypoxia were indistinguishable from their control counterparts in adulthood.

The present study was designed: 1) to prove hypothesis that the increasing sensitivity to hypoxia may result from enhancing demands of the neonatal brain for ATP; 2) to explore whether any parameter of cerebral energy metabolism may be utilized as a diagnostic and/or prognostic tool for newborns surviving severe hypoxic-ischemic insult. We applied *in vivo* phosphorus (^{31}P) magnetic resonance spectroscopy (MRS) to follow high-energy phosphates, adenosine triphosphate (ATP) and phosphocreatine (PCr), within the brain of 1-5 day old rat pups. PCr/ATP reflected well developmental changes in energy metabolism, and P_i/ATP was suitable for recording brain hypoxia. Phosphorylation potential PCr/P_i revealed both the developmental changes and the effect of hypoxia. Finally, increasing P_i/ATP or decreasing PCr/P_i indicated the onset of cerebral hypoxia, while their reversal reflected the efficiency of posthypoxic recovery.

Acknowledgement: This work was partially supported by the grants of VEGA (No. 2/4127/04 & 2/5052/25) and of Slovak State Program (project No. 2003SP200280203).

Friday, September 15, 2006

Lecture room L3
10:00 – 12:40

Section 10 TEACHING BIOCHEMISTRY AND MOLECULAR BIOLOGY

Chairs: Katarína Mikušová, Anton Horváth

L 10.1

BIOCHEMICAL ENCYCLOPAEDIA: NEW DEVELOPMENT OF THE STUDYING MATERIAL

Milan Kodíček, Miloslav Nič, Jiří Jirát, Jiří Znamenáček

Institute of Chemical Technology, 166 28 Praha6, Czech Republic

During the last period we have developed "Biochemical encyclopaedia" that provides basic learning and studying aid used by students of the Institute of Chemical Technology, Prague. It is now available both in the printed form sold together with CD-ROM (M. Kodíček: Biochemické pojmy – výkladový slovník. Vydavatelství VŠCHT Praha, 2004) and on web address: http://www.vscht.cz/eds/knihy/uid_es-002/ebook.obsah.htm.

The Encyclopaedia summarizes the most important terms used in the basic biochemical courses. The terms are structured in four levels as taught in our school; this tailoring to particular groups makes the Encyclopaedia useful also for students who attend lectures of biochemistry as part of "non-biochemical" studies, such as medicine or pharmacology. The Encyclopaedia contains 1100 entries, 57 pictures and schemes and 108 structural formulas and chemical equations. Further more, the electronic versions have special procedures that can help students when they are preparing for a biochemical examination.

During this summer we have started to prepare the higher version of the Encyclopaedia. We want to add new items and pictures, English equivalents of all terms and EC numbers of all enzymes (with electronic connections to enzyme catalogue). Some new electronic facilities will also be available.

The main aim of our presentation at this conference is to offer active cooperation in preparation of higher version of our Encyclopaedia to the Czech and Slovak biochemical teachers´ community.

L 10.2

TRENDS IN EDUCATION OF BIOCHEMISTRY ON THE ŠAFARIK UNIVERSITY

Viktor Víglaský

Department of Biochemistry, Institute of Chemistry, Faculty of Sciences, P.J. Šafárik University, Košice, Slovakia

Biochemistry is one of the fast developing disciplines of biosciences. Novel approaches, procedures and different techniques must be implemented also into the education process. Biochemistry is a multidisciplinary subject standing among chemistry molecular biology, pharmacology, medicine biophysics and biotechnology. A main goal of education processes of student is focused to give integral information from all of related disciplines. Proceedings in molecular biology during last two decades are in sign of rapidly developing various scale of techniques e.g. amplification methods of nucleic acids (PCR, RT PCR), systematic evolution of ligands by exponential enrichment (SELEX), separation techniques of cells, subcelular structures nucleic acids and proteins (electrophoretic methods, FACS, flow cytometry), immunoflorescent detection techniques (ELISA, RIA, DNA chips), *in vitro* translation systems, RNA interference etc. Students oriented on the biochemistry subject at our faculty have to pass a lecture covering main trends and techniques in molecular biology and biochemistry including the practice in laboratory. They get extraordinary skills in the area of spectrophotometric measurements; CD spectroscopy, absorption UV-VIS spectroscopy and fluorescent spectroscopy. Research is focused on the protein and nucleic conformational stability and their complexes with low molecular ligands.

To increase the motivation of students, there is possibility to attend also other laboratories in abroad to obtain deeper knowledge and improve their language skills.

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L 10.3

TEACHING BIOCHEMISTRY AT FACULTY OF SCIENCE IN BRNO

Vladimír Mikeš

Department of Biochemistry, Fakulty of Science, Masaryk University, Brno, Czech Republic

Increasing volume of new information necessitates to improve the quality of teaching. We are permenently confronted with the problemes how to teach, how to control the knowledge level of students. The information that could be found in Internet represents an alternative database source for students. In spite of the advantage of the interactive teaching the basic lecture remains the less laborious pedagogical and information ressource for students. The contribution compares handicaps and advantage of classical vs. new forms of theaching, the ways of testing the knowledge level. A new electronic texbook is also presented.

L 10.4

TEACHING OF MEDICAL BIOCHEMISTRY FOR STUDENTS FROM FACULTY OF NATURAL SCIENCES AND FACULTY OF PHYSICAL EDUCATION AND SPORT, COMENIUS UNIVERSITY, BRATISLAVA.

Branislav Líška, Marta Brechtlová, Ján Podhradský, Lukáč Halčák, Monika Ďurfinová

Ústav lekárskej chémie, biochémie a klinickej biochémie LF UK, Bratislava

Our department provides an education of medical biochemistry for students of the 3rd and 4th year from Faculty of Natural Sciences (FNSci) and students of the 1st or 2nd year from Faculty of Physical Education and Sport (FPES), according to the credit system of study at Comenius University.

We have been teaching medical biochemistry at FNSci for many years. The subject – Biochemistry of Physiological Functions was taught for 2 hours per week during 1 semester and finished by a final examination. Contents of the subject were the functional and organ biochemistry. The Department of Biochemistry of FNSci asked for extending of the subject after requests of their students. In the first phase, the Biochemistry of Physiological Functions was broken up into the part I (4 hours per week) and the part II (2 hours per week). The part II lectures explain the regulation of biochemical processes at the level of organism by means of nerve system and hormones. Later, the subject - Basic Pathobiochemistry and Clinical Biochemistry was added, i.e. 2 hours per week in the winter semester and 4 hours per week in the summer semester.

Teaching at FPES differs by the content of the subject and its orientation. The subject – Basic Functional Biochemistry for students of FPES involves also fundamentals of biochemistry in short form. The part of organ biochemistry is focused on course and regulation of metabolic and functional processes, mainly of the skeletal muscle as well as the myocardium and their supply of substrates and oxygen accordig to their work. The subject is taught for 2 hours per week and is finished by a final written test.

L 10.5

TEACHING OF MOLECULAR BIOLOGY AT THE FACULTY OF BIOTECHNOLOGY AND FOOD SCIENCES SAU IN NITRA

Zdenka Gálová, Želmíra Gregáňová

Department of Biochemistry and Biotechnology, FBFS SAU in Nitra

The subject *Molecular biology* is taught separately, but in coordination with different theoretical subjects as a chemistry, biochemistry, microbiology and genetics. The students of the Faculty of Biotechnology and Food Sciences SAU in Nitra deepen knowledge at the molecular level within specific subjects as a *Molecular biology*, *Methods and technologies of gene manipulation, Biotechnology in plant production, Genomics and genetic engineering*. The subject *Molecular biology* is compulsory subject for students of the 5st semester of the study branches *Applied biology* and *Biotechnology*. The subject has 60 hours per semester (2 h lecture and 2 h practicum/week). Lectures are orientated on explanation of the biomacromolecule's structure, gene expression and its regulation at the molecular level, mutations, construction of cloned systems. In the subjects *Methods and technologies of gene manipulation* (60 hours, 2/2, 6 ECTS, the 8st semester) and *Biotechnology in plant production* (60 hours, 2/2, 6 ECTS, the 9st semester) students broaden their previous knowledge into the applied aspects. All three subjects include also practical exercises and seminars. At the practical exercises students achieve practical laboratory skills in the isolation of proteins and DNA from the wheat grain, separate them by the PAGE or agarose gels and evaluate obtained electrophoretic profiles. In the 8st semester they isolate and cleave plasmid DNA with restriction enzymes, perform PCR reaction and in the 9st class mentioned methods applied into the plants and microorganisms. Upon these subjects continues subject *Genomics and genetic engineering*.

L 10.6

THE NEWEST ACHIEVEMENTS OF MOLECULAR BIOLOGY IN PRACTICE – STRUCTURE AND CONTENT OF THE SUBJECT: BIOTECHNOLOGY1, 2, 3 AND 4.

Danka Valková[1], Elena Hlinková[2], Anton Horváth[3], Marta Kollárová[3] and Ján Turňa[1]

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ABSTRACT

The study profile Biotechnology and the content of subject BIOTECHNOLOGY 1, 2, 3 and 4 reflect the trend of modern, technologically advanced society development. The level of development can be easily recognized by the tools utilized in this process.

Application of the newest achievements of genetics, biochemistry and molecular biology in practice resulted in upheaval of classical biotechnology – the use of living organisms in industrial practice to advanced, rationally designed biological systems used worldwide in the modern biotechnology.

Creation of genetically modified organisms represents the basic tool of molecular biotechnology and their applications in industrial practice. It was made possible, since the realization of genetic information has been known, at first on the prokaryotic cell level.

However, since the year 2000 many higher plants and animal have been modified with the promising use in modern biotechnology practice and in the field of biomedicine it is also the human being itself as a target of gene therapy and other molecular biotechnology applications. The student has to learn the principles of gene expression from basic microbial level, through the lower eukaryotic, suspension insect cell cultures including, to plants and animals, ending with the genetically ruled ontogenesis of higher organisms including. Bioethics together with legislature is also a part of the subject content.

Therefore, three departments of the Faculty of Natural Sciences of the Comenius University in Bratislava participate in the teaching of this subject. It is taught at the Magister level of the study, because of its complexity, the Bachelor level of basic knowledge of genetics, biochemistry and molecular biology is required as a standard prerequisite. However, in Bachelor programs both in chemistry and biology, the Basic Biotechnology subject is taught for common orientation and also for the student interest stimulation to take the BIOTECHNOLOGY 1,2,3 and 4 course in the Magister program.

L 10.7

LABORATORY WORKSHOPS AS MEANS OF MOTIVATION OF HIGH SCHOOL STUDENTS FOR EXPERIMENTAL BIOLOGY

Miroslava Slaninová[1], Eliška Gálová[1], Andrea Ševčovičová[1], Katarína Mikušová[2], Gabriela Gavurníková[2], Jozef Nosek[2] a Ľubomír Tomáška[1]

Departments of [1]Genetics and [2]Biochemistry, Comenius University, Faculty of Natural Sciences, Mlynská dolina, 842 15 Bratislava

A general problem of decrease in interest of high school students for science education in Slovakia is amplified by local-specific 'phenotypes' including culturally inherited preference of conformity and discrimination of originality and talent. In this context it is not surprising that Slovak universities are very passive towards talented high school students. This lack of an assertive attitude might eventually lead to a massive exodus of gifted young individuals and the Slovak universities will be left out with average and marginal importance. To avoid this pessimistic outcome, the implementation of a systematic search for talents, their recruitment and motivation, is a necessity. The specific aim of our project is to organize one-week workshops for 10-15 high-school students (15-16 years of age), where they are exposed to several topics of the contemporary genetics, molecular biology and biochemistry. The students are selected for the workshop based on (i) recommendations of their teachers, and (ii) letters of intends they are required to write in order to apply. The selected students are divided into 4 groups (2-3 students per group). The personnel involved in the project prepare four one-day laboratory courses, ranging from functional complementation test through DNA isolation, transformation to analysis of gene expression. Each student performs the experiment with an assistance of the assigned teacher. The students are obliged to write down notes into laboratory notebook and generate conclusions and hypotheses resulting from their experiments. We believe that the workshop represents a powerful tool for attracting young talents into the field of life sciences.

This project is supported by Grant KEGA 3/3021/05 and Grant ESF SOP- ĽZ 2005/1-101

Friday, September 15, 2006

Lecture room L3
15:00 – 18:00

Section 4 CELL REGULATIONS AND TRANSFER OF SIGNALS II

Chairs: Ján Kormanec, Radim Černý

L 4.6

NUCLEAR ACTIN AND MYOSIN I ARE REQUIRED FOR RNA POLYMERASE I TRANSCRIPTION

Vlada V. Philimonenko[1], Jian Zhao[2], Sebastian Iben[2], Hana Dingová[1], Katarína Kyselá[1], Michal Kahle[1], Hanswalter Zentgraf[3], Wilma A. Hofmann[4], Primal de Lanerolle[4], Pavel Hozák[1] and Ingrid Grummt[2]

[1]Institute of Experimental Medicine Prague, [2]Division of Molecular Biology of the Cell II, DKFZ Heidelberg, [3]Applied Tumorvirology, DKFZ Heidelberg, [4]University of Illinois at Chicago

The presence of actin and nuclear myosin I (NMI) in the nucleus suggests a role for these motor proteins in nuclear functions. We have investigated the role of actin and nuclear myosin I (NMI) in the transcription of ribosomal RNA genes (rDNA). Both proteins are associated with rDNA and are required for RNA polymerase I (Pol I) transcription. Microinjection of antibodies against actin or NMI, as well as short interfering RNA-mediated depletion of NMI, decreased Pol I transcription *in vivo*, whereas overexpression of NMI augmented pre-rRNA synthesis. *In vitro*, recombinant NMI activated Pol I transcription, and antibodies to NMI or actin

inhibited Pol I transcription both on naked DNA and pre-assembled chromatin templates. Whereas actin associated with Pol I, NMI bound to Pol I through the transcription-initiation factor TIF-IA. The association with Pol I requires phosphorylation of TIF-IA at Ser 649 by RSK kinase, indicating a role for NMI in the growth-dependent regulation of rRNA synthesis.

L 4.7

THE ROLE OF TWO ALTERNATIVE SIGMA FACTORS, SigB AND SigH, IN REGULATION OF DIFFERENTIATION AND STRESS RESPONSE IN *STREPTOMYCES COELICOLOR* A3(2)

Vladislava Mazuráková*, *Beatrica Ševčíková*, *Ján Kormanec
Institute of Molecular Biology, Centre of Excellence for Molecular Medicine, Slovak Academy of Sciences, Dúbravská cesta 21, 84551 Bratislava, Slovakia

Streptomyces coelicolor is gram-positive soil bacterium that undergoes a complex cycle of morphological differentiation dependent upon its ability to respond to changes in the environment, especially nutrient limitation and generation of turgor pressure. Genes involved in osmoadaptation and regulation of differentiation include those encoding alternative sigma factors belonging to the SigB family. In *S. coelicolor* two of them, SigB and SigH, have dual role in salt stress-response and morphological differentiation. *SigH* and *sigB* are part of operons, which contain genes encode homologue of anti-sigma factor and protein with unknown function. To clarify the *in vivo* function of *sigB* and *sigH* operons, stable null operon mutants were prepared. Their phenotype together with transcription and translation analysis of expression of *sigH* and *sigB* indicate SigB priority role in salt stress response and SigH in regulation of differentiation. The results also indicated a complicated regulation of both operons in relation to stress response and differentiation in *S. coelicolor*. In order to identify SigB and SigH regulons, previously developed two-plasmid method was used. Genes of these regulons encode proteins involved in primary and secondary metabolism including osmoadaptation and differentiation of *Streptomyces*. Expression from SigB-dependent promoters was induced by hyperosmotic stress with early inducing kinetic; from SigH-dependent promoters was induced by differentiation. These results indicated a role of SigB in osmotic stress response, while SigH in differentiation of *S. coelicolor*.

Acknowledgement. This work was supported by the VEGA grant 2/6010/26 from Slovak Academy of Sciences.

L 4.8

MECHANISMS OF HORMONAL HOMEOSTASIS IN PLANTS

Klára Hoyerová, Václav Motyka, Petre Dobrev, Miroslav Kamínek

Institute of Experimental Botany, AS CR, Praha, Czech Republic

Two classes of phytohormones, auxins and cytokinins, play a key role in regulation of cell division and differentiation. While rapid changes in auxin/cytokinin levels resulting from expression of specific genes involved in auxin and cytokinin biosynthesis can induce specific morphogenic responses (as e.g. initiation of specific organ formation) the subsequent re-establishment of hormonal homeostasis is essential for further progress of induced events.

We shall demonstrate how endogenous cytokinin levels are regulated by overexpression of genes involved in utilization cytokinin side chain precursors derived from mevalonic pathway (farnesyl diphosphate synthase, AtFPS1L) and cytokinin biosynthesis (isopentenyltransferase, AtIPT3) and how excessive physiologically active cytokinins are subsequently down-regulated to re-establish homeostasis of active cytokinins and auxin/cytokinin ratios. Operation of mechanisms involved in maintenance of cytokinin homeostasis that include conversion of active cytokinins to inactive N-glucosides and storage O-glucosides, cytokinin degradation by cytokinin oxidase/dehydrogenase and their immobilization by binding to specific proteins will be demonstrated. Potential exploitation of an autoregulatory system controlling cytokinin levels in transgenic wheat for improvement of plant productivity will be presented.

L 4.9

BASAL CALCIUM INFLUX IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES

Boris Lakatoš[1], Jana Slováková[1], Karin Kaiserová[1], Jozef Orlický[2], Ľudovít Varečka[1]

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Ca^{2+} plays a fundamental role as a second messenger in regulating numerous cellular functions. The mechanism(s) responsible for the increase in $[Ca^{2+}]_i$ during antigen or mitogen induced stimulation of lymphocytes has been widely investigated, by contrast, little is known about the processes underlying Ca^{2+} homeostasis and transport in resting cells in spite of the fact that changes in $[Ca^{2+}]_i$ are associated with variety of diseases. Here we report the basic parameters of $^{45}Ca^{2+}$ influx into isolated human blood lymphocytes and possible regulation pathways of this process.

The basal $^{45}Ca^{2+}$ influx into resting human blood lymphocytes shows biphasic kinetics with first rapid phase followed by the second long-lasting and markedly slower phase. This transport shows signs of saturability and reaches maximal values at 37°C and pH_{ex} 7.2. In the regulation of basal $^{45}Ca^{2+}$ influx is involved PKC as show results with PKC activator phorbol-12-myristate-13-acetate (PMA) and PKC inhibitor Ro-31-8220, and partially also c-AMP pathway is implied in this process.

This work was supported by the grant VEGA, nr.2/3188/23 and grant APVT 51-013802.

L 4.10

CAMP-MEDIATED SIGNALING OF *BORDETELLA* ADENYLATE CYCLASE TOXIN INDUCES MEMRANE RUFFLING OF MYELOID MONOCYTES

Jana Kamanová[1], Oľga Kofronová[1], Harald Genth[2], Jana Vojtová[1], Irena Linhartová[1], Ingo Just[2] and Peter Šebo[1]

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The action of many bacterial toxins perturbs homeostasis of cytoskeletal structures and alters morphology of host cells, thereby facilitating pathogen survival within the host. The whooping cough agent, *Bordetella pertussis,* secretes an adenylate cyclase toxin (ACT or CyaA) that targets and paralyzes host phagocytes expressing the $\alpha_M\beta_2$ integrin receptor CD11b/CD18. The toxin acts by delivering a catalytic adenylate cyclase (AC) domain into the cytosol of target cells, where the AC enzyme binds intracellular calmodulin and subverts cell signaling by unregulated conversion of ATP to cAMP. In parallel, ACT can permeabilize cellular membrane by forming small cation-selective pores.

We show here that ACT-catalyzed elevation of cytosolic cAMP in myeloid monocytic cells induces massive actin, as well as tubulin cytoskeleton rearrangements and formation of transient sheet-like cell membrane extensions, commonly referred to as membrane ruffles. Surprisingly, the ruffling i**n**duced by ACT is accompanied by inhibition of cell macropinocytic activity. The signaling mechanisms underlying the toxin-induced ruffling, as well as its biological role are under investigation, the status of which will be presented.

L 4.11

THE ROLE OF PHOSPHORYLATION IN CYCLIN DEPENDENT KINASE 2 AND 5 REGULATION. A COMPUTER SIMULATION STUDY.

Iveta Bártová[1,2], Michal Otyepka[1], Zdeněk Kříž[2], and Jaroslav Koča[2]

[1]Department of Physical Chemistry and Center for Biomolecules and Complex Molecular Systems, Palacky University, tr. Svobody 26, 771 46 Olomouc, Czech Republic, [2]National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

Cyclin-dependent kinases (CDKs) control progression of the cell cycle, apoptosis, transcription, and differentiation in neuronal cells. CDK consists of two subunits, a catalytic subunit kinase and regulatory protein cyclin. Several CDKs (CDK1, CDK2, CDK4 and CDK6) show a dual mechanism of activation based on cyclin binding and phosphorylation of the activation loop. CDK5 is a unique member of the CDK family, as it is not activated by cyclin. Instead, CDK5 activity is triggered by p35 and p39, proteins whose expression is limited to neurons and to a few other cell types [1]. Association of CDK5 with p35 or p39 is enough to full CDK5 activation [2, 3]. Additionally, phosphorylation of the glycine-rich loop (G-loop) is inhibitory for CDK2 or CDK1 but it seems to be stimulatory for CDK5 [4]. The phosphorylation of Thr14 and Tyr15 in the G-loop is an important element of CDK regulation machinery. Structural aspects of that were recently studied on CDK using molecular dynamics [5, 6].

References

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Friday, September 15, 2006

Lecture room L4
10:00 – 16:30

Section 11 MEW METHODS I

Chairs: Zdenka Sulová, Attila Ziegelhőffer

CP 1

MERCK LIFE SCIENCE INTRODUCE PRODUCTS FOR RESEARCH IN GENOMICS & PROTEOMICS.

Adam Andráško, Lucia Babjaková, Soňa Bernátová, Miloš Čebík

Merck spol. s.r.o., LSLD

Merck company will introduce actual product range offer for Proteomics and genomics research, e.g. sample preparation, nucleic acid isolation and purification, extraction, purification and fractionisation of proteins, electrophoresis, molecular imaging, PCR, Elisa, protein microarrays and others.

Company Merck spol s.r.o. is a producer of its own products (Merck Biosciences) and a distributor of well-known LifeScience research product's producers (Eppendorf, Macherey – Nagel, Consort, Fermentas, Kodak, Genekam, AssayPro, Vilber Lourmat, Air Liquide, BioTek, Binder, Brand, Heidolph, H+P, Lauda, Ohaus, Vacuubrand, Werner and many others).

CP 2

SNAP-TAG: USE OF A SINGLE PROTEIN TAG FOR PROTEIN DETECTION, LABELING AND IMMOBILIZATION

Dr. Lucinda Gedge

SNAP-tag is a versatile self labeling protein tag that labels itself covalently and specifically with a wide range of fluorescent or affinity substrates. Proteins fused to the SNAP-tag are easily expressed in a variety of common expression systems and can be labeled in cells or in solution. Substrates for labeling of the SNAP-tag are derivates of benzyl purines or benzyl pyrimidines and attach only once per SNAP-tag fusion protein. This self labeling reaction is highly specific, because SNAP-tag substrates are stable in biochemical conditions and no other protein reacts with this class of substance. There are no limitations to the nature of the chemical compound that can be linked to SNAP-tag, thus it can be used in a broad variety of experimental settings.

SNAP-tag can be used in cells for protein trafficking and relocation experiments, selective labeling of receptors in the plasma membrane, receptor internalization assays, and pulse-chase labeling. Applications range from dynamic labeling in living cells to the use in fixed, permeabilized cells. In addition SNAP-tag can be used in solution for interaction assays, including microtiter plate assays and FRET or TR-FRET assays. SNAP-tag further allows immobilization of proteins on surfaces for pull downs or purification. The presentation will cover these application areas, with a focus on cellular labelling.

A list of relevant publications is available on www.covalys.com

ROCHE APPLIED SCIENCE SOLUTIONS FOR REAL-TIME PCR

***Dalimil Žůrek*[1,2]**

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[2]Roche, s.r.o., Diagnostics Division Lazaretská 8, 811 08 Bratislava

Real-time PCR is often used in the scientific community for differently Applications as Genotyping, Gene Detection or Gene Expression. The complete process of generating data for this kind of analysis consists of many steps starting from sample to analysis. The received data are influenced by biological variations governed by the laws of nature which cannot always be controlled. Nevertheless, a reduction of technical variation is the achievable goal, reached by using workflows that show lowest inter- and intra-assay variances.

In real-time PCR, the PCR reaction is monitored via indirect detection methods, using fluorescent dyes or probe-based formats, which help monitor the amplification process. As a consequence, this necessitates optimization of real-time PCR-Instruments for homogeneity and accuracy in both processes: amplification and detection. The accuracy of amplification is often contrary to speed and homogeneity. Flexibility in the usage of assay format or fluorescent dye requires highly specific optical units.

The Roche LightCycler® 2.0 System Family sets the standard for rapid, sensitive and accurate real-time PCR. With LightCycler® 480 System, we will introduce an additional instrument in the market─ adding to the already well-accepted performance of LightCycler® 2.0 System on a multiwell-plate based platform. In addition to delivering speed and accuracy, the system is highly versatile. The flexibility of the optical system enables using most current dyes, also in multiplex. Exchangeable 96- or 384-multiwell plate block facilitates different throughput adaptations.

The highly flexible software supports multitude-assay formats and applications for quantification as well as melting-curve analysis. The algorithms used to characterize genes qualitatively and quantitatively have been optimized extensively, thereby facilitating the analysis of Data received from multiple Application. New reagents including new type of LNA (locked nucleic acid) Hydrolysis Probes – UPL have been developed with the goal of having economical but high quality solutions, which could also been adapted to other instrumentation as been provided with Roche Applied Science.

CP 4

PROMEGA CELL BASED ASSAY SYSTEMS – HOW TO SEE STILL INVISIBLE PROCESSES?

RNDr. Martin Janitor, PhD.

Lambda Life a.s., Bojnická 20, 841 03 Bratislava

Choosing a cell viability or cytotoxicity assay from among the many different options available can be a challenging task. Picking the best assay format to suit particular needs requires an understanding of what each assay is measuring as an endpoint, of how the measurement correlates with cell viability, and of what the limitations of the assay chemistries are. Improved simplicity of homogeneous methods and correlation with *in vivo* toxicity data have contributed to the increased use of cell-based assays as alternatives for animal testing in toxicology laboratories. There are a variety of ways to measure cell viability or cytotoxicity *in vitro* at the end of an experimental treatment period. As improved assay methods have been developed, there has been a trend to move away from radioactive or cumbersome methods and adopt simplified homogeneous methods that can be easily automated. The use of cell-based assays has grown in recent years to become an integral part of both basic research and the drug discovery process. Valuable information not obtainable in biochemical assays, such as compound permeability, stability, and target availability, can be gathered by using these systems.

With the advent of convenient and scalable homogeneous viability assays, it is now possible to gain information relating to cell toxicities from sizable compound libraries in multiple well models or panels before testing those new chemical entities in specific functional assays or animal models.

AUTOMATED CELL COUNTING AND FLOW CYTOMETRY ANALYSYS-MICROCYTOMETRY USING GUAVA TECHNOLOGIES PLATFORMS

Dr.Heidi Onderková

We hereby describe novel capillary based microcytometry method allowing absolute cells counting without beads. Cell count and viability assays are performed on instruments-flow cytometers including exchangable capillary. Results present are consistent with traditional methods.

Optimized kits,applications and assays include:

Guava ViaCount Assay-cell viability
Annexin V binding (Nexin Assay)
Multiple caspase activity (MultiCaspase)
DNA strand breakage (TUNEL)
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Guava Express Assay
Guava Cell Cycle Assay
CD4/CD8 Absolute Countin´g
Cell Tracking
Elispot

Guava platform presents benchtop instrumentation for personalized flow cytometry without sheath fluid, laser alignment . Instruments are standard tubes and plates compatible.

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CP 6

CELL LAB QUANTA, A COMBINATION OF FLOW CYTOMETRY AND THE COULTER PRINCIPLE

Roman Vlček

Beckman Coulter

Cell Lab Quanta™ SC is an advanced, yet cost effective flow cytometer with 3color, side scatter and cell size measurements. Side scatter and Coulter volume make size analysis and fluorescence measurements more precise

The gold-standard “Coulter Principle” for cell sizing and counting is the industry most highly regarded technique for precision and reliability. Volume measurements are not affected by shape, color, or refractive index

Multiple excitation wavelengths (nm), including UV from a Mercury (Hg) arc lamp and laser, provide flexible fluorochrome selection enabling applications typically only possible through expensive complicated systems. The Quanta SC system is optimized for excitation at 366, 405, 435, and 488nm.

Friday, September 15, 2006

Lecture room L4
16:50 – 18:50

Section 11 MEW METHODS II

Chairs: Ľudovít Varečka, Ladislav Bumba

L 11.1

FLUORESCENCE SPECTRAL MATRICES

Katarína Dubayová, Jaroslav Kušnír

Ústav lekárskej chémie, biochémie a klinickej biochémie,
Lekárska fakulta UPJŠ v Košiciach

The massive progres in bioinformatics and chemometrics in last years enables to apply new ideas and philosophy to the study of chemical composition and function of complex living systems The until recently unimaginable spectral analysis of a complex mixtures as one individuum, today becomes real (metabolomic/metabonomic profile analysis).

The application of 3-D fluorescence spectral techniques for the monitoring of complicated systems (biological or industrial mixtures, cellular mixtues or tissues, mitochondria, body fluids...) has found its stabile place in biochemical analysis. Defined system of fluorophores (individual for each mixture) enables to express sample originality in the form of fluorescence "fingerprints"- fluorescence spectral matrices. Different parameters involved in spectral measurements make possible to create two main types of fluorescence matrices – excitation-emission and synchronous (CW or CE) fluorescence matrices. The problem of nonlinearity of fluorescence and concentration of fluorophores is exclusively solved by fluorescence concentration matrices.

Generaly, all fluorescence matrices represent a complex qualitative and quantitative profiles of an analyzed system. Their comparison with a "standard matrix" (via pattern recognition techniques) displaces very effectively the anomalies in the profile. An application of various chemometric methods, artificial neuronal networks (ANN) or other mathematical apparatus is necessary for a precise evaluation and interpretation of a such profile analysis

L 11.2

TOWARDS STRUCTURAL AND FUNCTIONAL ASPECTS OF PLANT CHLOROPLAST AND CHLOROPLAST INNER MEMBRANES

Ladislav Bumba[1,2] and František Vácha[2]

[1] Inst. Microbiology, Videnska 1083, 142 20 Praha 4, [2] Inst. Plant Molecular Biology, Branisovska 31, 370 05 Ceske Budejovice

Progress in various fields of microscopy techniques brought up enormous possibilities to study the photosynthesis from the level of chloroplasts to the individual pigment–protein complexes embedded in the thylakoid membrane. The aim of this lecture is to present recent developments in the photosynthesis research obtained using such highly advanced techniques. Two areas of microscopy techniques covering optical microscopy and electron microscopy are discussed. Whereas the electron microscopy is used in photosynthesis mainly for structural studies, the optical microscopy is used also for functional studies.

The first part of the lecture is focused on the ultrastructure of plant chloroplasts studied by a single-molecule spectroscopy setup at a temperature of 77K and exploring spatial location of photosystems. Two chloroplast thylakoid membrane regions were visualized by fluorescence microscopy and detected at different wavelengths. The size of these regions and the spatial resolution of the microscope allowed us to measure their chlorophyll fluorescence emission spectra of these membrane domains. (Vacha *et al.* (2006) J. Lumin. *in press*).

The second part covers the use of negative staining and electron microscopy followed with single particle image analysis in structural studies of Photosystem II and Photosystem I pigment–protein complexes. It will be summarized information about the structure of these membrane complexes as well as its arrangement and interactions with their antenna proteins in thylakoid membranes of the most photosynthetic organisms. Results on subunit organization, with the use of immunogold labeling of 6x His tagged protein with Ni^{2+}–nitrilo triacetic acid Nanogold are compared with the data obtained by X-ray crystallography (Vacha *et al.* (2005) Micron **36**, 483–502).

L 11.3

MICRODIALYSIS AS A NEW TECHNIQUE FOR CYTOKINES DETECTION

Michaela Vítková, Ján Polák, Eva Klimčáková, Michaela Kováčiková, Jindra Hejnová, Zuzana Kováčová, Magda Bajzová, Vladimír Štich

3. LF, Univerzita Karlova, Praha

Microdialysis is a technique to monitor the chemistry of the extracellular space in living tissue. This method is based on the transport of substances from the extracellular space to microdialysate catheter through the membrane with various sizes of pores and allows repeated sampling of interstitial fluid and infusion of effector molecules into the tissue without influencing whole body function. The novelty of this technique is a determination of interstitial concentrations of cytokines and other proteins.

In our studies, we investigate the changes in protein levels involved in the metabolism of adipose tissue (e.g. IL-6) with microdialysis techniques using 100kDa catheters. For analysis of proteins in dialysate we use ELISA method. Simultaneously with ELISA, we have tested a new multiplex immunochemical technique using Luminex 100. The advantage of this analytical method is that only a very little amount of sample can be used for analysis of a number of proteins (up to 25 proteins in 50 μl of sample), which is essential in microdialysis (the microdialysate catheters are generally perfused by speed of l μl/min, thus the sample volumes are very low).

In our experiments, we proved the possibility to determine IL-6 in dialysate of adipose tissue in concentrations significantly higher when compared to its circulating plasma concentrations. Furthermore, dynamic changes in the regulations of protein secretions in adipose tissue, it means the changes in protein concentration in response to physiological stimulation during various interventions were observed. In conclusion, the microdialysis appears to be a suitable technique to investigate secretion of proteins by adipose tissue in vivo.

L 11.4

CAVER: A PROGRAM FOR MOLECULAR SPELEOLOGY.

Pavel Banáš[1], Martin Petřek[2], Michal Otyepka[1], Pavlína Košinová[1] and Jiří Damborský[3]

[1]Department of Physical Chemistry and Center for Biomolecules and Complex Molecular Systems, Palacky University, tr. Svobody 26, 771 46 Olomouc, Czech Republic

[2]National Centre for Biomolecular Research, Masaryk University, Kamenice 5/A4, 625 00 Brno, Czech Republic

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The biomacromolecules are pretty complicated chemical systems with many clefts, voids, pockets, and tunnels. In many cases, the sites of interest can be deeply buried in interior of the biomacromolecules and typically connected with molecular surface by several tunnels. The main aim of this study was to develop new software for rapid, accurate and fully automated identification of paths leading out from biomolecular clefts, pockets or cavities in dynamic and static structures.

The developed program CAVER[1] identifies and visualizes routes from the interior of the biomacromolecule outside. CAVER algorithm is sufficiently robust to allow the analysis of any molecular system, including proteins, nucleic acids or inorganic materials. Calculations can be performed using discrete structures from crystallographic analysis and NMR experiments as well as with trajectories from molecular dynamics simulations. The fully functional program is available as a stand-alone version and as plug-in for the molecular modeling program PyMol (http://loschmidt.chemi.muni.cz/caver/). Many examples of molecular speleology assisted by CAVER will be presented.

(1) Petřek M., Otyepka M., Banáš P., Košinová P., Koča J., Damborský J.: BMC Bioinformatics 7: 316, 2006.

Poster Section

Section 1

GENOMICS

PO 1.1

CHEMOKINE BINDING PROTEIN ENCODED BY M3 GENE OF MURINE HERPESVIRUS 72

Petra Belvončíková, Iveta Vančová Iveta, Valéria Hájnická, Marcela Kúdelová, Ingeborg Režuchová
Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovak Republic

Viral chemokine binding proteins represent an interesting class of antichemokines agents encoded by poxviruses and herpesviruses. One of them is encoded by lymphotropic Muride herpesvirus - 4 (MuHV-4) currently serving as an attractive experimental model for study on pathogenesis of human gammaherpesviruses (EBV and KSHV). Till now, three strains of MuHV-4 – MHV 68, MHV 72 and MHV 76 are the most intensively studied *in vivo* as well as *in vitro*. M3 gene identified in the genome of strain MHV 68 was found encoding for a distinct secreted viral chemokine binding protein-III (vCKBPIII) with high affinity to a broad range of mouse and of human chemokines thus blocking chemokine signaling.

In this study, M3 gene as well as M3 protein of strain MHV72 was investigated and compared to those of strain MHV 68. Primary structure analysis of MHV72 M3 gene revealed one codon changing mutation (from Asp to Gly) situated near the chemokine binding site and causing changes in the predicted secondary structure of the MHV72 M3 protein. The mutation found resulted in lowered hydrophilicity and also surface exposure of this region. We used baculovirus expression vector system as a tool to characterize MHV 72 M3 protein properties. Its ability to bind both mouse and human chemokines was proved. For three human chemokines - huMCP1, huIL8, and huRANTES, about two-times higher affinity was determined, while binding of huEotaxin and huMIP1α was similar (but not the same) as that of M3 protein of strain MHV 68.

Properties and functions characterization of M3 protein of MuHV-4, suggested as an important regulator of host immune response, could uncover new mechanisms of viral pathogenesis that may lead to new treatments or prevention strategies for virus-associated diseases.

PO 1.2

COMPARATIVE GENOMICS OF MOUSE HERPESVIRUS STRAINS ŠUMAVA AND MHV68

Jana Blaškovičová[1], Juraj Kopáček[1], Jela Mistríková[2], Miroslava Šúpolíková[2]

[1]Institute of Virology SAS, Bratislava, [2]Department of Microbiology and Virology, Faculty of Natural Sciences, Comenius University Bratislava

Mouse herpesvirus isolate Šumava (MHV-Šumava) is an oncogenic gammaherpesvirus which infects wild rodents and can readily infect experimental mice. MHV-Šumava is an isolate of strain MHV-68 which is closely related to human gammaherpesviruses such as Epstein-Barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV) and Herpesvirus Saimiri (HVS). MHV-Šumava differs from the other MHV isolates (MHV-68, MHV-72 and MHV-76) in some virological and pathogenetical features. To identify differences of MHV-Šumava from other MHV isolates on molecular basis, we analyzed its genome and searched for potential aberrations.

Our results indicate that MHV-Šumava is naturally occurring deletion mutant of MHV-68 that lacks 9,304 bp of the left end of the unique portion of the genome encoding nonessential pathogenesis-related genes. MHV-Šumava lacks the M1, M2, M3 genes, and a part of the M4 gene. Sequence analysis shows 96-100% homology of MHV-Šumava with MHV-68 and MHV-76 in the genome parts upstream and downstream from the deletion site of MHV-Šumava. The presence of another naturally occurring mutant suggests that this may be the common feature of MHV strains.

Supported by VEGA 1/2272/05

PO 1.3

THE GENOME OF μ1/6, A *STREPTOMYCES AUREOFACIENS* INFECTING PHAGE

Jarmila Farkašovská, Luboš Kľučár, Andrej Godány

Institute of Molecular Biology, Slovak Academy of Sciences, Bratislava

Bacteriophages are omnipresent in the enviroment and they are thought to play major roles in the ecological balance of microbial life, in bacterial diversity and bacterial pathogenesis.

We have focused our present work on the phage μ1/6 which has a narrow host range for industrially important *Streptomyces aureofaciens* strains producing tetracycline. The entire double-stranded DNA genome of the *Streptomyces aureofaciens* phage μ1/6 was sequenced. Its size is 38194 bp with an overall molar G+C content of 71.19%. Fifty-two potential open reading frames were identified, divided into two oppositely transcribed regions. In the left arm of the μ1/6 genome, we identified putative integrase and possible regulation proteins. The rightwards transcribed region contains genes organized into apparently four functional units responsible for: (i) DNA replication, (ii) DNA packaging and head assembly, (iii) tail assembly, and (iv) lysis. Comparative analysis with three complete genomes of streptomycete phages revealed resemblance with respect to the organization of their genes into functional moduls. Further, our results indicate that μ1/6 and streptomycete phages ΦC31 and ΦBT1 share only local protein similarities but the organization of head assembly genes seems to be conserved among them. Closer relationship was revealed between μ1/6 and VWB, covering regions probably responsible for lysogeny, replication and lysis. Many putative proteins of μ1/6 show homologies to proteins encoded by phages from diverse bacterial hosts. This protein homology argues for occurance of extensive horizontal genetic transfer among these bacteriophages resulting in mosaicsm of their genomes.

This work was supported by the VEGA grant no. 2/5070/25.

PO 1.4

POLYMORPHISMS AND MUTATIONS IN TUMOR SUPPESSOR GENES *P53* AND *BRCA* IN BREAST CANCER.

Mária Franeková[1], Pavol Žúbor[2], Andrea Štanclová[3], Tatiana Bohušová[4], Dušan Dobrota[4], Martin Péč [1], Peter Račay[4]

[1]Department of Biology; [2]Department of Obstetrics and Gynaecology; [3]Department of Pathological Anatomy; [4]Department of Biochemistry; Comenius University in Bratislava, Jessenius Faculty of Medicine, Martin

Breast cancer is the most common malignant disease of the women. Tumour suppressor genes represent one from the anticancer defence mechanisms. Products of these genes are activated by stress signals that cause the DNA damage. After activation they are involved in various processes as the cell cycle arrest, apoptosis or DNA repair mechanisms. *p53*, *BRCA1* and *BRCA2* are the important tumour suppressor genes. Polymorphisms and the mutations of these genes can influence the protein tumour suppressor function.

Many polymorphisms of the *p53* gene were observed. Well-known are the polymorphisms MspI in intron 6 and BstUI in exon 4 that can influence the function of the p53 protein. Both polymorphisms are supposed to be associated with development of cancer, including breast cancer.

Mutations in *BRCA* genes are responsible for the autosomal dominant inherited pattern of breast cancer susceptibility. Hundreds of different mutations in *BRCA1* and *BRCA2* genes have been described, for example frameshift mutations BRCA2 6174delT and BRCA1 5382insC that belong to the most frequent mutations in middle Europe.

In Slovak population the association of these genetic alterations with breast cancer risk was also observed especially among women younger 50 years.

This work was supported in part by grants no. aAV/1106/2004, UK/24/2004, UK/41/2005 and 2005/14-MFN-06.

PO 1.5

PLASMID pAG20 FROM *Acetobacter aceti* 3620.

Jozef Grones[1] and Miroslava Kretová[2]

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Bacteria of the genus *Acetobacter* are Gram-negative obligatory aerobic bacteria characterised by strong activities to oxidize alcohols and wide range of sugars. They are non-pathogenic, commonly available easily cultivable in laboratory condition grow at low pH, able to oxides different carbon substrates into organic acids and contain large scale of plasmid DNAs. In the bacterial strains of *Acetobacter aceti* CCM 3620 was identified by standard procedure purified plasmid pAG20. The DNA sequence of small cryptic plasmid pAG20 in *Acetobacter aceti* was determined at 3 064 bp with 51.6 % GC pairs. The plasmid encoded 186 amino acids protein which is important for a plasmid replication in Gram-negative bacteria except *Escherichia coli.* Two 21 bp large direct repeat sequence 1 and two 13 bp direct repeat sequence 2 were determined in regulation region upstream from gene encoded Rep protein. Vector pAG24 with kanamycin gene and two deletion derivates pAG25 and pAG26 without *rep* gene from plasmid pAG20 were construed. Plasmid pAG24 is replicated in a broad host range like *Escherichia coli, Acetobacter pasteurianus, Acetobacter aceti, Comanomonas* spp., *Serratia marcescens,* and *Shigella* spp..

PO 1.6

QUANTITATIVE ANALYSIS OF GENE EXPRESSION LEVELS IN HEP-2 CELLS AFTER INDUCTION OF APOPTOSIS.

Dita Královcová

Department of Biological and Biochemical Sciences, Faculty of Chemical technology, University of Pardubice

Apoptosis is the cell suicide mechanism that metazoan organisms have evolved to eliminate redundant, damaged or infected cells. This genetic program is vital for normal development, for maintenance of tissue homeostasis, and for effective immune system.

Apoptosis was induced by etoposide in Human laryngeal cell line Hep-2 during 0, 6 and 12 h. Etoposide belongs to the most commonly used class of anticancer drugs. The drug acts by inhibiting the ability of topoisomerase II to ligate cleaved DNA molecules. The changes in expression levels of two genes (p53 and DFFB) during etoposide treatment were evaluated by real-time reverse transcriptase-polymerase chain reaction (RT-PCR). The expression level of p53 significantly increased after 6 h as well as 12 h treatment with etoposide in Hep-2 cells. The expression level of DFFB significantly increased after 6 h treatment with etoposide.

PO 1.7

MENINGOCOCCAL ADHESION SUPPRESSES PRO-APOPTOTIC GENE EXPRESSION AND PROMOTES EXPRESSION OF GENES SUPPORTING EARLY EMBRYONIC AND CYTOPROTECTIVE SIGNALING OF HUMAN ENDOTHELIAL CELLS.

Irena Linhartová[1], Marek Basler[1], Jeffrey Ichikawa[2], Vladimír Peličic[3], Radim Osičkа[1], Stephen Lory[2], Xavier Nassif[3] and Peter Šebo[1]

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[3]*INSERM U570, Faculté de Médecine* René Descartes*, 156 rue de Vaugirard, 75015 Paris, France.*

Neisseria meningitidis colonizes human nasopharynx and occasionally causes lethal or damaging septicemia and meningitis. Here we examined the adherence-mediated signaling of meningococci to human cells by comparing gene expression profiles of human umbilical vein endothelial cells (HUVEC) infected by adherent wild-type, *frpC*-deficient mutant, or the non-adherent (ΔpilD) *N. meningitidis.* Pili-mediated adhesion of meningococci resulted in alterations of expression levels of human genes known to regulate apoptosis, cell proliferation, inflammatory response, adhesion and genes for signaling pathway proteins such as TGF-β/Smad, Wnt/β-catenin, and Notch/Jagged. This reveals that adhering piliated meningocci manipulate host signaling pathways controlling cell proliferation while establishing a commensal relationship.

PO 1.8

Recombinant Nucleocapsid Protein of Porcine Circovirus Type 2.

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Post-weaning multisystemic wasting syndrome (PMWS) is now a well-established disease of swine herds, causing important economic losses in many European countries, North America and Asia. PMWS is linked to the emergence of a new porcine circovirus type 2 (PCV2) that is a small non-enveloped circular single-stranded DNA virus in the *Circoviridae* family. PCV2 has two major open reading frames (ORFs). ORF1 encodes the viral replicase and is less variable, while ORF2 encodes the viral capsid protein (Cap) and displays a higher rate of variation. This implies that ORF2 may determine the type-specific features of PCV2. Simple and reliable diagnostic methods are sorely needed for detection of PCV2-specific antibodies and monitoring of PCV2 infection. In this study, we aimed to develop a modified direct enzyme-linked immunosorbent assay (ELISA) for detection of PCV2 using the recombinant capsid protein Cap as an antigen. The *cap* gene was cloned using an *E. coli* expression vector yielding Cap protein fused to a C-terminal 6xHIS affinity tag (Cap-6xHIS). No expression of of Cap-6xHIS was, however, observed. It is shown that this was due to rare codon usage within the *cap* gene. When the codons of *cap* gene were optimised for *E. coli,* the protein was produced at high levels. The purified protein has further been successfuly used to develop an ELISA for serological detection of PCV2 infection in pig farms.

PO 1.9

BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF NOVEL *Glu-1Dy* LOCI ALLELE IN HEXAPLOID WHEAT (*Triticum aestivum* L.)

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Abstract

The wheat (*Triticum aestivum* L.) contains six genes encoding high molecular weight (HMW) subunits of glutenin. Each of the loci *Glu-A1*, *Glu-B1* and *Glu-D1* contains tightly linked the genes x and y, which are related but have subtle differences in their structures and properties. We analyze old wheat varieties and landraces of the Carpathian Basin in Central Europe and we found one new allele which is encoded by *Glu-D1* loci. The present findings were substantiated by reserved phase high performance liquid chromatography (RP-HPLC) and by size-exclusion high-performance liquid chromatography (SE-HPLC) analysis. We used published primers for DNA identification of known alleles encoded by *Glu-D1* loci and we got any signal, what it suggests that our new allele has some changes insides of the coding region. The detailed analysis of the coding regions of the new allelic variant is in progress. We would like to underline that the last findings of new allele of *T. aestivum* encoded by *Glu-D1* loci was published approximately 17 years ago!

This work was supported by APVV from project 27-014504.

PO 1.10

SEQUENCE VARIABILITY OF S-alk(en)yl-L-CYSTEINE SULFOXIDE LYASE FROM GARLIC (*Allium sativum* L.) CLONES.

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Plants of the genus *Allium* gain their characteristic taste and odor from compounds containing several sulfur atoms. A prominent role in both traditional medicine and interhuman relationships accounts to garlic. Garlic (*Allium sativum* L.) is well-know vegetable. Garlic belongs to one of the oldest cultivated plant species. Garlic is used often in Czech cooking. The key enzymatic reaction preceding the generation of the smells is the production of a chemically reactive sulfenic acid which then spontaneously reacts to form healt promoting substances. Alliinase is the key enzyme to be involved, with full name S-alk(en)yl-L-cysteine sulfoxide lyase. The garlic enzyme cleaves S-allyl-L-cysteine sulfoxide in a beta-elimination-deamination reaction. Although importanceof the enzyme, up to now no complete genomic DNA sequence is available in the public databases. cDNA sequences are accessible only. We designed primers and amplified whole genomic sequences from different clones of garlic representing basic morphological types – bolting, semi-bolted and non bolting types, Czech germplasm and exotic germplasm. The amplified sequences were cloned (three independed amplifications per each clone by the use of proof-reading polymerase) into plasmids, transformed into bacteria and resulting recombinant plasmid were subsequetly sequenced. Number of introns and exons were identified and sequences were compared. We found SNPs in exons and introns. Frequency of SNPs is being verified by SNaPshot assay. Results of the work will be discussed.

PO 1.11

„BIOLOGICAL COST“ OF ACQUIRED ANTIBIOTIC RESISTANCE

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The rise of antimicrobial resistance in bacteria poses a growing challenge in the fields of medicine and public health. Dissemination of antibiotic resistance is mediated by clonal spread of a particular resistant strain and/or by the spread of resistance genes. Although bacteria can become resistant to antibiotics due to mutation of chromosomal genes, this is a relatively rare event. Much more probable is that a non-resistant cell will gain resistance through lateral gene transfer, which can occur at a rate of one in ten organisms. The latter involves the horizontal transfer of mobile genetic elements such as plasmids and transposons. Both these elements use cell machinery to propagate within the cell and thus give rise to additional biological cost associated with the replication and maintenance of the elements.

In our work “biological cost” of acquired antibiotic resistance in *Escherichia coli* was analysed based on the relative rates of growth, survival and competitive performance of antibiotic-sensitive and -resistant strains. Tetracycline resistant strain (R0/11) showed lower growth rate and fitness compared to isogenic antibiotic-sensitive strain. The cost of resistance was ameliorated by transfer function as no lowered growth rate and fitness were observed after transfer of resistance to laboratory *E. coli* strain.

Our data indicates that biological cost of acquired antibiotic resistance is greatly ameliorated by other function(s) encoded by mobile gene element mediating resistance transfer.

PO 1.12

GENETIC POLYMORPHISM OF GLUTATHIONE-S-TRANSFERASE P1 AND PROSTATE CANCER

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Prostate cancer is the third commonly diagnosed solid tumor in men by age 50. In the metabolism of numerous potential carcinogens are involved biotransformation enzymes, glutathione S-transferases (GSTs). There is evidence that suggests that detoxification enzymes may play a role in the formation of prostate cancer. In the present study, we investigated the association of GSTP1 polymorphisms with susceptibility to prostate cancer in the Slovak population. GSTP1 has a polymorphic site at codon 105 (exon 5), where an adenosine-to-guanosine (A-G) transition causes an Ile-to-Val substitution (Ile105Val) and produces a variant enzyme with lower activity and less capability of effective detoxification.
GSTP1 Ile105Val polymorphism was detected by PCR-RFLP using peripheral blood DNA. For GSTP1, the data were suggestive of trend of increasing risk with higher numbers of codon 105 valine alleles compared with isoleucine alleles. Our findings suggested that genetic variability in members of the GST gene family may be associated with an increased susceptibility to prostate cancer.

This work was supported by grants UK/264/2006, MVTS Bil/ČR/SR/UK/06 and AV 4/0013/05.

PO 1.13

MOLECULAR CHARACTERIZATION OF PRIMARY COLORECTAL CANCERS WITH PROGRESSIVE METASTATIC PHENOTYPE BY OLIGONUCLEOTIDE MICROARRAYS

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PURPOSE: Colorectal cancer (CRC) is one of the most common malignancies. Unfortunately a significant proportion of surgically cured patiens in the early stage of the disease develop progression and die from the disease. This study aimed to find individual up/down-regulated genes associated with progression and metastatic potential of colorectal cancers using low-density oligonucleotide microarrays. Molecular characterization of patients at high risk of cancer progression may assist to oncologists in treatment decision by selecting those patients who will need adjuvant chemotherapy. PATIENTS AND METHODS: Patients who underwent surgical resection were divided into different prognostic groups by disease-free survival (DFS>36 month – good prognosis; DFS<36 month – bad prognosis). Total RNA was extracted from each frozen tumor specimen and gene expression profiles were obtained using a human oligonucleotide microrray (SuperArray) spotted with 128 genes known to be involved in metastasis. Genes with expression levels showing at least a 2-fold difference were identified as differentially expressed. RESULTS: Characteristic gene expression profiles were obtained from tumor specimens of prognostically different patients with CRC. Expression analysis identified 21 differentially expressed genes (19 up-regulated, 2 down-regulated) in primary tumors of patients who had progressive disease (DFS<36). The functional categories of up-regulated genes belonged to cell cycle (MYC, HRAS, TP53), adhesion molecules (cadherins CDH8, CDH11, CDH19; molecule CD44; catenins CTNNA1, CTNNB1; chemokine CXCR4), matrix metalloproteinases (MMP7, MMP9, MMP10, MMP11, MMP13) and prometastatic factors such as growth factor IGF1, metastasis associated protein MTA1, transcription factor ETV4 (e.g. COX-2 and MMP-7 promoters activation) and scaffolding protein CAV1. The lower expression levels showed genes encoding negative cell proliferation regulator NME1 and plasminogen activator PLAUR. All of these expression differences are consistent with previous reports and molecular and cellular aspects of cancer progression and metastasis. CONCLUSIONS: Our preliminary data suggest that oligonucleotide microarray technology should contribute to a better understanding of the progression of colorectal cancers, and facilitate prediction of their metastatic potential. Analyzing of gene expression data from larger group of CRC patients will enable us to identify distinct prognostic subsets of patients based on molecular characteristics in the near future.

This work was supported by IGA MZ ČR NR/9076 – 4

PO 1.14

ASSESMENT AND DESIGN OF MOLECULAR GENOTYPING METHODS FOR DISCRIMINATION OF SALMONELLA STRAINS

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Salmonella enterica subsp. *enterica* represents one of the most frequent food pathogen causing enterititis and represents a major healthcare and economic problem. Even highly developed countries have reported considerably high number of infection cases in recent years, mainly due to emergence and spread of multiresistant *Salmonella* variants such as the phage type DT104 with combined resistance to 5 antibiotics. *Salmonella* represents a considerably diverse genus, for which phenotype based methods are capable of discriminating several thousands of serotypes and phage types. Precise typing of salmonellas is of great importance for epidemiological studies but also for food industry and healthcare. For these needs, phenotype based methods proved insufficient and unsuitable. Genotype based molecular methods offer significantly more detailed and precise information with epidemiological relevance.

We assessed several techniques for genotyping of *Salmonella* strains, among which multi-locus variability of tandem repeats analysis MLVA provided the greatest discriminatory power. Salmonellas are however subjected to a variability mechanism, which is mediated by lysogenic bacteriophages. Relatively high number of varying phage of salmonellas were used involved in our studies. Several phage specific gene targets were selected and used for screening of strains, with promising variability observed. Due to significant decrease in time costs, increase in discriminatory power and reliability of considered molecular methods when compared to phenotyping methods we expect their wider use in future industrial and healthcare applications.

PO 1.15

PRIMARY STRUCTURE OF THE GENOME OF LYMPHOCYTIC CHORIOMENINGITIS VIRUS STRAIN MX

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Lymphocytic choriomeningitis virus (LCMV) is a member of an Arenaviridae family and belongs to Old World group. Arenaviruses contain two segments of single stranded genomic RNA with dual polarity. The large RNA segment (L) encodes a virus polymerase (L) and a small Z protein (ZP) containing a zinc-binding RING domain. The small RNA segment (S) is coding for a nucleoprotein (NP) and a glycoprotein precursor (GPC) that is post-translationally cleaved to the external glycoprotein GP1 and the transmembrane glycoprotein GP2. Arenaviruses utilize a so-called ambisense replication strategy.

Arenaviruses are unique viruses that utilize several interesting molecular mechanisms and serve as important experimental systems for the investigations into virus-host relationships. LCMV is a prototype member of the Arenaviridae family and is used as an excellent model for the study of acute and persistent infections and of the role of the immune system during the virus infection.

In the past, our group had contributed to identification of a new MX strain of LCMV and determination of the primary structure of two virus genes NP and ZP. Here we present the sequences of additional MX LCMV genes. Using RT PCR we obtained cDNAs encoding glycoprotein (GP) and L protein and determined their primary structure. Comparative analysis with the known GP and L sequences from the other LCMV strains revealed that MX strain is phylogenetically most related to LCMV strains WE and Armstrong.

Supported by VEGA (2/5081/26)

PO 1.16

MACRONUCLEAR DNA ORGANIZATION IN SOIL PROTOZOAN *OXYTRICHA* SPP.

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Soil protozoan belonging to the family of hypotrichous ciliates *Oxytrichidae* was isolated in pure culture and analyzed. While morphological studies suggested that this protozoan is *Ochytricha* like, phylogenetic analysis of 18S rDNA indicated that it is closely related to *Halteria* genus. Similar to other oxytrichs, it carried in its cells two kinds of nuclei: a diploid micronucleus and a macronucleus with highly processed macronuclear DNA in the form of low molecular weight molecules with an average size of approximately 2200 base pairs. Random gene-sized chromosomes from our protozoan were cloned and sequenced. Sequence analysis has shown that each of them contained inverted terminal repeats of the telomere sequence 3' – dG_4T_4 – 5' which is perfectly conserved in all *Oxytricha* spp. macronuclear chromosome ends. All chromosomes examined shared identical structure with AT rich (nearly 100 %) leader and trailer sequences and single open reading frame. Canonical polyadenylation signal could be detected in some trailer sequences. The sequences obtained showed typical ciliate codon usage with TAA and TAG codons for glutamine, whereas in other organisms these are stop codons. In some of the clones the coding sequence was interrupted by short "in frame" intervening sequences, a typical intron feature of *O. trifallax* and other ciliates. Results from the genetic studies if this ciliate open the way to the investigation of genetic exchange between different soil organisms under natural conditions.

L 1.17

TELLURITE RESISTANCE GENES AND FITNESS OF THE UROPATHOGENIC E. coli CELLS

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The presence of tellurite resistance (***ter***) genes operons has been reported in several different human pathogens in spite of the fact that tellurium and its soluble salts are rare in nature and are no longer in use as antimicrobial agents. We cloned ***terW,Z,A-F*** genes from an uropathogenic ***E. coli*** isolate into pBluescript plasmid and introduced this construct into other tellurite sensitive uropathogenic ***E. coli*** isolates. The presence of the ***ter*** genes resulted in the increased level of potassium tellurite resistance as well as the level of resistance to oxidative stress mediated by hydrogen peroxide. These new strains were shown in the in vitro macrophage test to have prolonged ability to withstand the aggressive environment of phagolysosomes, the basic mechanism of the constitutive antibacterial defense of organism.

We therefore propose that the contribution of the ***ter*** genes to oxidative stress resistance and thus to fitness of bacteria is at least one reason for their presence within a broad range of pathogenic microorganisms as an important virulence factor. The presence of such operon on a large conjugative plasmid pTE53 represents an important tool for the evolution of new pathogens.

PO 1.18

ANALYSIS OF MURINE HERPESVIRUS STRAIN 72 AND 4556 (MHV72 AND MHV4556) GENOMES REVEALS GENES DIFFERENT IN PRIMARY STRUCTURE: PARTIAL RESULTS

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Murine herpesvirus (MHV) 68 established as *Murid herpesvirus 4* (MuHV4) is the best-characterized murine herpesvirus shown closely related to human gammaherpesviruses – KSHV and EBV. All MHV isolates (60, 68, 72, 76, 78, 4556, 5682, and ŠUM) originate from free-living murine rodents wrapped at the territory of Slovakia and Bohemia. Here, two MHV strains isolated from different murine species, MHV72 (*C. glareolus*) and MHV4556 (*A. flavicollis*) were one-plaque purified and their genomes/genes were investigated by restriction mapping and by primary structure analyses. To search for MHV specific gene block - M1, M2, M3, M4, and viral tRNAs (not found in strain MHV76), *Bam*HI, *Eco*RI, *Hind*III, *Sac*I and *Sph*I restriction maps of MHV72 genome were analysed. Southern blot analysis of MHV72 *EcoR*I B fragment digested with *Sph*I proved the full length of this genome region. Furthermore, the primary structure analyses confirmed the presence of M3 and M4 genes in MHV4556 genome. To assess the differences in MHV72/MHV4556 genes, the primary structure of 14/10 genes of MHV72 and of MHV4556 **w**as determined. We identified mutations unique for MHV72 genes (MK3, M3, and M7) and those unique for MHV4556 genes (MK3, M4, and gB). Some from mutations found give a rise to a new restriction site what takes chance for strain specific identification of either MHV72 or MHV4556 strain.

This work was supported by VEGA, the joint grant agency of Slovak Ministry of Education and Slovak Academy of Sciences (#2/4121/04) and the Slovak Research and Development Agency under the contract No. APVV-51-005005.

PO 1.19

COMPARISON OF HUMAN PAPILLOMAVIRUS 16 DISTRIBUTION IN SMEARS FROM TWO DIFFERENT CERVIX REGIONS OF ONCO-GYNAECOLOGICAL PATIENTS FROM WEST SLOVAKIA

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Human papillomavirus (HPV) infections are associated with benign and malignant lesions of cutaneous/mucosal epithelia. From nearly 100 HPV genotypes, a subset of HPV genotypes defined as high-risk types has been detected in cervical carcinomas/precursor lesions to point to them as a pre-requisite for cancer development. Far the most predominant is HPV16, being found mostly by PCR method in 54.4%/69.7% of cervical carcinomas worldwide/in Europe. Here, we present the first study on concurrent HPV infection and high-risk types distribution in exocervix and in endocervix. In our interest was to compare the HPV infection results gained by cytology and by PCR. At first, paired 102 cervical smears were investigated in PCR with consensus primers GP5/6 allowing detect at least six HPVs (6, 11, 16, 18, 31, 33). From paired cytological smears, at least at one cervical region 28 (54.9%) cases and at both regions 19 cases (37.2%) were positive. Then, the distribution of high-risk types 16 and 18 was revealed by specific PCR. At least one cervical region was found positive in 16 (57.2%) cases only for HPV16 and in 10 (35.7%) cases only for HPV18, respectively. Both HPV types were identified in paired cervical smears of seven (25.0%) cases but in one cervical region only. No patient was double infected at both cervical regions. For all samples, HPV16/18 prevalence found in this study was 43%. HPV distribution analyses confirmed more frequent double infection in exocervix, but higher prevalence of HPV16 in endocervix.

This work was supported by National program of Research and Development #SP 51/028 08 00/028 08 03, VEGA #2/4121/04 and by the Slovak Research and Development Agency under the contract No. APVV-51-005005.

Section 2

PROTEOMICS AND ENZYMOLOGY

PO 2.1

THE IDENTIFICATION OF ALBUMIN AND GLOBULIN FRACTION OF PROTEINS AND GLYCOPROTEINS ISOLATED FROM BARLEY GRAINS

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Protein and glycoprotein amount and composition have crucial influence on the suitability of grains of individual barley (*Hordeum Vulgare*) cultivars for its final uses (e.g. malt production).

In this work, the protein and glycoprotein composition of TRIS and water extracts are compared. Concanavalin A (Con*A*) lectin chromatography following by SDS–PAGE and gel IEF were used for the simplification of these complex samples. The gels were stained with Coomassie Brilliant Blue R 250 and proteins in the gel bands were identified according to the standard proteomic protocol using trypsin digestion and measurement of peptides masses by MALDI-TOF/TOF MS. Peptide masses and fragment MS/MS patterns were submitted to the web-accessible searching programs Mascot using SwissProt or NCBInr as searching databases. The attention was concentrated on the bands belonging to the proteins bound during on Con*A* chromatography thus potential high-mannose and hybrid types of *N*-glycoproteins.

The identified proteins include β-amylase, β-Glucosidase, Tryptophan decarboxylase, Protein Z, rRNA *N*-glycosidase, Endochitinase, etc.. The most interesting protein was Embryo globulin that was found in several bands (55 kDa, 36 kDa, 25 kDa, 21 kDa and 14 kDa). The pI values of proteins obtained by use of IEF provide good view of possible posttranslational modifications.

Acknowledgement: This work was supported by the Ministry of Education, Youth and Sports, Czech Republic (Research Centre No. 1M6215648902).

PO 2.2

STUDY OF WATER-SOLUBLE PROTEINS FROM BARLEY BY GEL ELECTROPHORESIS AND MALDI MASS SPECTROMETRY

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This study was undertaken to investigate and explain the effect of malting process on protein composition and posttranslational modification(s) between two different barley cultivars (Jersey and Tolar). For this purpose gel electrophoresis (both 1-D and 2-D) and matrix assisted laser desorption/ionization mass spectrometry were used. It is well known that the protein composition of barley determines the cultivar quality in terms of malting and brewing beer. Mainly water-soluble proteins contained in barley play crucial role in both malting and brewing processes and therefore our research was focused on them.

Based on the analyses of the protein pattern no relevant differences between Jersey and Tolar varieties were observed. More significant changes between grain and malt were observed which showed the influence of malting process to posttranslational modification of some proteins. The measured mass spectra confirmed that several proteins like NLTP1 and NLTP2 which are responsible for the formation of head foam were glycated.

Acknowledgement

This work was supported by the Research Centre for Study of Extract Compounds of Barley and Hop 1M6215648902.

PO 2.3

XENOBIOTIC INFLUENCE ON *ARABIDOPSIS THALIANA* PROTEOME

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Explosives belong to the dangerous soil and water pollutants, which are toxic or mutagenic to many life forms. Phytoremediation is emerging as an efficient treatment technology that uses plants to bioremediate pollutants from soil and water environments. At the present time, phytoremediation is still a nascent technology that seeks to exploit the metabolic capabilities and growth habits of higher plants: delivering a cheap, soft and safe biological treatment that is applicable to specific contaminated sites and wastewaters is a relatively recent focus. We have examined the ability of plants to remove and degrade xenobiotic aromatic nitrocompounds and nitroesters from contaminated waste water by *in vitro* cultures of *Arabidopsis thaliana*. This study focuses on the proteome analysis of *Arabidopsis thaliana* by two-dimensional gel electrophoresis. The phytoremediation proteome is given the hundreds of enzymes already identified as a participating in bacterial remediation processes. The aim of the study is to find and identify the changes in *Arabidopsis thaliana* proteome incurred by xenobitic influence.

Acknowledgement: *The study is supported by projects 1P05OC042 and 1P0ME730.*

PO 2.4

A NOVEL CADMIUM-BINDING PROTEIN FROM *Escherichia coli*

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Cells of *Escherichia coli* increase greatly the synthesis of a small primarily cytoplasmic protein YodA as soon as the cell growth rate falls below the maximal growth rate supported by cadmium exposure after which the mature product is exported to the periplasm. According to proposed a three-dimensional model for this receptor, YodA may be a metal-binding protein.

In the present work, to be ensure that YodA is able to bind metal *in vivo* conditions, we have constructed a mutant of *E. coli* with an insertional inactivation of the *yodA* gene. In experiments to test the ability of both, wild type and *yodA* mutant *E. coli* cells to bind cadmium, we have used gamma-labeled [^{109}Cd]. While wild type *E. coli* strain was able to bind a metal, Δ*yodA* mutant strain failed to do so. In addition, analysis of such a mutant demonstrated that it grows at a rate distinguishable from that of the isogenic parent in the presence of cadmium ions. However, challenging cells with hydrogen peroxide and additional metals such as zinc, copper, cobalt and nickel did not significantly affect on growth rate of the mutant. This growth phenotype was found to be the result of the loss of its ability to bind cadmium. These results suggest that the role of YodA protein may be to decrease of the concentration level of cadmium ions in *E. coli* cells during cadmium stress by its ability to bind heavy metal.

Acknowledgements: *This work was supported by VEGA Grant No. 2/4075/04 and APVV No. 51-0248-05.*

PO 2.5

STRESS PROTEINS IN THE CYTOPLASMIC MEMBRANE OF *BACILLUS SUBTILIS*

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Environmental stimuli induce the synthesis of both specific and general stress proteins in Bacillus subtilis. The aim of the study was to characterize stress proteomes of the cytoplasmic membrane of *Bacillus subtilis 168 trp$_{2}$-* exposed to acidic pH and ethanol. Although these stress factors impair the cell function in a specific manner, they share the ability to denature proteins. Therefore, specific and general stress proteins in the membranes were examined.

Both ethanol (3% v/v) and pH 5.0 increase the doubling time T of *B. subtilis* from 17 min to 25 min. Cytoplasmic membranes isolated from these cells were subjected to an optimized 2-D PAGE analysis. Two alternative methods of protein detection were used, i.e. silver staining and ^{35}S-Methionine puls labeling. In each experiment 4 parallel gels were prepared and about 400 protein spots were quantified using Molecular Imager scanner and PD Quest software. The stress induced proteins were identified by MALDI-TOF MS. In the silver stained gels five ethanol-specific stress proteins were detected as well as five acid-specific proteins. Two of these proteins found in both proteomes were identified as general stress proteins responsible for stress signalling; the specific stress proteins are involved in lipid metabolism and in the detoxification capacity of the cell. Three of the silver stained stress proteins were detected also in the radiolabeled gels. These data suggest the pattern of both the permanently and transiently produced stress proteins.

PO 2.6

BIOLOGICAL VARIABILITY OF SURFACE IMMUNOGENIC PROTEINS IN *Streptococcus agalactiae*

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Streptococcus agalactiae or group B (GBS) streptococcus is the leading cause of life threatening bacterial infections in newborns. GBS has also emerged as an important pathogen among adults, especially with chronic diseases (diabetes, cirrhosis and malignances) and immunodeficiencies. GBS is also the leading cause of mastitis in cows. Mastitis is the disease that affects milk quality and ultimately farm profitability. Some studies based on molecular biological characterization of human and bovine isolates suggested close relationship between them. However, bovine GBS isolates have higher chromosomal diversity and human GBS isolates have higher level of allelic diversity. The diversity of GBS strains has also been studied at the level of the distribution of functional traits such as virulence factors and antigens. The latter hold promise in using as vaccine components, an alternative for disease prevention. Some GBS surface proteins, such as R protein, the α and β subunits of the C protein, Rib protein, and alpha-like protein family have been investigated as potential vaccine candidates.

In this study a diversity of alpha-like protein family between bovine GBS isolates were characterized by PCR analysis and DNA sequence comparison.

PO 2.7

DIFFERENTIAL EXPRESSION OF *COXIELLA BURNETII* PROTEINS IN VIRULENT PHASE I AND AVIRULENT PHASE II

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Coxiella burnetii, an obligate intracellular parasite of eucaryotic cells, is the etiological agent of Q fever. The disease is a widespread zoonosis and is endemic throughout the world. *C. burnetii* is extremely resistant to harsh environmental conditions due to spore formation, it readily becomes airborne, and it is highly infectious for humans. For these properties, it is at the list of biological warfare (BW) agents in "Category B". The human infection is acquired most often by inhalation of contaminated aerosols, or less frequently, by drinking infected milk, though infection through skin trauma, sexual contact or mother to foetus transmission should also be considered. The acute form of Q fever is characterized as a flue-like illness or atypical pneumonia, or less frequently as granulomatous hepatitis with a significant incidence of neurological complications. Persistent infections may lead to chronic form of the disease that affects between 5 and 11 percent of those who contract acute Q fever. It may be associated with endocarditis.

C. burnetii proteins represent the major immunoreactive antigens in the serological diagnosis of Q fever. In spite of the fact that the complete genome of *C. burnetii* has recently been published, there is a lack of information on protein composition and structure. Proteomic methods are promising tool for obtaining new data in this respect and can be utilized for both diagnosis and prophylactic purposes. Our investigations have focused on the identification of differentially expressed *C. burnetii* proteins in virulent phase I and avirulent phase II as they were resolved by two-dimensional electrophoresis (2-DE).

Supported by APVT(51-032804) and VEGA(5030/25).

PO 2.8

ISOLATION OF IMMUNOGLOBULIN E (IgE) FOR DETECTION OF ALLERGOGENIC EPITOPES USING BIOAFFINITY MAGNETIC REACTORS

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Isolation of IgE is one of the pivotal steps for development of specific immunosorbent used for targeted detection and isolation of immunogenic epitopes of food allergens within epitope mapping techniques epitope extraction and epitope excision.

Human IgE is multifunction four-chain glycoprotein (Mw 190,000) with relatively high content of carbohydrates (~ 12%). Although the concentration of IgE in physiological human serum is extremely low (up to 480 ng/ml, i. e. 200 IU/ml), it has unique biological activities mainly in etiology of allergic diseases. Increased contents of IgE in serum is considered as one of the main markers of atopic reaction.

For minimal concentration of IgE in human serum in relation to other proteins is necessary, isolation to be enough effective and specific. Abundant proteins such as albumin and IgG have to be removed at first. The specific monoclonal anti-IgE antibodies and/or Concanavalin A immobilized on the magnetic particles SiMAG (Chemicell, Germany) and/or Estapor (Merck, France) were utilized for isolation and purification of serum IgE. Utilization of magnetic carriers has plenty of advantages and provides proper tool for rapid and simple detection of main allergogenic epitopes.

Financial support for this work was provided by the Ministry of Education, Youth and Sports of the Czech Republic (project MSM 0021627502) and by the Czech Science Foundation (projects No. 203/05/0241 and No. 203/05/2106).

PO 2.9

SEARCH FOR THE PROTEINS INTERACTING WITH THE NUCLEOPROTEIN OF LYMPHOCYTIC CHORIOMENINGITIS VIRUS STRAIN MX

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Lymphocytic choriomeningitis virus (LCMV) is an arenavirus that can develop a long-lasting persistent infection without disturbing vital functions of infected cells. It is an enveloped virus containing two RNA segments L and S, which encode nucleoprotein (NP), glycoprotein (GP), RNA polymerase (L protein) and Z protein (ZP). NP associates with the viral genomic RNA and L protein to form the viral ribonucleoprotein (RNP). GP-C is precursor of two virion glycoproteins, GP-1 and GP-2, which form spikes on the virion envelope and mediate cell entry. ZP is a small RING finger protein. It is a structural component of the virus that plays a role in transcriptional regulation and control of proteosynthesis. Persisting LCMV may exert its effects via cooperation with the cellular proteins. Ribosomal P protein and eIF-4E can be incorporated into virions and physically interact with ZP. ZP also binds with promyelocytic leukemia protein (PML), which is pro-apoptotic. Translocation of PML bodies to the cytoplasm during arenavirus infection may be involved in the antiapoptotic effect of the virus.

The purpose of our study has been to find new interaction partners of LCMV proteins. Using a pull-down assay with HeLa cells extracts, we detected a 68 kDa protein interacting with GST-NP. This protein has been identified by electrospray sequence mapping as cytokeratin 1. Further experiments to confirm the binding and elucidate its biological significance are in progress.

Supported by VEGA (2/5081/26).

PO 2.10

STRUCTURE-FUNCTION STUDY OF HYPOXIA-REGULATED CARBONIC ANHYDRASE IX USING IN VITRO MUTAGENESIS AND SPECIFIC SULFONAMIDE INHIBITORS

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Carbonic anhydrase IX (CA IX) is a cancer-associated transmembrane protein which consists of N-terminal proteoglycan-like region (PG), carbonic anhydrase domain (CA), transmembrane segment (TM) and short intracellular tail (IC). To determine the function of CA IX domains, we prepared series of CA IX variants with mutations and deletions in different parts of the protein and expressed them in MDCK cells. The transfected cells were analyzed for their hypoxia-induced capacity to acidify extracellular environment, bind CA IX-selective sulfonamide and destabilize cell-cell adhesion.

Despite the correct cellular localization of all tested CA IX mutants and deletion variants, only MDCK cells expressing wild type CA IX and PG deletion variant were able to decrease extracellular pH under hypoxia. Deletion of CA domain, changes in amino acid composition or shortening of IC tail led to loss of acidification capacity. The mutant CA IX proteins also showed reduced or lost capacity of cell dissociation. Finally, we demonstrated that the hypoxic MDCK cells that express the wild type CA IX protein can bind and accumulate FITC-labeled CA IX-selective inhibitor, whereas no inhibitor accumulation was observed with CA-deletion variant and some IC mutants of CA IX. Our results show that both extracellular and intracellular domains are important for the proper function of CA IX.

Supported by APVV (51-0248-05) and EUROXY (LSHC-CT-2003-502932)

PO 2.11

HUMORAL CHANGES IN PLEURAL SPACE ASOCIATED WITH CANCER, INFLAMMATION AND INJURY

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Analysis of pleural fluids provides a unique oppurtunity to study secretory products of genes associated with cancer, inflammation and injury. We performed a multifactorial analysis including in addition to current effusion analytes components of proteolytic system, markers of inflammation and adhesion molecules, in 90 effusion samples of different origin. We also analyzed tens of pleural fluid samples collected after thoracoscopy and talc-induced pleurisy representing acute injury/inflammation. We found a powerful humoral response, involving most parameters measured, in parainflammatory and talc-induced pleurisy exudates, a markedly lower reaction in majority of paraneoplastic exudates, and almost negligible in transudates caused by haemodynamic disorders. Minor portion of tumor-associated fluids, however, displayed parainflammatory patterns. Interestingly, this subgroup included majority of metastatic tumors. A significant correlation among a great deal of reacting markers suggests a coordinated, inflammatory type response, both in malignant and inflammatory effusions, differing in the degree of expression only.

This work was supported by the Ministry of Health, Grant No NR 7891-3, and by the Research Project MSM 0021620819, The Czech Republic.

PO 2.12

PROTEOME CHANGES IN CHRONIC LEUKEMIA CELLS JURL-MK1 INDUCED BY IMATINIB MESYLATE TREATMENT

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Chronic myelogenous leukemia (CML) is a malignant cancer of the bone marrow which is characterized by the occurence of an oncogene bcr-abl in CML cells. Imatinib mesylate® (Gleevec) is currently the leading drug for CML treatment; it works by blocking an abnormal BCR-ABL kinase activity. By biochemical means including methods of proteomic analysis we studied the mechanism of cytotoxic effects of Imatinib mesylate on the CML derived cell line JURL-MK1.

Treatment with Imatinib mesylate (1μM) induced G1/G0 phase arrest within 24h in JURL-MK1 cells, followed by a decrease of Ki-67 expression and induction of apoptosis. DNA fragmentation is achieved within 48h monitored by TUNEL assay. Expression of erythroid differentiation marker, glycophorin A, increased in parallel with the proceeding apoptosis.

The proteomic analysis (2-D electrophoresis combined with MALDI-TOF mass spectrometry) revealed several proteins that were affected by Imatinib mesylate treatment (1μM, 48h). These proteins included 14-3-3 proteins, chaperone HSP70, proliferating cell nuclear antigen (PCNA) and tropomyosin (in more detail see report by Pluskalová et al.).

The work was supported by grant VZ 00023736 of the Ministry of Health, Czech Republic.

PO 2.13

HOW IMATINIB MESYLATE DOES OPERATE IN CML-DERIVED CELL LINE JURL-MK1?

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Chronic myelogenous leukemia (CML) is a serious malignancy of hematopoietic stem cell. Imatinib mesylate® (Novartis Pharma AG, Basel) represents the most efficient drug in CML therapy to date. We deal with a study of molecular pathways affected by Imatinib based on the proteomic approach in a CML-derived cell line JURL-MK1 (see report by Grebeňová et al).

The well known effect of Imatinib mesylate® is the inhibition of the oncogenic kinase Bcr-Abl. Nevertheless, we noticed two other important features involved in the regulation of Bcr-Abl after Imatinib treatment. Bcr-Abl bears the anti-apoptotic properties partially due to its cytoplasmic localization. We revealed a cleavage of the protein 14-3-3 responsible for the nuclear localization of several client proteins including Bcr-Abl. We suppose the change of cellular localization of Bcr-Abl due to Imatinib treatment, which contributed to apoptotic induction. The second observed event is related to the stability of Bcr-Abl protein. We detected suppression of Hsp90 and p23 proteins, which form a multichaperonic complex essential for Bcr-Abl stability. Thus, Imatinib affects not only activity, but also the localization and expression of Bcr-Abl.

Further on, we revealed other proteins affected by Imatinib treatment. We detected suppression of the proliferating cell nuclear antigen (PCNA) which is involved in the regulation of cell proliferation, as well as the decreased protein level of tropomyosin which contributes to the cytoskeleton stability.

The work is supported by grant VZ 00023736 of the Ministry of Health, Czech Republic.

PO 2.14

ANTIMICROBIAL AND PHYSIOLOGICAL ACTIVITY OF SOME EXOGENOUS HONEYBEE PROTEINS AND PEPTIDES.

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Honeybee, similarly to other insects, response to bacterial infection by inducing higher expression of the genes coding for antimicrobial peptides, which are subsequently secreted into haemolymph. A special case of defence of honeybee are antimicrobial proteins and peptides present in royal jelly (RJ), the honeybee larval food. We have characterized new antimicrobial RJ proteins and peptides, which have a crucial defensive role in the first phase of infection of *Paenibacillus l. larvae*, the honeybee pathogen which cause honeybee disease - American fouldbrood and we have found out the antifungal activity of the peptides as well. Thus antimicrobial peptides could be an important tool for study of molecular mechanisms of larval infection and for identification of candidate genes of antimicrobial resistance of honeybee. Our preliminary experiments indicate potential biological activity of the RJ proteins and peptides as stimulators of tumour necrosis factor-alpha (TNF-alpha) in mouse macrophages and haemolytic activity. The release of cytokines by mouse macrophages mediated by the RJ-proteins could explain the broad therapeutic properties of honeybee products. In these processes, TNF-alpha could play a pivotal role as the factor regulating cell proliferation and inflammation as was observed in other organisms.

Acknowledgements: This work was supported by EU 6RP – BeeShop, No. FOOD-CT-2006-022568; VEGA grant Agency of Ministry of Education of Slovak Republic and Slovak Academy of Sciences, No. 2/4059/04 and Max-Planck Society for Partner Group of Slovak Academy of Sciences.

PO 2.15

LARVAL PROTEINASES OF HOUSEFLY *MUSCA DOMESTICA*. Endo- and exopeptidolytic activity into natural and chromogenic substrates

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As protein degradation is essential physiological process, also in insects, considerable effort was made by many authors to elucidate which type of peptidolytic enzymes are involved in this process. All four classes of digestive proteinases which have been identified in vertebrates also occur in insects. Many authors have reported, that predominant activity belongs to serine proteinases of two different subclasses, trypsin-like and chymotrypsin-like and weaker aspartyl proteinase.

In the present paper the distribution of enzyme activities into homogenates and supernatant fractions of larvae of *Musca domestica* in three different larval stadium was determined. All assayed enzymes were present in higher concentration in soluble supernatant fraction. Unexpectedly the major part of peptidolytic activity was of aminopeptidase nature, substrate L-Leu-p-NA. Practically no activity was found for elastolytic activity, substrate Suc-$(Ala)_3$-p-NA. Trypsin-like and chymotrypsin-like activities determined to specific chromogenic substrates, when compared with aminopeptidase activity, were markedly lower. Enzymatic activities determined into all types of class specific substrates were apparently highest in first larval stadium. The enzymes showed maximal activity in the pH range 8-9 with both types of chromogenic substrates and natural hemoglobin too and enzymes were fully inactive below pH 3. With hemoglobin there was second peak in acidic pH with maximum around pH 4.

Acknowledgement: *This work was supported by the research grant from Slovak Grant Agency APVT, research project no. APVT-20-007404*

PO 2.16

PROPERTIES OF PARTIALLY PURIFIED LARVAL AMINOPEPTIDASES OF HOUSEFLY MUSCA DOMESTICA

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Proteinases and peptidases in the larval form of flies are involved in digestion of proteins in food and also in degradation of intracellular proteins. Many authors have been reported, that they belong to serine proteinases of trypsin- and chymotrypsin-like type. In our work we have partially purified aminopeptidases in first larval stadium of housefly Musca domestica by ammonium sulfate precipitation and ultrafiltration on Centriflo membrane cones. By the use of substrate L-Leu-p-NA, which is specific chromogenic substrate for leucin aminopeptidase we ascertained K_m value (1.61×10^{-4} mol.l^{-1}). We studied inhibitory effects of some metals and specific enzyme inhibitors (Trypsin inhibitor from soybean, Phenylmethanesulfonyl fluoride (PMSF), Tosyl-L-phenylalanine chlormethyl ketone (TPCK), Nα-Tosyl-L-lysine chlormethyl ketone (TLCK), Chymostatin, p-Chloromercurybenzoic acid, Iodoacetamide). The enzyme activity was totally inhibited by some bivalent metal ions (Pb, Zn), markedly inhibited by heavy metals (Co, Cd), but was not practically influenced by other assayed cations (Ca, Ba). Mg and Mn had slight inhibitory effects. Inhibitory effect was observed in case of p-chloromercurybenzoic acid (2% of inhibition), TLCK (6% of inhibition) and EDTA (62% of inhibition). From our results on inhibitory effects of some metals and specific enzyme inhibitors we can suppose that prevalent amounts of peptidolytic enzymes present in first larval stadium of housefly are of aminopeptidase nature belonging to metalloproteinase group. These results supposed that aminopeptidases of insect nature are similar to vertebrate aminopeptidases.

Acknowledgement: *This work was supported by APVT grant APVT-20-007404*

PO 2.17

LIPOXYGENASE ACTIVITY OF POPPY SEEDLINGS AND CELL CULTURES OF *PAPAVER SOMNIFERUM* L.

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Lipoxygenases (LOXs, EC 1.13.11.12) are a class of widespread dioxygenases with important role in the octadecanoid pathway activated at distinct stages of development and during the interaction of plants with pathogens, insects, or abiotic stress.

LOX activities during the early stages of germination of *Papaver somniferum* L. (*Papaveraceae*) have been analyzed. The highest LOX activity was observed on the fourth day of germination. By using 4-days-old poppy seedlings LOX was identified in the 100.000 x g supernatant and also in the microsomal fraction. Two LOX isoforms were isolated from 100.000 x g supernatant of poppy seedlings using ammonium sulphate precipitation followed by gel filtration (Sephadex G-150), ion exchange chromatography (HA-Ultrogel) and hydrophobic chromatography (Phenyl-Sepharose CL-4B). Percoll density gradient was used for chloroplast separation and after their disruption, LOX was isolated by size-exclusion and hydrophobic chromatography.

The influence of enzyme in response to treatment of cell suspension cultures of *Papaver somniferum* L. with abiotic elicitor (methyl jasmonate) was also investigated. The effect of 10, 100 and 1000 μM concentrations of methyl jasmonate was tested. The elicitor was in contact with the culture for a period of 2, 4, 6, 8, 24 and 48 hours. Elicitation of cell suspension cultures triggered the markedly induction of LOX, so this process point to the activation of plant defensive responses.

PO 2.18

POLYPHENOL OXIDASE FROM LATEX OF COMMON CELANDINE (*Chelidonium majus*).

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Chelidonium majus is a source of several benzophenanthridine alkaloids, mainly sanguinarine and chelerythrine. Their biosynthesis begins with the condensation of dopamine and 4-hydroxyphenyl-acetaldehyde to form the first alkaloid intermediate, (S)-norcoclaurine. Polyphenol oxidase (PPO) is assumed to be involved in the formation of dopamine from tyrosine.

PPO was isolated from the latex of common celandine. The latex was collected from stems of full-developed plants in an equal volume of 0,5 M manitol/phosphate buffer. After centrifugation, 1000g organelle fraction and supernatant were obtained. The PPO activity was detected in organelle fraction predominantly. The enzyme was purified using gel filtration (Sephadex G-150) and affinity chromatography (*p*-amino-benzoic acid coupled to Sepharose *CL-4B*). The enzyme was purified 87-fold and analyzed by SDS-PAGE. One band corresponding to Mr 64 kDa was obtained. The purified latex PPO exhibited high diphenolase activity in comparison with almost unmeasurable monophenolase activity.

Using polymerase chain reaction the 600 bp long fragment of the PPO gene was prepared and cloned. The homology is high for polyphenol oxidases from other plants. Six conserved hystidyl residues from Cu-binding domains were identified.

PO 2.19

THE EFFECT OF BIOTIC STRESS ON THE ACTIVITY OF NADP-MALIC ENZYME IN PLANTS FROM *NICOTIANA* GENUS

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The influence of biotic stress caused by viral infection on the activity and isoenzyme content of NADP-malic enzyme EC 1.1.1.40 (NADP-ME) in plants from *Nicotiana* genus was studied. The biological role of NADP-ME, apart from being involved in C_4 and CAM photosynthesis, is regulation of cytoplasmatic pH, providing NADPH for lipid and secondary metabolites biosynthesis.

The NADP-ME activity was 5-times higher in leaves of plants *Nicotiana tabacum* L., cv. Petit Havana, SR1 infected by potato virus Y (PVY^{NTN}) as compared to healthy control plants. The NADP-ME activity was also enhanced in roots of these plants, but only 1.4-times. There was only one isoform of NADP-ME in leaves, stems and roots of *Nicotiana tabacum* detected after non-denaturating electrophoresis. Different results were obtained from similar experiment with plants *Nicotiana benthamiana* and transgenic plants *Nicotiana benthamiana* carrying gene for viral non-structural protein HC-Pro, which is responsible to the pathogenicity and can change sensitivity to viral infection. No significant increase in the NADP-ME activity in leaves of these plants was found. However NADP-ME in leaves and stems was present in 2 isoforms. Only in roots of these plants the activity of NADP-ME was enhanced twice as compared to healthy controls. In this study, no differences between non-transgenic and HC-Pro plants were obtained.

Acknowledgement: *This work was supported by grant: Grant Agency of the Charles University 428/2004*

PO 2.20

CHARACTERISATION OF EXOPOLYGALACTURONASE AND OLIGOGALACTURONATE HYDROLASE FROM PARSLEY ROOTS

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Two pectic enzymes, exoPG (exopolygalacturonase) and OGH (oligogalacturonate hydrolase), were isolated from parsley roots and partly purified. Both enzymes were further characterized and inbetween compared. In case of exoPG pH optimum were determined at 5.2 and temperature optimum nearby 70°C. For OGH optima were determined at pH 4.2 and 60°C. Single band, obtained from the SDS-PAGE, shows identical Mr (55.3kDa). Determined isoelectric points are almost the same, for exoPG the pI value was found at 5.3 and for OGH nearby 5.2. Both exohydrolases cleave glycosidic α-1,4-bonds of D-galacturonan at its non-reducing end yielding D-galactopyranuronic acid as the only reaction product. Comparison of initial rates of reactions with various substrates of different degree of polymerization (DP) shows, that the affinity of both enzymes to the substrates increases with the increasing degree of substrate polymerization. In the case of exoPG the polymeric substrate is the most suitable and it is cleaved fastest while dimer is hydrolased very slowly. On the other hand, OGH prefers oligomers with lower DP and the polymer is splitted slowest, thereby this enzyme is supposed to be an oligogalacturonate hydrolase. Edman degradation also demonstrates the inherence of both ezymes, whereas N-terminal amino acid of main exoPG is threonin and of the OGH is alanin.

This work was supported by grants from the Slovak Grant Agency for Sciences (VEGA) No.2/6133/26, No.2/4133/04 and by grant No.II/2/2005 from the Slovak Academy of Sciences to Centre of Excellence GLYCOBIOS. Financial support from Ministry of Education, Youth and Sports under MSM 021630501 is greatly appreciated.

PO 2.21

TWO FORMS OF XTH IN PARSLEY ROOTS

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In higher plants, cells are surrounded by a wall, which defines the cell's shape and thereby contributes to the structural integrity and morphology of the entire plant. One of most prominent cell-wall associated enzymes in higher plants is xyloglucan endotransglycosylase/hydrolase (XTH, EC 2.4.1.207). Enzymes of XTH family react with the polysaccharide xyloglucan (XG) by random cleavage of the β-1,4-polyglucose backbone of the donor molecule and transferring the created potential reducing end to the molecule of water (hydrolysis) or to hydroxyl group at C-4 on the non-reducing end of another XG molecule or XG-derived oligosaccharide (transglycosylation). In this work, XTH was partially purified and characterized from parsley roots (*Petroselinum crispum* cv. "*Olomoucká dlouhá*"). At least two forms with different isoelectric points and pH optima were found. The two forms had the same molecular weight about 33 kDa. For the form with pH optimum 5.8 the K_M values for xyloglucan-derived oligosaccharide (XGOs) as acceptor and XG as donor were established. The measured data showed higher affinity for XGOs than XG and indicated sequential mechanism of enzyme reaction. Increasing concentrations of XGOs increased the hydrolytic activity of XTH and enzyme attacked XG polymer more randomly. The other form was inactive in basic region but it was stable in a broad pH range as well as the first form.

This work was supported in parts by grants from the Slovak Grant Agency for Sciences (VEGA) no.2/6133/26 and no.2/4133/04 and by grant No. II/2/2005 from the Slovak Academy of Sciences to Centre of Excellence GLYCOBIOS.

PO 2.22

IMMUNOCHEMICAL ANALYSIS OF PLANT NITRIC OXIDE SYNTHASE

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The signalling role of NO has been demonstrated in vital plant development processes like germination, growth, stomata movement, maturation, senescence and also in plant responses to external stress stimuli. However, the available research knowledge on the exact sources and localization of NO production in plant cells and organs is still very limited. Only very recently, the only known plant analogue of animal nitric oxide synthase (NOS), has been discovered and characterized in Arabidopsis. In our experiment we aimed to prepare specific polyclonal antibodies to AtNOS protein. We have selected two peptides from the published AtNOS protein sequence on the base of immunogenic parameters predicted by DNASTAR Lasergene software. The synthetised peptides were covalently linked to keyhole limpet hemocyanine and injected intramusculary and/or intradermally to the rabbits. The anti-AtNOS antibodies were partially purified from the rabbit serum by affinity chromatography on columns with covalently linked AtNOS peptides. Partially purified anti-AtNOS antibodies were used to identify AtNOS in tissue extracts of Arabidopsis and NOS-like protein in other plant species after SDS-PAGE electrophoresis and blotting. The identity of immunopositive proteins was further investigated by the MALDI-TOF-MS analysis.

This work was supported by the grant MSM 6198959215 from the Ministry of Education, Youth and Sports of the Czech Republic.

PO 2.23

THE INHIBITION OF COPPER DIAMINE OXIDASE BY REACTIVE NITROGEN SPECIES

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Copper amine oxidases (Cu-AO, amine oxygen oxidoreductase deaminating, copper-containing; EC 1.4.3.6) belong to a widespread class of enzymes involved in the catabolism of biogenic amines and polyamines. The oxidation of polyamines by Cu-AO may generate other biologically active substances, such as aldehydes, ammonia and hydrogen peroxide, in cell signalling, growth and stress response. Cu-AO exist as dimers, each subunit contains one tightly bound Cu(II) and one 6-hydroxydopa quinone (TPQ) prosthetic group. Recently, an irreversible inactivation of lentil seedlings Cu-AO by adducts of polyamines and nitric oxide (NO) called 'NONOates' has been reported, presumably through the attack by NO of the Cu(I)-semiquinolamine radical catalytic intermediate in the active site of Cu-AO. We attempted to characterize in more detail the mechanism of the enzyme inhibition and the reaction products. We determined enzyme inhibition parameters on incubation of purified lentil and pea Cu-AO with NO-donors spermine-NONOate and MAHMA-NONOate. Spectral changes corresponding to the covalent modification of TPQ group by NO were recorded. The product of the reaction was isolated from NO-modified Cu-AO by enzyme digestion and further analysis by reverse-phase HPLC and mass spectrometry. Obtained results aim to bring more knowledge on the possible relation between NO as ubiquitous plant signalling molecule and the regulation of plant polyamines catabolism.

This work was supported by the grant MSM 6198959215 from the Ministry of Education, Youth and Sports of the Czech Republic.

PO 2.24

DETERMINATION OF HYDROGEN PEROXIDE IN PLANT EXTRACTS BY AMPEROMETRIC SENSOR

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The production of reactive oxygen species (ROS), particularly H_2O_2, appears to be ubiquitous defense mechanism of eucaryotes. H_2O_2 is comparatively more stable in comparison with another ROS, e.g. superoxide radical anion or hydroperoxyl radical, and can cross biological membranes. Several roles for hydrogen peroxide during pathogen infection of plants have been proposed: as direct antimicrobial agent, as activator of defense genes, as agent for crosslinking substances to limit pathogen infection and as trigger of the hypersensitive response, cell death and salicylic acid production. Colorimetric methods are more frequently used for detection of H_2O_2 in plant extracts. Our new method for the determination of hydrogen peroxide concentration uses a peroxide electrode (Pt-electrode +650 mV versus Ag/AgCl) equipped with cellulose acetate membrane serving as a barrier for the elimination of some interfering substances of plant extract that could oxidize and affect electrode signal. The H_2O_2 was extracted by homogenization of plant material with 0.1 M hydrochloric acid.

This work was supported by the grants MSM 6198959216 and MSM 6198959215 from the Ministry of Education, Youth and Sports of the Czech Republic.

PO 2.25

THE IMPACT OF SOME PLANT HORMONES ON THE METABOLISM OF PROLINE IN WHEAT

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This paper reports the results of experiments in investigating the effect of 24-epibrassinolide (24-epi) and cytokinin (benzylaminopurine-BAP) on the activity of glutamate kinase (EC 2.7.2.11), chlorophyll and proline content in leaves of spring wheat. Proline plays an important role in plants in the regulation of their metabolism during stresses. The experiments were carried out in field and the preparations were applied in a spray in concentration 10^{-6} M. The enzyme glutamate kinase (GK) starts the biosynthesis of proline from glutamate and its activity was determined by the modified hydroxamate method[1,2]. Chlorophyll content was assessed spectrophotometrically from acetonic extract.

The activity of GK grew up to 132.5 % (24-epi) and 181.5 % (BAP) in comparison with the control plants. Both preparations have stimulating effect on the chlorophyll content.

Amino acid analysis of plant extracts was carried out by AccQ*Tag method[3]. HPLC system with a fluorescence detection was used in the measurements. Reversed-phase HPLC with a gradient elution was optimized for the determination of proline. The treatment with 24-epi and BAP caused rapid increase of proline concentration in plants.

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This work was supported by the grant MSM 6046070901 from the Ministry of Education, Youth and Sports, Czech Republic.

PO 2.26

STREPTOMYCETES EXTRACELLULAR NUCLEASES EXPRESSED IN *E. COLI*

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Streptomyces sp. are Gram-positive filamentous bacteria adapted for growth in soil by morphological differentiation involved in their life cycle. Filaments of substrate mycelium in vegetative phase often lack cross-walls and have several copies of chromosome. As the colony ages, a characteristic aerial mycelium is formed, which subsequently fragments and sporulates. This is similar to the growth and differentiation of fungi and from the morphological and metabolic points of view, *Streptomyces sp.* can be considered as boundary organisms. In addition, the onset of aerial mycelium formation is occurring with a noticeable dying of the substrate hyphae. This fact supports the hypothesis that the aerial mycelium reuses material first assimilated into the substrate mycelium. The DNA of the substrate mycelium should be recycled by means of deoxyribonucleases. As *Streptomyces sp.* are common in soil, where a large rank of organic polymers have to be utilized, they are generally considered to be distinguished producers of hydrolytic enzymes. These include non-specific extracellular nucleases, similar to that fulfilled by other degradative enzymes. We have focused on determination and closer characterization of these enzymes. This study is oriented to molecular-biological characterization of extracellular nucleases from *S. coelicolor* A3(2) and *S. aureofaciens* B96.

PO 2.27

MALATE DEHYDROGENASE FROM *STREPTOMYCES AUREOFACIENS*

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The malate dehydrogenase (MDH) was purified from the strain of *Streptomyces aureofaciens* to electrophoretic homogeneity. The molecular mass of native MDH was estimated as 70 kDa, which composed of two 38 kDa subunits. It showed a strong specificity for NADH and was much more efficient for the reduction of oxaloacetate than for the oxidation of malate, with a pH optimum around 8. *S. aureofaciens* MDH has a typical N-terminal „signature" sequence specific for *Actinomycetales* and a „MDH-type" NAD^+-binding motif GAAGXXG. The *S. aureofaciens* MDH showed a low specificity to NADPH coenzyme too, the specific activity did not excess of 1.5 % the specific activity with NADH coenzyme. These functional characterization of *S. aureofaciens* MDH shows, that enzyme is very similar in many respects to other bacterial MDHs with the notable exception of a lack of inhibition by excess oxaloacetate and a low NADPH specificity.

Purified *S. aureofaciens* MDH was crystallized either in the absence or in the presence of NADH and NADPH coenzymes by hanging-drop vapor-diffusion method. An X-ray study showed, that MDH crystals belong to space group C222 with unit-cell parameters a = 102.2 Å, b = 509.6 Å, c = 51.9 Å, $\alpha = \beta = \gamma = 90$ degree; MDH-NADH crystals to space group C2 with unit-cell parameters a = 103.7 Å, b = 523.7 Å, c = 105.4 Å, $\alpha = \gamma = 90$ degree, $\beta = 101.14$ degree; and MDH-NADPH crystals to space group $C222_1$ with unit-cell parameters a = b = 72 Å, c = 520 Å, $\alpha = \beta = \gamma = 90$ degree. The crystal of native MDH diffracted to 2.0 Å resolution. *This work was supported by the Grant Agency of the Slovak Republic (VEGA), grant No. 1/3240/06.*

PO 2.28

REGULATION OF THE PHOSPHATIDYLGLYCEROLPHOSPHATE SYNTHASE IN KLUYVEROMYCES LACTIS

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The *KlPGS1* gene encodes the phosphatidylglycerolphosphate synthase (PGPS), the essential enzyme in the biosynthesis of mitochondrial anionic phospholipids cardiolipin (CL) and phosphatidylglycerol (PG) in the yeast *Kluyveromyces lactis*.

In this study we analysed the regulation of the *KlPGS1* expression. It is known that the biosynthesis of cardiolipin occurs solely in the mitochondria and in the yeast *Saccharomyces cerevisiae* is regulated by factors affecting mitochondrial development (carbon source, growth phase) and phospholipid precursors inositol and choline. To determine how the expression of the *KlPGS1* gene is regulated, the total mRNA was isolated and hybridisated with ^{32}P-labelled *PGS1* probe. mRNA was isolated from the cells which were grown i) in an inositol-free synthetic glucose or glycerol/ethanol medium and ii) in synthetic glucose medium in the presence or absence of the inositol and choline. The intensity of signals in the cells grown i) under the different growth phase (mid-log phase, early stationary phase, later stationary phase) and ii) in the early stationary phase was determined.

Our results from Northern blot method showed, that the expression of *KlPGS1* gene was constitutive. It was not regulated by the factors affecting the mitochondrial development nor by phospholipid precursors inositol and choline.

PO 2.29

SCREENING FOR CATALASE-PEROXIDASE GENES IN ENVIRONMENTAL SAMPLES OF BACTERIA.

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Catalase-peroxidases are protective enzymes involved in the defence of cells against various forms of oxidative stress. They belong to the superfamily of bacterial, fungal, and plant heme peroxidases and are widely distributed among bacteria and fungi. The structure of *katG* gene coding for catalase-peroxidase is complex since it was formed by gene duplication of an ancestral peroxidase gene. Only the N-terminal domain is responsible for catalytic activity in decomposing hydrogen peroxide and oxidising various substrates. We have designed degenerated oligonucleotide primers for screening of *katG* genes in the environmental samples of bacteria isolated from soil contaminated with crude oil, sludge of a wastewater treatment plant, or soil of old mines. The screening was performed via PCR on DNA samples isolated from cultivated bacteria. Both forward and reverse primers were designed in the N-terminal catalytic domain. They are located around the essential parts of *katG* gene coding for distal and proximal ligands of the prosthetic heme group. The obtained PCR products were cloned in TOPO vector and sequenced. We have found novel genes coding for catalase-peroxidase from four Gram-positive and three Gram-negative bacteria and compare them with the sequences available in databases in multiple sequence alignment. Selected genes will be cloned in expression vectors to allow the investigation of their protective function against oxidative stress.

Our research was supported by grants number APVT-51-024804 and VEGA 2/5069/25

PO 2.30

PURIFICATION OF BACTERIAL ALKALINE PEPTIDASE BY CHROMATOGRAPHICAL METHODS

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The aim of this work was purification of peptidase obtained by the submerged cultivation of the production *Bacillus licheniformis* strain L-5. We obtained the crude enzyme preparation by removing bacterial culture and by precipitation of enzyme by acetone cooled to -18^{o}C. The enzyme obtained was purified by different chromatographical methods on various carriers: by gel filtration on a Sephadex G-25 and a Sephadex G-100, by ionexchange chromatography on an ECTEOLA-cellulose and by affinity chromatography on a *p*-aminobenzamidine-Sepharose and a bacitracin-Sepharose. By gel filtration on a Sephadex G-25 we removed the low-molecular substances and we obtained 1.55-fold purified peptidase with the enzyme yield of 48.3% and on a Sephadex G-100 column we achieved 14.33-fold purification of enzyme with the 13.33% yield. In case of the application of chromatography oo ECTEOLA-cellulose we achieved approximately 18-fold purification and the yield was 10%. When we used the affinity chromatography method for the purposes of purification of enzyme purified by gel and ionexchange chromatography, we obtained on a *p*-aminobenzamidine-Sepharose homogeneous peptidase on the basis of the results of electrophoresis in a SDS-PAGE. This enzyme was 58-fold purified and the yield was 4.89%. We achieved the highest degree of purification, 63.16, and the yield of 5.04%, by affinity chromatography on a bacitracin-Sepharose. The molecular weight of the purified enzyme preparation determined by the SDS-PAGE method was approximately 29 kDa. The enzyme preparation of alkaline Ser-peptidase prepared like this is suitable for feed and food applications.

PO 2.31

DIFFERENCE IN ENZYMATIC AND NONENZYMATIC DEHALOGENATION OF 1,2-DICHLOROETHANE AND 1,2-DIBROMOETHANE

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1,2-Dichloroethane (DCE) and 1,2-dibromoethane (DBE) are toxic and mutagenic compounds. They are also rather resistant to biodegradation and persist in the environment[1]. Nevertheless, several bacterial cultures that are able to use DCE as the only carbon and halogen source have been isolated. The most efficient catalysis has been observed with the haloalkane dehalogenase DhlA from *Xanthobacter autotrophicus* GJ10 and substantially lower activity with the haloalkane dehalogenase LinB from *Sphingomonas paucimobilis* UT26[2]. Interestingly, DhlA shows nearly the same catalytic activity with both DCE and DBE, while LinB converts DBE six orders of magnitude faster then DCE.

The different reactivity of DCE and DBE was examined by quantum mechanical and combined QM/MM calculations, by molecular dynamics simulations of enzyme-substrate complexes and kinetic analysis. The results show that the different catalytic activity of LinB with DCE and DBE cannot be simply explained by the difference in the activation barriers of respective reactions, but the active site flexibility, solvation effects and solvent dynamics must also be taken into account.

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Section 3

MEMBRANE BIOCHEMISTRY AND BIOENERGETICS

PO 3.1

DCCD RESISTANT MUTANT OF METHANOTHERMOBACTER THERMAUTOTROPHICUM : BIOCHEMICAL CHARACTERIZATION

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The methanogenic archaea are a group of strictly anaerobic organisms that posses the unique feature of producing methane de novo by the process of methanogenesis. In this process, redox reactions of methanogenesis drive the formation of primary electrochemical gradients of protons and sodium ions across a membrane. Both of these gradients can be directly coupled to ATP synthesis via two specific, H^+- and Na^+- dependent processes. In genome of *M. thermautotrophicum* was identified only A_1A_o type ATPase. Based on biochemical and inhibitory studies it is believed that this ATPase is H^+-translocating in cells of *M. thermautorophicum*. Interestingly, under specific conditions, Na^+ -driven ATP synthesis can be observed. These findings have raised one essential question. Is Na^+ - dependent ATP synthesis mediated via A_1A_o ATPase ? This discrepancy we are endeavouring to resolve by an isolation and characterisation of various mutants with some lessions in bioenergetic subsystems.

In this work we report the isolation and biochemical characterization of DCCD (N,N'-Dicyclohexylcarbodiimide) resistant mutant. The study of this mutant could help us to better understand the function and relationship H^+ and Na^+ bioenergetic subsystems.

Acknowledgements:

This investigation was supported in part from the Science and Technology Assistance Agency (Slovak Republic) APVT-51-024904 and by a Research Grant VEGA 2/6025/26 from the Slovak Academy of Sciences

PO 3.2

BIOCHEMICAL CHARACTERIZATION OF A MUTANT OF THE Methanothemobacter thermautotrophicus deficient in Na^+/H^+ *ANTIPORTER*

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The existence of Na^+/H^+ antiporter as an universal device for linking the H^+ cycle with Na^+ cycle was confirmed in some methanogens and this antiporter might be the basic regulatory element in the complex bioenergetic strategy of these cells. To understand the function and interrelationship of H^+, Na^+ energetic subsystems and Na^+/H^+ antiporter, an amiloride-resistant mutant with diminished Na^+/H^+ antiporter activity was isolated from *M. thermautotrophicus*. Mutant cells exhibited higher production of methane than wild-type cells. Na^+/H^+ antiporter activity was considerably diminished in the mutant cells. The ATP synthesis driven either by potassium diffussion potential in the presence of sodium ions or by methanogenesis was considerably higher in the mutant cells. An abundant 670 kDa membrane-associated protein that is present only in the mutant strain was identified as a coenzyme F_{420} reducing hydrogenase by MALDI-TOF MS. Western blotting and subsequently immunodection of proteins of membrane vesicles of the wild-type and amiloride-resistant mutant with antibodies against subunit A and subunit B of archaeal A_1A_0 ATPase have not exhibited any changes. SDS PAGE of a chloroform/methanol extraction which yielded only two proteins, subunit D (MtrD) of the Na(+)-translocating methyltetrahydromethanopterin:coenzyme M methyltransferase and the proteolipid of the A_1A_0 ATPase has shown several different bands of proteins in the mutant cells. These results show that the impairment of the Na^+/H^+ antiporter system in the amiloride-resistant mutant of *M. thermautotrophicus* is accompanied by changes in a few membrane associated proteins.

Acknowledgement:
This work was supported in part from the Science and Technology Assistance Agency (Slovak Republic) APVT –51-024904 and by Research grant VEGA 2/3047/23 from the Slovak Academy of Sciences.

PO 3.3

NADH DEHYDROGENASE OF TRYPANOSOMATIDS

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Complex I is least characterized enzyme complex of respiratory chain of trypanosomatids. Data about its activity and sensitivity to inhibitors are contradictory in the literature up to this date. We have spectrophotometricaly measured NADH:ubiqinone 2 and NADH:ferricyanide dehydrogenase activity in mitochondrial lysates of *P. serpens* and *T. brucei* procyclics. Different sensitivity of both activities in both mitochondrial lysates to rotenone, piericidin and diphenyl iodonium was observed. We have isolated NADH dehydrogenase activity from both lysates by native polyacrylamide electrophoresis (PAGE) and we had detected its activity by histochemical staining directly in the gel. Two different high molecular weight NADH dehydrogenase activities were demonstrated in mitochondrial lysates of *P. serpens* but only one in mitochondrial lysates of *T. brucei*. 2nd dimension PAGE performed in denaturing conditions demonstrated that both NADH dehydrogenase activities of *P. serpens* have multisubunits composition. One subunit present in both active bands of *P. serpens* crossreacts with anibodies against 39 kDa subunit of bovine complex I. Several of the subunits were analyzed by MALDI and one of them contains a domain typical for NADH:ubiquinone oxidoreductase subunit. On the basis of presented results scheme of respiratory chains of *P. serpens* and *T. brucei* were completed.

PO 3.4

THE ROLE OF IDEBENONE IN ACTIVATION OF GLYCEROLPHOSPHATE OXIDATION IN BROWN ADIPOSE TISSUE MITOCHONDRIA

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Coenzyme Q (CoQ) as an obligatory component of the mitochondrial respiratory chain participates in the oxidative phosphorylation process. Beneficial effects of CoQ were observed in animal experiments or human therapy when the synthesis of CoQ was decreased. Because CoQ is highly hydrophobic, synthetic analogues with lower hydrophobicity were prepared and idebenone (hydroxydecyl-ubiquinone) was found to be very effective in the replacement therapy. The aim of our study was to test to which extent idebenone can activate glycerolphosphate (GP) oxidation measured as oxygen uptake and GP oxidoreductase activities (GP cytochrome c oxidoreductase or GP-dichlorophenol-indophenol oxidoreductase). We found the increase of GP-dependent oxygen uptake as well as the activation of above mentioned enzyme activities. This indicates that idebenone may be used for the activation of GP shuttle and the acceleration of glycolysis by increased NADH reoxidation. It might be important in the case when mitochondrial Complex I is impaired and energy production must be maintained.

This work was supported by GACR (305/04/0500), Ministry of Education, Youth and Sport (1M6798582302) and Joint Project in the Framework of Czech-Italian Scientific and Technological Cooperation (43/B 14).

PO 3.5

THE EFFECT OF GLYCINE UPTAKE ON SYNAPTIC PLASTICITY: A COMPUTATIONAL MODEL

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Glycine transporters are members of the Na+/Cl- dependent transporter gene family and play crucial roles in regulating inhibitory as well as excitatory neurotransmission. In our previous work we suggested that N-terminal domain of glycine transporter GlyT1b could be modified by calpain protease and that such modification results in significant change of transporter activity (1). So far we have been unable to identify specific pathway which would activate such modification *in vivo*. Since overall activation of calpain protease has many side effects including toxicity, we are currently trying to asses this process by mathematical model. As a first step we developed mathematical model of glycine transporter GlyT1b function, which accurately describes the time course of the glycine uptake in several experimental conditions. For a model of transporter we used conventional kinetic equation for the various state system. For the prediction of glycine uptake we used combination of electrochemical, continuity and rate equation. Model accuracy was preliminary tested on HEK293 expressing glycine/Na/Cl cotransporter (GLYT1b). The results will be used for modeling of both intact and truncated GlyT1b activity in brain tissues and their potential physiological/pathological impact on brain function.

1., Neurochemical Research, 30, 1093–1100 (2005)

Supported by SAS VEGA 2/4054/24.

PO 3.6

PORE-FORMING AND ENZYMATIC ACTIVITIES OF *BORDETELLA* ADENYLATE CYCLASE TOXIN SYNERGIZE IN PROMOTING LYSIS OF MONOCYTES

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Bordetella adenylate cyclase toxin-hemolysin (CyaA) targets myeloid phagocytes expressing the $\alpha_M\beta_2$ integrin (CD11b/CD18) and delivers into their cytosol an adenylate cyclase (AC) enzyme that converts ATP into cAMP. In parallel, CyaA acts as a hemolysin, forming small membrane pores. Using specific mutations, we dissected the contributions of the two activities to cytolytic potency of CyaA on J774A.1 murine monocytes. The capacity of AC to penetrate cells and deplete cytosolic ATP was essential for promoting lysis and the enzymatically inactive but fully hemolytic CyaA-AC^- toxoid exhibited a fifteen-fold lower cytolytic capacity on J774A.1 cells than intact CyaA. Moreover, a two or four-fold drop of specific hemolytic activity of the CyaA-E570Q and CyaA-E581P mutants was overpowered by an intact capacity to dissipate cytosolic ATP into cAMP, allowing the less hemolytic proteins to promote lysis of J774A.1 cells as efficiently as intact CyaA. However, an increased hemolytic activity, due to lysine substitutions of glutamates 509, 516 and 581 in the pore-forming domain, conferred on AC^- toxoids a correspondingly enhanced cytolytic potency. Moreover, a three-fold increase in hemolytic activity could override a four-fold drop in capacity to convert cellular ATP to cAMP, conferring on the CyaA-E581K construct an overall two-fold increased cytolytic potency. Hence, although appearing auxiliary in cytolytic action of the toxin on nucleated cells, the pore-forming activity can synergize with ATP-depleting activity of the cell-invasive AC enzyme and complement its action towards maximal cytotoxicity.

PO 3.7

ROLE OF THE ELECTROCHEMICAL H^+-GRADIENT IN TRANSMEMBRANE CA^{2+} FLUXES IN VEGETATIVE MYCELIUM *TRICHODERMA VIRIDE* ADAPTED TO DIFFERENT CA^{2+} CONCENTRATIONS.

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The aim of this work was to study the changes of the properties of the $^{45}Ca^{2+}$ transport in submerged mycelia of *Trichoderma viride* adapted to different extracellular Ca^{2+}concentrations ($[Ca^{2+}]_o$). Results showed that the adaptation to the low $[Ca^{2+}]_o$ did not cause changes in kinetic parameters of the $^{45}Ca^{2+}$ influx but the adaptation to high $[Ca^{2+}]_o$ increased the $K_M(Ca^{2+})$. The V_{max} of the $^{45}Ca^{2+}$ influx decreased with the age of (non-adapted) mycelia with the concomitant decrease of the $K_M(Ca^{2+})$ but the latest change was prevented in mycelia adapted to high Ca^{2+}.

The uncoupler (3,3',4',5- tetrachloro salicylanilide, TCS) (30 μM) stimulated of both $^{45}Ca^{2+}$ influx and efflux from fresh non-adapted mycelia *T.viride*. However adaptation to the high $[Ca^{2+}]_o$ decreased the stimulation by the uncoupler as compared to the control, and the Ca^{2+} chelator EGTA, which stimulated it. In the aged mycelia, the stimulation by TCS of the $^{45}Ca^{2+}$ influx faded away, in parallel with the activity of the H^+-ATPase. The $^{45}Ca^{2+}$ efflux from mycelia was affected by TCS in a similar way as the $^{45}Ca^{2+}$ influx. The results demonstrate the adaptive responses of transport processes participating in the mycelial Ca^{2+} homeostasis and ageing are in agreement with a notion that both Ca^{2+}-influx and -efflux are coupled by the H^+-homeostasis at the plasma membrane.

PO 3.8

ROLE OF N-TERMINAL REGION IN DOPAMINE TRANSPORTER REGULATION

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Dopamine transporter (DAT) belongs to the Na+/Cl- dependent transporter gene family and plays critical role in synaptic dopamine clearance. DAT is also site of action of several drugs including amphetamine and cocaine, which elicit psychomotor behavior associated with euphoria, self-reward. Transporter molecule comprise of 12 transmembrane domains, N and C-terminal domains, both oriented to the cytoplasm. Role of transporter N-terminal domain is largely unknown. Despite the effect on transporter uncoupled currents, modification of outer DATN-terminal sequences has not been associated with direct effect on transporter activity. In our experiments, we have investigated whether deletion of inner DATN-terminal sequences will cause transporter instability or change in transporter intrinsic properties. For this purpose we deleted N-terminal sequences and simultaneously introduced sequence elements artificially increasing the surface expression of deleted DAT. Experiments in HEK293 cells revealed no difference in transporter affinity but normalization of transporting activity to equal surface expression revealed several fold differences in maximal transport rate (Vmax). Above indicate that deletion of inner DAT-N-terminal sequences influenced transporter Vmax but interestingly this change was not because of differences in total number of surface localized transporters.

Supported by SAS VEGA 2/4054/24.

PO 3.9

BRAIN MITOCHONDRIA FUNCTION IN SPONTANEOUS HYPERTENSION – EFFECT OF TREATMENT WITH LOSARTAN AND PRAZOSIN

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Increased production of free radicals and impairment of mitochondrial function are important factors in pathogenesis of hypertension. This study examined impact of hypertension on respiratory chain function and antioxidant levels in brain mitochondria and their influence by blockade of angiotensin (AT1)-receptors or alpha-1 adrenoreceptors.

Spontaneously hypertensive rats (SHR) received losartan (10 mg/kg) or prazosin (20 mg/kg) from age 4 to 9 weeks. Blood pressure increase in SHR was partly reduced by the therapy. The glutamate-supported respiration, rate of ATP production, ADP:O, concentrations of α-tocopherol, coenzyme Q_9 and coenzyme Q_{10} were decreased in brain mitochondria of SHR when compared to Wistar rats. The succinate-supported function and cytochrome c oxidase activity were not affected either by hypertension or by therapy. Both losartan and prazosin treatment improved glutamate-supported respiration, rate of ATP production and mitochondrial level of coenzyme Q_{10}. Concentrations of α-tocopherol and coenzyme Q_9 increased in group treated with losartan.

The impairment of energy production and decreased level of lipid-soluble antioxidants in brain mitochondria could contribute to increased vulnerability of brain tissue in hypertension. Both antihypertensives used had beneficial effect on function of brain mitochondria and their antioxidant content.

Supported by grants VEGA No 1/3442/06 and 2/6139/26.

PO 3.10

MEMRANE PHOSPHOPROTEOME ANALYSIS OF BACILUS SUBTILIS UNDER ETHANOL STRESS

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Ethanol as a potent membrane perturbing agent has various effects on Bacillus subtilis: inhibits growth, protein synthesis and blocks adaptive synthesis of membrane fatty acids after the cold shock. Therefore, the role of the cytoplasmic membrane in alcohol sensing was analysed.

To study the dynamics of protein phosphorylation in bacteria grown under control and ethanol (3% v/v) stress conditions, the phosphate transfer from γ-[^{32}P]ATP to proteins of membrane fraction was used. Cytoplasmic membranes isolated from the bacteria in mid-exponential phase were subjected to *in vitro* protein kinase assay followed by SDS-PAGE. Then, the membrane proteins were analyzed with PhosphorImager and ImageQuant software program. In the control cells phosphorylation of three proteins (95 kDa, 81 kDa and 75 kDa) was detected. Interestingly, in the cells exposed to ethanol stress for 30 min, the phosphorylation levels of the 81 kDa and 75 kDa proteins were 3-fold increased when compared with the control cells, whereas the phosporylation of the 95 kDa protein was 2-fold reduced. These three differentially phosphorylated proteins were identified with MALDI –TOF MS system for protein sequencing as pre-protein translocase SecA 95 kDa, elongation factor EF-G 77 kDa and heat shock molecular chaperon DnaK – 63kDa. The role of EF-G in ethanol stress signalling in cells with reduced protein synthesis is discussed.

PO 3.11

PERMEABILITY PROPERTIES OF RAT CARDIAC RYANODINE RECEPTOR

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Ryanodine receptor (RYR) is the major intracellular Ca^{2+} release channel required for excitation-contraction coupling in cardiac and skeletal muscle. The channel controls Ca^{2+} flux from the storage site in the sarcoplasmic reticulum to active site on the contractile apparatus. Crystallographic structure of the RYR channel is currently unavailable, therefore the architecture of the conductive pore involved in ion handling can be probed only indirectly by examining permeation properties of the channel using various ions as a charge carrier. Till now, all available evidence is compatible with the proposal that the conduction pathway of the RYR channel can be occupied by only one ion at a time. The purpose of our study was to re-examine this conclusion under asymmetrical ionic conditions which have not yet been tested. RYR channels were isolated from the rat heart and reconstituted into planar lipid membrane. We revealed that the zero-current potential determined under bi-ionic conditions showed clear concentration dependence, when $[Li^+]/[Ca^{2+}]$ ratio was held constant at 12:1. Furthermore, the zero-current potential did not show minima or maxima with varying Ba^{2+}/Ca^{2+} mole fraction. In the light of barrier model of ion permeation through the channel, the concentration dependence of the zero-current potential is one of the characteristics predicted for multi-ion channels. Thus, our results weaken for the first time the hypothesis about the single-ion nature of the RYR channel conduction pathway.

PO 3.12

THE INTERACTION OF MITOCHONDRIA WITH THE CYTOTOXIC CHALCONES INVESTIGATED BY FLUORESCENCE POLARIZATION

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Effect on mitochondrial outer membrane of six selected chalcones and synthetic cyclic analogues *E*-2-(X-benzylidene)-1-benzosuberones, and -indanones were investigated by fluorescence spectroscopy. Excitation and emission fluorescence spectra of each compound were recorded in respiration medium. Interaction of the compounds with the outer mitochondrial membrane was investigated by recording their fluorescence polarization in the presence of rat liver mitochondria during 24 hours experiment. It was found that the ring size as well as the position of the substituents has significant effect on fluorescence characteristics of the compounds. It was found that *E*-2-(4'-metylbenzylidene)-1-indanone displayed a continuous increase of fluorescence polarization signal in the presence of mitochondria - a similar polarization pattern to that of the cytotoxic cyclic chalcone analogue *E*-2(4'-metoxy-benzylidene)-1-benzosuberone and the analogous E-2-(4'-hydroxybenzylidene)-1-benzosuberone. A different pattern of interaction with the mitochondrial outer membrane from that observed for the 4-metylchalcone and the *E*-2-(4',6'-dimetoxybenzylidene)-1-indanone. 4-Methoxychalcone was fluorescent but similar to E-2-(4'-methoxybenzylidene)-1-indanone showed little FP change on interaction with the outer mitochondrial membrane.

Keywords: Chalcone, cyclic chalcone analogues, cytotoxicity, mitochondrial outer membrane, fluorescence excitation and emission spectra, fluorescence polarization

PO 3.13

ROLE OF ABC-TRANSPORTERS IN STEROL HOMEOSTASIS

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Uptake of sterol molecules is in yeast *S. cerevisiae* accompanied by at least two interesting phenomena's i) under normal growth conditions sterol uptake is blocked although the synthesis of ergosterol, typical yeast sterol, is energetically very expensive ii) two PDR pumps, proteins generally implicated in export of molecules out of the cells, are involved in sterol internalization. Neither of the two phenomena's was entirely elucidated yet. In this study we are investigating the later phenomena participation of PDR pumps in sterol uptake. Wilcox *et al* (2002) identified two paralogues, Aus1p and Pdr11p involved in sterol internalization in yeast *S. cerevisiae*. They are members of the ATP-binding cassette transporter superfamily. Both proteins are expressed in anaerobic conditions when cells depend on sterol uptake from the environment since ergosterol synthesis is prevented under these circumstances. In addition sterol uptake could be activated in aerobic conditions in *upc2-1* genetic background. Absence of sterol uptake in *upc2-1 aus1 pdr11* triple mutant and non-viability of *aus1 pdr11* in anaerobiosis indicate participation of both proteins in sterol import. In the recent paper Li and Prinz (2004) showed that Aus1p and Pdr11p mediate nonvesicular movement of plasma membrane (PM) sterol to the endoplasmatic reticulum. We will present data showing that absence as well as overexpression of *AUS1* lead to changes in sterol composition in cells where sterol uptake is not induced. These data indicate that function of Aus1p might be more complex.

Acknowledgements: This work was supported by grant APVT-51-029504.

PO 3.14

DIFFERENCES IN PREDISPOSITION TO OBESITY BETWEEN MOUSE STRAINS C57BL/6J AND A/J: THE ROLE OF MUSCLE AND ADIPOSE TISSUE METABOLISM

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Background and Aims: Adult C57BL/6J (B6) and A/J mice differ in susceptibility to high fat (HF) diet-induced obesity with the former strain exhibiting higher propensity to obesity. This difference could be explained by relatively high induction of uncoupling protein 1 (UCP1) - mediated thermogenesis by HF diet in brown fat of A/J. HF diet also induces leptin, this induction is stronger in A/J than in B6, and leptin may augment lipid oxidation in muscle by stimulating of AMP-activated protein kinase (AMPK). Therefore, the goal of this study was to characterize possible involvement of muscle non-shivering thermogenesis in the differential response of two genotypes to HF diet, particularly in the propensity to obesity.

Material and methods: To eliminate the effects of cold, male B6 and A/J mice were born and kept at thermoneutral temperature of 30 ºC. At the age of 4 wk, mice were weaned onto either standard (ST) or HF diet. At the age of 6 wk, animals were either subjected to in-vivo measurements - indirect calorimetry (Somedic; Sweden) or sacrificed and dissected tissues were analyzed – muscle endogenous oxygen consumption (Oroboros Oxygraph), plasma leptin concentrations (Mouse Leptin Ria Kit, LINCO research), and gene expression in muscles (qRT-PCR).

Results: HF diet induced significantly higher increase in plasma leptin levels in A/J than in B6 mice. In oxidative muscle of A/J but not B6 mice, HF diet induced increase of respiration. Also slightly higher values of metabolic rate were observed in the former genotype. Muscle gene expression data will be presented

Conclusion: Muscle non-shivering thermogenesis could be induced in A/J but not B6 mice by HF diet and could contribute to the resistance to obesity. The leptin – AMPK regulatory cascade may be involved.

PO 3.15

BONGKREKIC ACID AND ATRACTYLOSIDE INHIBITS CHLORIDE CHANNELS FROM MITOCHONDRIAL MEMBRANES OF RAT HEART

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Aim of the work was to characterize the effect of bongkrekic acid (BKA), atractyloside (ATR) and carboxyatractyloside (CAT) on single channel properties of chloride channels from mitochondria. Mitochondrial membranes isolated from a rat heart muscle, were incorporated into the lipid membrane bilayer (BLM) and single chloride channel currents were measured in 250/50 mM KCl *cis*/*trans* solutions. The control single chloride channel properties were: Open probabiliyty 0.80-0.95, conductance from 104 to 172 pS, reversal potential from -14 to -30 mV. BKA (1- 100 μM), ATR and CAT (5-100 μM) inhibited the chloride channels in dose dependent manner. The inhibitory effect of the BKA, ATR and CAT was pronounced from the *trans* side of a BLM and it increased with time and at negative voltages (*trans*-*cis*). These compounds did not influence the single channel amplitude, but decreased open dwell time of channels. The inhibitory effect of BKA, ATR and CAT on the mitochondrial chloride channel may contribute to explanation of some of their cellular or subcellular effects.

Section 4

CELL REGULATIONS AND TRANSFER OF SIGNALS

PO 4.1

LINEAR DRIFTING OF p53 FOR ITS CONSENSUS SEQUENCE

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The most important biological function of the tumour suppressor p53 protein is its sequence specific binding to DNA. Using a competition assay we investigated the effect of DNA length on p53 protein binding to p53 target sequences. We found that the p53 consensus sequence in longer DNA fragments is a considerably better target than the same sequence in shorter DNA fragments. Quantitative electrophoretic mobility shift assay of different complexes shows a direct correlation between the binding affinity of p53 protein and the DNA length. Agarose gels are very suitable for gel-shift analysis and offer more opportunities for resolution between p53 sequence specific and non-specific binding. We conclude that not only the type of sequences but also the length of the DNA molecule can be an important determinant of p53 DNA binding. GAČR 301/04/P025, GAAV. B500040502.

PO 4.2

SEQUENCE SPECIFIC DNA BINDING OF P53 CAN BE ENHANCED BY DNA SUPERCOILING

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We investigated the effect of DNA negative supercoiling on the DNA sequence specific binding of human wild-type p53 protein. We found that supercoiled pBluescript DNAs with inserted p53 target sequences were stronger competitors than a mixture of scDNA pBluescript with the given 20-mer target ODN. scDNAs were always better competitors than their linearized forms. Two DNAs with extruded cruciforms within the target sequence were the best competitors; removal of the cruciforms resulted in a decrease of competitor strength. We conclude that cruciforms and DNA bends contribute to the enhancement of p53 sequence specific binding (SSDB) to scDNA and that the DNA supercoiling is an important determinant in the p53 SSDB which may play a significant role in the complex p53 regulatory network. GAČR 301/04/P025, GAAV. B500040502.

PO 4.3

TRANSCRIPTIONAL REGULATION OF S100P GENE CODING FOR THE CANCER-RELATED CALCIUM-BINDING PROTEIN INVOLVES EGF- AND HORMONE-DEPENDENT PATHWAYS

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S100P is a calcium-binding protein with a proposed role in various cellular processes including malignant transformation. S100P has been detected in carcinomas derived from different tissues including the breast, pancreas, prostate, colon etc. Expression of S100P was correlated with the loss of hormone dependence and resistance to chemotherapeutic drugs. Nevertheless, data on molecular mechanisms governing S100P gene expression have not been published so far.

In order to elucidate the factors/pathways involved in the regulation of S100P gene transcription in tumor cells, we have initiated the functional study of the 5' upstream genomic region. We have generated several reporter constructs in which the luciferase gene was placed under the control of different genomic fragments derived from this region. The constructs were transiently co-transfected with the reference renilla plasmid into human carcinoma cells lines and their transcriptional activities were evaluated using the dual luciferase assay. Analysis of the transfected cells treated with hormones, growth factors and inhibitors of signal transduction pathways revealed the dependence of S100P transcription on EGF-induced signaling and hormone stimulation, and helped to identify the regulatory regions mediating the transcriptional response of S100P gene to these pathways.

Supported by VEGA (2/5082/25).

PO 4.4

HYPOXIA MIMETICS INDUCE DIFFERENTIAL RESPONSES IN RAT IMMORTALIZED RAT2TK⁻ CELLS AND RAT TRANSFORMED BP6 CELLS

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Hypoxia inducible factor 1 (HIF-1) is the main regulator of molecular responses to hypoxia. Under normoxic conditions its α subunit is hydroxylated on specific proline residues by prolyl hydroxylases (PHDs) and destroyed in the proteasome. PHDs require oxygen, Fe^{2+}, and 2-oxoglutarate for their activity. In hypoxia, HIF-1α is stabilized and transported to the nucleus where it dimerizes with the β subunit and initiates the transcription of hypoxia-inducible genes. Hypoxia-like response can by triggered by hypoxia mimetics, such as desferrioxaxamine (DFO) and dimethyloxalylglycine (DMOG).

We focused on finding alternative mechanisms of HIF-1 induction in the presence of oxygen that could link hypoxia to mitochondrial signals. Our aim was to analyze the effects of hypoxia mimetics and compounds that interfere with metabolic pathways on the expression of *Ca9* and other hypoxia-regulated genes in rat cell lines.

These compounds differentially induced gene expression depending on cell type and presence of HIF-α subunits *in vitro*. *In vivo* inducibility of certain genes by systemic hypoxia suggests a possibility to use them as indicators for decreased oxygen delivery due to ischemia and metabolic disturbances in a rat model.

Supported by EUROXY (LSHC-CT-2003-502932).

PO 4.5

CARBONIC ANHYDRASE IX IS INDUCED BY ACIDOSIS INDEPENDENTLY OF HYPOXIA IN HUMAN GLIOBLASTOMA CELLS.

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Abstract

Acidosis and hypoxia are main regulatory factors influencing cellular signaling in most solid tumors. Both of them participate to distinct adaptive changes leading to aggressive cell phenotype. CA IX is predominantly expressed in tumors with poor prognosis and is strongly induced by hypoxia. Here we investigated whether extracellular acidosis in absence of hypoxia can influence CA IX expression in two human glioblastoma cell lines 8-MG-BA and 42-MG-BA. We found that extracellular acidosis (pH 6,4) contributes to the increased level of CA IX protein, mRNA and the activity of *CA9* promoter. Increased level of CA IX protein persisted in conditions, when pericellular hypoxia was abrogated with continuous stirring, as revealed by western blot analysis and luciferase assay. These observations strongly suggest that acidosis induces CA IX expression independently of pericellular hypoxia and that both factors have additive effect. Altogether, our results suggest that acidosis increases the CA IX expression via a hypoxia-independent mechanism.

PO 4.6

METABOLISM OF GABA IN FILAMENTOUS FUNGI

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The role of γ-aminobutyric acid (GABA) in filamentous fungi is still not known. It was suggested that it has a developmental function. We have observed the time course of GABA production in filamentous fungi grown in liquid and on agar media and we measured the activities of some enzymes that may be connected to the metabolism of GABA. We also tried to identify changes in GABA levels during growth on alternative substrates e.g. organic acids.

Our results show that glutamate decarboxylase activity corresponds to the rise in the GABA levels reaching a maximum between 24 – 48 h. Also aminotransferase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase activities were highest after 24 h followed by a drop in subsequent days. GABA was not released into the medium at any stage of growth and its intracellular level decreased rapidly after transferring fungus onto a medium with citrate. Although yeast *Saccharomyces cerevisiae* has all necessary enzymes and it is able to utilize GABA, it does not produce detectable levels of GABA.

This work was supported by Slovak State Programme (Project No. 2003SP200280203) and Slovak Grant Agency VEGA (grant No. 1/2334/05)

PO 4.7

INDUCTION AND REPRESSION OF PROTEIN P21 IN LYMPHOCYTES TREATED WITH ACTINOMYCIN D AND N-BUTYRIC ACID.

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Intercalating into rDNA the actinomycin D (AMD) provokes an arrest of rRNA synthesis and a translocation of ribosomal proteins. Inhibition of activity of oncoprotein MDM2 caused by ribosomal protein L11 shields p53 and p21 against the proteasomal degradation, induces growth arrest and drives cells towards the apoptosis. Inhibiting histone deacetylases the butyrate (BUT) favorizes the acetylated structure of chromatin, potentiates p21 transcription, diminishes the transcription of p53 and induces p53 independent apoptosis. Studying a simultaneous effect of AMD and BUT on human lymphocytes we found that the presence of BUT in AMD treated cultures caused variations in cellular levels of proapoptotic protein p53, growth inhibitor p21 and apoptotic marker PARP. Within 7 hours following the BUT and AMD addition the p53 transcription decreased, p21 amount temporary augmented and then the level of p21 rapidly decreased. The diminution of p21 was accompanied by degradation of PARP and accumulation of p53 introduced by AMD mediated inhibition of MDM2. Inhibition of caspases by z-VAD-fmk abrogated the destruction of PARP but didn´t abrogated the destruction of p21. The BUT treated cultures added with proteasome inhibitor MG132 augmented p21 and p53 levels despite PARP digestion. We suggest that the BUT induced the MDM2 independent, p21 specific proteolytic cleavage followed by caspase dependent p21 digestion. Conclusion: The down-regulation of expression of tumor suppressor p53 by BUT is balanced by temporary up-regulation of intracellular level of growth inhibitor p21. Following this effect, butyrate mobilizes MDM2 independent but MG132-inhibitable proteinase(s) and specific cleavage of p21 – an inhibitor of caspase-3. The degradation of p21 by increasing activity of caspase-3, results finally in the accelerated apoptotic cell death.

Supported by grant VZ 00023736 from the MHCR.

PO 4.8

WHAT IS THE ROLE OF LEPTIN IN ACUTE STRESS?

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Leptin is recently identified polypeptide hormone, which is an integral component of the system regulating body fat stores. It was demonstrated that leptin can inhibit hypothalamic CRH release by the direct or indirect feedback actions, but the secretion of ACTH from the pituitary was not influenced. In addition to this feedback inhibitory actions on the hypothalamus it was shown that activation of HPA axis by stress stimulates the secretion of leptin. The aim of this study was the correlation of plasma levels of leptin, ACTH and corticosterone in Wistar and Lewis rats after restraint stress with prevalence of emotional (IMO) or physical component (IMO+C) of action (1). As biochemical parameters of stress response we estimated plasma levels of hormones at 60th and 120th min after the onset of one-hour lasting stress. A sandwich ELISA of leptin based on combination of polyclonal antibody coated microtiter plate, biotin-labeled polyclonal antibody, and streptavidin-HRP conjugate was used. In Wistar rats the corticosterone increase was larger in 60th min interval of emotional stress and after the physical stress. Plasma levels of leptin revealed only small changes between controls and stress groups. In Lewis strain, with deficient activity of HPA axis, the levels of stress hormones were lower than in Wistar rats, and leptin level was very significantly increased after physical stress at 60^{th} min. This finding may be considered as a compensatory mechanism in animals with blunted HPA activity. In summary, we did not demonstrate the direct correlation between levels of stress hormones and leptin under stress conditions. Further studies are necessary to elucidate the regulatory role of leptin in stress responses.

Supported by MSM 0021620806 and GAČR 309/06/0121.

1. Klenerová V. et al.: Behav. Brain Res. 136: 21-29, 2002.

PO 4.9

INVOLVEMENT OF ADENOSINE RECEPTORS IN ACTIVATION OF ANTIVIRAL IMMUNE RESPONSES BY ACYCLIC NUCLEOSIDE PHOSPHONATES

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Antiviral acyclic nucleoside phoshonates (ANPs) are synthetic analogues of natural nucleotide monophoshates. Purine derivatives represent counterparts of AMP, and after intracellular mono- and diphoshorylation they become analogues of ADP and ATP. We have found previously that beside their antimetabolic mode of antiviral action, ANPs also possess remarkable immunostimulatory potential that may participate on the over-all antiviral effectiveness.

The present data show that secretion of cytokines induced by distinct ANPs depends partially on activation of P_1 purinoceptors. With exception of chemokine MIP-1α/CCL3, the effects were inhibited by antagonists of adenosine A_1, A_{2B}, (not by adenosine A_{2A} receptor antagonist), and A_3 receptors. The adenosine A_1 receptor antagonist inhibited cytokines TNF-α, IL-10, and chemokine RANTES/CCL5; adenosine A_{2B} receptor antagonist inhibited TNF-α and RANTES, and adenosine A_3 receptor antagonist inhibited IL-10 and RANTES. The high-output production of nitric oxide depended mainly on activation of adenosine A_1 receptor. It may be concluded that different cytokines have distinct requirements for activation of the members of the P_1 family of purinoceptors.

The work was supported from the Centrum for New Antivirals and Antineoplastics (No. 1M6138896301).

PO 4.10

INDUCTION OF CELL DEATH AND ADHESIVE PROPERTY CHANGES IN COLON EPITHELIAL CELLS USING NONADHERENT CULTIVATION

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The maintainance of balance between proliferation, differentiation and apoptosis of the cells is typical for the health colon epithelium, and this balance can be disrupted during the carcinogenesis. Colonic epithelial cells are shed into intestinal lumen at the top of the crypt and die by detachment-induced apoptosis (anoikis). Cytokinetics and adhesive properties of the colon cells could be affected by dietary factors (e.g. fatty acids) or naturally occuring endogenous factors, such as cytokines (e.g. TNF family).

We have studied the effects of short-chain fatty acid butyrate and apoptotic inducers from TNF family (TNF-α, Fas ligand and TRAIL) on cytokinetic parameters and adhesive properties of the normal (human fetal colon cell line - FHC) and cancer (human adenocarcinoma cell line – HT-29) colon cells in association with anoikis induction. To mediate anoikis, we established the model of nonadherent cell cultivation. Results of cytokinetic experiments have indicated that the FHC cells are more sensitive to TNF family than HT-29 cells. Moreover, nonadherent cultivation increased cell death induction by TNF family treatment in the HT-29 cells. The butyrate-mediated inhibition of proliferation was lower in nonadherent cells versus the adherent cells. Simultaneously, FHC cells were more sensitive to the butyrate effect than HT-29 cells. In the HT-29 cells, we observed the high level of butyrate-mediated cell death compared to FHC cells during both adherent and nonadherent model of cultivation. In addition, expression of apoptotic proteins and adhesive molecules and kinases were examined by western blott analysis.

PO 4.11

NEW TARGETS FOR SALMONELLA TREATMENT: RPOE REGULON OF *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM

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Salmonella species are intracellular bacterial pathogens causing a spectrum of diseases in human and animals. The RpoE sigma factor is critical to *Salmonella typhimurium* virulence and stress response. Therefore, identification and characterization of the *S. typhimurium* RpoE regulon would reveal new genes involved in the virulence of *S. typhimurium.* The products of these genes may be used as new targets to design compounds inhibiting their activity, which may be used as novel therapeutics for salmonella infections. Expression of *S. typhimurium rpoE* is directed by three promoters, including one, *rpoEp3*, directly recognized by RpoE. The *rpoEp3* promoter was greatly induced in the stationary phase and after cold shock. Using this promoter we optimized the *E. coli* two-plasmid system for identification of *S. typhimurium* RpoE-dependent genes. After screening the *S. typhimurium* library, positive clones containing *S. typhimurium* RpoE-dependent promoters were identified and the promoters located by S1-nuclease mapping. All the promoters contained sequences similar to the consensus sequence ggAACtt-N_{15}-gTCtaA. The RpoE-dependent genes encoded proteins of primary metabolism, LPS and phospholipids biogenesis, regulatory proteins and periplasmic folding factors. Expression of several genes was verified in *S. typhimurium* wild type and *rpoE* mutant..

Acknowledgement. This work was supported by Science and Technology Assistance Agency under the contract No. APVT-51-012004, the VEGA grant 2/6010/26 from Slovak Academy of Sciences, and the Wellcome Trust grant 065027/Z/01/Z.

PO 4.12

CHANGES OF CELL LIPIDS IN HUMAN COLONIC CELL LINES AFTER TREATMENT WITH FATTY ACIDS.

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Epithelial colonic cells are exposed to lipid compounds of the diet like essential polyunsaturated fatty acids (PUFAs) and butyrate - the short-chain fatty acid produced by microbial fermentation of fibre. Signals induced by these factors are integrated inside the cells and may have significant effects on cell metabolism and kinetics.

We investigated the response of human colonic cell lines derived from normal fetal tissue (FHC) and colon adenocarcinoma (HT-29, HCT116, SW620) to treatment with sodium butyrate (NaBt, 1-3mM) and PUFAs (50 microM) of n-6 (arachidonic acid, AA) or n-3 series (docosahexaenoic acid, DHA). Flow cytometry was the main methodology used. Depending on the level of transformation, the cells responded differently to the agents used both individually and in combination. Modulation of proliferation (cell number, cell cycle) and apoptotic parameters (subG0/G1 population, membrane mitochondrial potential - TMRE fluorescence, nuclear morphology - DAPI staining) were associated with changes of membrane lipid packing (merocyanine 540 fluorescence), expression of fatty acid transporter protein (anti-FAT/CD36 monoclonal antibody), accumulation of lipid droplets in cytoplasm (Nile red fluorescence), and reactive oxygen species production (dihydrorhodamine-123 fluorescence). We conclude that PUFAs as well as NaBt may cause significant changes in colonic cell lipids, influence oxidative metabolism and modulate colonic cell kinetics depending on the level of cell transformation.

Supported by grants No. 524/04/0895 GACR and No. 1QS500040507 IGA ASCR.

EXPRESSION OF INHIBITOR OF APOPTOSIS PROTEINS SURVIVIN AND XIAP IN LUNG CARCINOMA CELLS AND TISSUES

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Background. Failure of the apoptosome pathway in tumor cells may account for their escape from apoptosis. The inhibitor of apoptosis proteins survivin (Sur) and X-linked inhibitor of apoptosis (XIAP) may play an important role in suppression of this cell death pathway in tumors. In this work, to find out whether Sur and XIAP are upregulated in non-small cell lung carcinoma (NSCLC), we studied their expression on the level of mRNA and protein in NSCLC cell lines, NSCLC tissues and lungs. *Methods*. The expression of Sur and XIAP mRNAs was assayed by real time RT-PCR with using total RNA isolated from the cells and tissues. The levels of Sur and XIAP proteins were quantitated with sandwich ELISAs. *Results*. The expression of both Sur mRNA and protein was substantially higher in NSCLC cells and tissues as compared to the lungs. In fact, in 56 (63%) of 89 and 23 (85%) of 27 examined NSCLC patients the tumors had more than tenfold higher expression of both Sur mRNA and Sur protein, respectively, as compared to matched lungs. Although the expression of both XIAP mRNA and protein in NSCLC cells and tissues and lungs was not significantly different, their levels in NSCLC and lung tissues were significantly higher as compared to Sur mRNA and protein, respectively. *Conclusions*. The present study provides evidence that both Sur mRNA and protein are overexpressed in NSCLCs while XIAP mRNA and protein are strongly expressed in both NSCLCs and lungs. These data suggest that both Sur and XIAP and their complexes may be involved in apoptosome pathway suppression in NSCLC. This work was supported by research projects NR/7860-5 from the Ministry of Health, Czech Republic.

PO 4.14

ROLE OF MITOGEN-ACTIVATED PROTEIN KINASES (MAPK) IN EXPRESSION OF IMMUNOBIOLOGICA EFFECTS OF ACYCLIC NUCLEOSIDE PHOSPHONATES

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Acyclic nucleoside phosphonates (ANPs), synthetic analogues of natural nucleotides, are potent antivirotics. The major mechanism of their antiviral effects is the inhibition of DNA polymerases and of reverse transcriptases. We have shown recently that in addition to their antimetabolic mode of action, a number of ANPs also possess prominent immunostimulatory potential. The aim of the present work is to analyze participation of fundamental transduction pathways undermining the expression of immunobiological properties of ANPs. The experiments were performed *in vitro* using mouse resident peritoneal macrophages. The results demonstrate that the production of nitric oxide (NO), as well as the secretion of cytokines (TNF-α, IL-10), and chemokines (CCL5/RANTES, CCL3/MIP-1α) can be inhibited by pharmacological inhibitors specific for p38, ERK1/2, and JNK MAPKs. The effectiveness of individual MAPK inhibitors has been found to depend on the type of the immune response. Activation of all p38, ERK1/2, and JNK MAPKs by immunostimulatory ANPs has been confirmed by ELISA determination of phosphorylated forms of MAPKs.

The work was supported from the Centrum for New Antivirals and Antineoplastics (no. 1M6138896301).

PO 4.15

NON-GENOMIC INHIBITION OF HUMAN PURINERGIC $P2X_7$ RECEPTOR BY $1\alpha,25(OH)_2D3$

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Purinergic $P2X_7$ receptors intervene in lymphocyte activation and are responsible for multiple processes, including calcium influx and permeation of large cations. We investigated whether purinergic receptors are modulated by rapid, non genomic actions of the steroid hormone $1\alpha,25(OH)_2D3$.

First, using fluorescent probe Fluo-3, we examined the effect of $1\alpha,25(OH)_2D3$ on calcium rise induced by a specific agonist of $P2X_7$ receptors BzATP and/or by a voltage-dependent K^+ channel blocker 4Aminopyridine (4AP). $1\alpha,25(OH)_2D3$ prevented the calcium entry stimulated by BzATP. The hormone also significantly reduced 4AP - induced Ca^{2+} increase via $P2X_7$ channels.

Ability to form membrane pores that are permeable to high molecular mass dye up to 320 Da is a well described characteristic of the $P2X_7$ receptor in lymphocytes. We therefore analysed the effect of $1\alpha,25(OH)_2D3$ on the permeation of large molecules by questioning ethidium bromide (314 Da) uptake into human peripheral blood mononuclear cells. We confirmed that $1\alpha,25(OH)_2D3$ reduced both the BzATP and the 4AP-stimulated ethidium bromide fluorescence.

Obtained results demonstrate for the first time that $1\alpha,25(OH)_2D3$ is capable to inhibit the permeability of $P2X_7$ channel.

Supported by Slovak Research and Development Agency No APVT-21-019702.

PO 4.16

RAPID EFFECTS OF 1α,25(OH)$_2$D3 IN RESTING HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS.

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Steroid hormone 1α,25(OH)$_2$D3 acts via both long-term, genomic and rapid, non-genomic mechanisms, yet little is known about its rapid actions on intracellular calcium. Here, we examined nonenomic effects of 1α,25(OH)$_2$D3 on Ca^{2+} mobilization and entry in resting human peripheral blood mononuclear cells (PBMC) from healthy volunteers. 1α,25(OH)$_2$D3-induced biphasic increase in intracellular calcium concentration, determined by fluorescent probe Fluo-3. The initial 1α,25(OH)$_2$D3-stimulated calcium rise was sensitive to Thapsigargin indicating that it is resulting from calcium release from intracellular stores. An inhibitor of capacitative calcium entry (2-amino-ethyldiphenyl borate) significantly decreased $[Ca^{2+}]_i$ in PBMC treated with 1α,25(OH)$_2$D3 and abolished the biphasic response, while nifedipine had no effect on the 1α,25(OH)$_2$D3-induced calcium entry. These findings suggest that 1α,25(OH)$_2$D3 promotes two-step calcium response through calcium release from internal stores, followed by store refilling via calcium-release calcium-activated (CRAC), but not L-type calcium channels.

Supported by Slovak Research and Development Agency under the contract No APVT-21-033002 and No APVT-21-019702.

PO 4.17

INDUCTION OF EXPRESSION OF PTGF-β/NAG-1/MIC-1/GDF-15 IN PROSTATE AND COLON EPITHELIAL CELLS USING NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

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Colon and prostate cancer are leading cause of cancer death in the Western world. Antitumorigenic effects of nonsteroidal anti-inflammatory drugs (NSAIDs) are well established in several types of cancer disease. However, mechanisms driving these processes are not known in all details. Finding the biomolecular mechanisms associated with effects of NSAIDs in prostate and colon cancer is an important endeavor, and potentially will lead to novel treatment strategies. It has been shown that some effects of NSAIDs are independent on inhibition of activity of cyclooxygenases. Induction of PTGF-β/NAG-1/MIC-1/GDF-15, gene from transforming growth factor-β superfamily, is associated with pro-apoptotic and anti-tumorigenic effects of some NSAIDs.

In our study, we compared effects of selected NSAIDs on induction of PTGF-β protein in several cancer and non-cancerous prostate and colon epithelial cell lines. We found significant differences in response to treatment by NSAIDs and induction of PTGF-β protein between individual cell lines. Generally, only cells with intact p53 function were able to induce expression of PTGF-β protein after treatment by NSAIDs. This observation correlates with property of NSAIDs to inhibit proliferation and induce apoptosis in p53-wild-type cell lines. Our observation showed importance of p53 status in sensitivity to anti-proliferative and pro-apoptotic effects of NSAIDs.

PO 4.18

THE PRODUCTION OF NITRIC OXIDE AND THE ACTIVITY OF NO SYNTHASE DURING PATHOGENESIS IN TOMATO

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Very little information exists on resistance mechanisms in *Lycopersicon* spp. where some physiological and biochemical aspects play an important role. Three *Lycopersicon* spp. accessions differing in the level of resistance to biotrophic parasitic fungus *Oidium neolycopersici* L. Kiss (tomato powdery mildew) were studied. Recently a signaling role of NO has been demonstrated in plant germination, growth, stomata movement, maturation, senescence and plant responses to stress stimuli. The production of nitric oxide and the activity of NO synthase during pathogenesis in the tissue of *L. esculentum* cv. Amateur (susceptible control), *L. hirsutum* f. *glabratum* (LA 2128) (highly resistant) and *L. chmielewskii* (LA 2663) (moderately resistant) were investigated during the first 120 hours post inoculation (hpi). We determined NO production in plant tissue extract by oxyhemoglobin method and the amount of nitrite and nitrate by Griess reagent. Significant differences in NO production and nitrite/nitrate levels were observed in infected leaves of susceptible, moderately resistant and highly resistant Lycopersicon spp. accesssions during the first 120 hours post inoculation. We have also observed systemic response in the form of increased NO synthesis in plant leaves adjacent to inoculated leaves. The obtained results are discussed in respect to the role of NO in plant-pathogen interaction and plant defence mechanisms.

This work was supported by the grant MSM 6198959215 from the Ministry of Education, Youth and Sports of the Czech Republic.

PO 4.19

MULTIPLE RESPONSES OF RADISH AND TOBACCO LEAVES TO SALINITY AND DROUGHT STRESS

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There is a large variation in the responses of plants to various abiotic stresses. In this work, we studied the effects of salinity and drought stress on phytohormone (cytokinin, auxin and abscisic acid) levels and metabolism and on antioxidative enzymatic defence in radish (*Raphanus sativus* L.) and tobacco (*Nicotiana tabacum* L.) leaves. Radish and tobacco were intentionally chosen for experimental material as these two plant genera substantially differ in activities of cytokinin deactivation pathways (McGaw and Horgan 1985, *Biol Plant 27*, 180). While most of cytokinins are preferentially inactivated by the *N*-glucosylation leading to formation of physiologically inactive cytokinin *N*-glucosides in radish, the irreversible degradation to adenine (or adenosine) catalysed by cytokinin oxidase/dehydogenase represents the predominant way of cytokinin deactivation in tobacco.

Both radish and tobacco salt- and drought-stressed plants displayed similar phenotypic alterations and/or signs of wilting accompanied by a decrease in contents of chlorophyll *a* and *b*, β-carotene and xanthophylls. The exposure of the two plant species to salinity or drought resulted in the enhancement of ABA and auxin levels. Total cytokinin concentrations increased in radish but tended to decrease in tobacco under adverse environmental conditions. No marked or only slight variation in activities of antioxidative enzymes in response to abiotic stress were found in radish, while significant changes were observed in tobacco. Analogies and differences between radish and tobacco plants in their responses to salinity and drought will be discussed.

PO 4.20

BH3-ONLY PROTEINS IN THE BCL-2 FAMILY AS STUDIED IN YEAST

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Proteins in the Bcl-2 family regulate programmed cell death in mammals by promoting the release of cytochrome *c* from mitochondria in response to various pro-apoptotic stimuli. While multi-domain pro-apoptotic members Bax and Bak are believed to be actual executioners of cytochrome *c* release, their activity is regulated by other members of the family, including anti-apototic members Bcl-XL and Bcl-2, as well as pro-apoptotic members referred to as 'BH3-only proteins'. Although yeast do not have homologues of Bcl-2 proteins, it's been shown that yeast can be used as a simple model system, in which to study the action of both multi-domain pro-apoptotic and anti-apoptotic members in the Bcl-2 family following their ectopic expression. In order to address basic questions as to the mechanisms that underlie the interactions of Bcl-2 family members, we have extended this model system by introducing representative 'BH3-only' members. Here we show that expression of these proteins induces cell death in yeast when Bax and Bcl-XL are coespressed indicating that their function can be reconstituted in yeast system. Results of these studies will be presented and discussed.

This work was supported by Science and Technology Assistance Agency under the contract No. APVT-20-012404 and by NATO under the contract No. RIG 981468.

PO 4.21

MODULATORY EFFECTS OF HYPOXIA ON VIRUS GENE EXPRESSION AND INFECTIOUS PROPERTIES

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Although mammalian systems are strictly aerobic, many processes have to occur in the presence of low or no oxygen. An important example is tumor formation. The key step in regulation by hypoxia seems to be the action of HIF proteins, which recognize the hypoxia-response element (HRE) in the promoters of responsive genes. This interaction enables to start signaling pathways and processes necessary for the cell survival. Viruses as intracellular parasites are also likely to be influenced by hypoxic conditions. We have studied effects of hypoxia on two different viruses – mouse gammaherpesvirus 68 (MHV68; a DNA virus) and lymphocytic choriomeningitis virus (LCMV; a RNA virus).

The genome of MHV68 contains a number of putative HRE elements. In the presence of low oxygen levels, virus seems to reactivate from persistently infected cells. Furthermore, the regulation of several putative promoters by hypoxia was tested, and ORF50 was found to be upregulated under these conditions.

In contrary to MHV68, LCMV genome contains no putative HRE elements. Yet, exposure of cells persistently infected with this virus to hypoxia results in markedly increased levels of the viral glycoprotein mRNA. In addition, increased amounts of infectious viral particles are released from persistently infected cells into the culture medium under hypoxic conditions.

We show that both viruses (MHV68 and LCMV) respond to hypoxic conditions, but their response seems to be mediated by different mechanisms.

Supported by VEGA 2/5080/26

PO 4.22

TRANSCRIPTION OF THE GENES ENCODING METALLOPROTEINASES AND THEIR INHIBITORS IN TUMOR CELLS EXPOSED TO HYPOXIA

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Hypoxia-induced adaptive changes in the transcription profile of tumor cells lead to acquisition of aggressive properties including the capability to degrade extracellular matrix (ECM), invade surrounding tissue and metastasize. ECM metabolism is principally regulated by metalloproteinases (MMPs), which are produced as inactive proenzymes and processed to active forms by removal of an amino-terminal propeptide. Proteolytic activity of MMPs is also regulated by tissue inhibitors (TIMPs). We used quantitative PCR (qPCR) to study the transcriptional levels of the selected repertoire of MMPs and TIMPs in five carcinoma cell lines (CGL1, CGL3, SiHa, C33a and HT29) and analyze their transcriptional responses to hypoxia. qPCR was performed on RNA isolated from the cells incubated for 24 h in an atmosphere containing 1% O_2 and from parallel normoxic samples (21% O_2). The cell lines showed clear differences in the inherent transcription levels of MMPs/TIMPs under normoxic conditions with generally higher expression of TIMPs compared to MMPs. Sensitivity to hypoxia was tested using CA9 and GLUT-1 genes, which are transcriptional targets of hypoxia-inducible factor (HIF). Despite functional HIF pathway, the cells lines responded differentially both with respect to the type of induced MMPs/TIMPs and to the magnitude of induction. The strongest response to hypoxia was observed in SiHa cells. Our data show that the transcriptional effects of hypoxia on MMPs/TIMPs clearly depend on the cellular context.

Supported by EUROXY (LSHC-CT-2003-502932).

PO 4.23

EXPRESSION OF *ZEA MAYS* PHOSPHATIDYLCHOLINE - SPECIFIC PHOSPHOLIPASE C GENES UNDER STRESS CONDITIONS

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Phospholipases are key enzymes of broad range of signal transduction pathways in animals as well as in plants. They operate as parts of the phospholipide signaling system that transduces signals (e.g. abiotic and biotic stresses) from the extracellular space to the cell. Phosphatidylcholine-specific phospholipase C (PC-PLC) is an enzyme, which catalyses hydrolysis of lipid phosphatidycholine into phosphocholine (PCho) and diacylglycerol (DAG), both potential second messengers. This enzyme is well described in bacteria less in animals. Recently PC-PLC was described in *Arabidopsis thaliana* as the enzyme that is expressed during phosphate starvation. We have found *in silico* four PC-PLC homologs in *Zea mays* genome. The subject of the presented work is study of the expression of these genes under both abiotic and biotic stresses. We have found by quantitative RT-PCR increased expression of genes *Zm*PC-PLC 1,3 and 4 after salt, aluminum and cold treatment. Currently we study effects of biotic stresses (mimicked by treatment of plants by benzothiadiazol, salicylic acid and jasmonic acid) on PCPLC genes. Such results indicate possible role of PC-PLC in stress related signal transduction events. We plan to confirm these observations with *A. thaliana* plants.

Acknowledgement: This work was supported by the Czech Ministry of Education, Youth and Sports, grant no. 1P05ME825.

PO 4.24

ROLE OF CIDEa PROTEIN IN VALINOMYCIN-INDUCED APOPTOSIS IN RAT NEONATAL CARDIOMYOCYTES

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CIDEa (cell death-inducing DFF-like effector a) is a novel apoptosis activator protein. It shares homology with the N-terminal region of DNA fragmentation factor (DFF-45). Under physiological conditions it is located in mitochondria. It is not clear what stimuli may trigger translocation of CIDEa from mitochondria to nucleus in order to start DNA fragmentation. We selected neonatal rat cardiomyocytes as a model to study the effect of variable stressful stimuli on CIDEa localization. TNFalpha, hydrogen peroxide, CCCP, A23187, valinomycin, and camptothecin were chosen because of their effect on membrane potential, ion gradients or apoptotic pathways. Valinomycin caused a 3-fold increase of caspase 3 activity, detected by specific oligopeptide cleavage, accompanied by 5-fold increase in DNA fragmentation, detected by comet assay, after 3 hours. Moreover, fluorescent in situ immunodetection of CIDEa in methanol-fixed cardiomyocytes showed nuclear staining in approximately 40% of cells after valinomycin treatment. All other stimuli displayed variable levels of apoptotic markers, but none affected CIDEa localization. These observations lead us to propose that CIDEa responds to potassium gradient collapse by nuclear translocation followed by DNA fragmentation.

(This research is supported by grants GACR 301/05/0221 and MSM 6198959216)

PO 4.25

MONOCLONAL ANTIBODY M20 BINDS TO CATALYTIC DOMAIN AND INDUCES INTERNALIZATION OF CANCER-RELATED CARBONIC ANHYDRASE IX INDEPENDENTLY OF HYPOXIA

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Receptor internalization is an important process regulating the abundance and/or signal transduction via cell surface proteins. It a particular impact on the outcome of immunotherapy with specific monoclonal antibodies (MAbs) against the cancer-related antigens. The internalizing MAbs can be exploited either to block the receptor-mediated transmission of the growth/survival signals or to deliver therapeutic drugs selectively to cancer cells. Carbonic anhydrase IX (CA IX) is a suitable target for cancer immunotherapy. It is a transmembrane protein expressed in various human tumors, but not in the corresponding normal tissues. Expression of CA IX is increased by hypoxia and correlates with cancer prognosis.

We have generated several MAbs against the extracellular part of CA IX. MAb M20 binds to the catalytic domain, as shown by CA IX deletion variants. Confocal analysis revealed that this MAb is also capable to induce internalization of CA IX. The internalization was not affected by hypoxia and low extracellular pH that are typical for tumor microenvironment. M20-mediated internalization also led to down-regulation of CA IX at the cell surface. Our results suggest that CA IX behaves as a signaling molecule and that properties of M20 are promising for its therapeutic application.

Supported by VEGA (2/6113/26) and EUROXY (LSHC-CT-2003-502932).

Section 5

XENOBIOCHEMISTRY

PO 5.1

AhR LIGANDS AFFECT BOTH POST-TRANSLATIONAL MODIFICATION OF CONNEXIN 43 AND GAP-JUNCTIONAL INTERCELLULAR COMMUNICATION

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Disruption of gap junctional intercellular communication (GJIC) and expression of proteins forming gap junctions, connexins, has been suggested to play a role in deregulation of cell proliferation during tumor promotion. The ligands of aryl hydrocarbon receptor (AhR) are potent liver tumor promoters that have been previously shown to disrupt GJIC and expression of connexin 32, a major connexin expressed in hepatocytes. The liver contains also a population of poorly differentiated liver oval progenitor cells that are considered to be important target of hepatocarcinogens, and which express principally connexin 43. AhR ligands are not known to induce acute inhibition of GJIC in this cell type and little is known about long-term effects of AhR ligands. However, we have previously shown that AhR ligands disrupt contact inhibition in rat liver epithelial "stem-like" WB-F344 cells, which led to increased accumulation of cells in S-phase of cell cycle and enhanced cell proliferation. Therefore, in the present study, we investigated the impact of model AhR ligands on GJIC and connexin 43 status in WB-F344 cell line. We found that treatment with TCDD or polycyclic aromatic hydrocarbons led gradual loss of P2 and P1 phosphorylated forms of connexin 43. Surprisingly, although GJIC has been partly reduced in treated cells, the inhibition never reached more than 50 %, as compared to the control. In conclusion, we provide evidence that AhR ligands disrupt connexin 43 status in this cell model, which might be linked to disruption of cell proliferation and tumor promoting effects of AhR ligands.

[Supported by grant No. 524/06/0517 from the Czech Science Foundation.]

PO 5.2

PLATINUM(IV) COMPLEX WITH ADAMANTYLAMINE OVERCOMES INTRINSIC RESISTANCE TO CISPLATIN IN OVARIAN CANCER CELLS

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Cisplatin is one of the most important anticancer drugs displaying clinical activity against wide variety of solid tumors. A major cause of treatment failure in ovarian cancer patients remains the resistance of cancer cells to cisplatin.

LA-12 is a novel platinum(IV) complex containing bulky hydrophobic ligand amantylamine. We studied the ability of LA-12 and its reduced counterpart with oxidation state Pt(II) – LA-9 to overcome intrinsic cisplatine resistance in ovarian cancer cell line SK-OV-3.

Concentrations able to inhibit the cell proliferation of 10 % and 50 % of cells, IC_{10} and IC_{50}, were determined. We found strong differences between effects of platinum(II) complexes cisplatin and LA-9 and platinum(IV) complex LA-12 on cytokinetics parameters. IC_{50} of LA-12 complex was 2,74 times lower than in case of a cisplatin.

There were no significant differences between effects of cisplatin and LA-9. Apoptosis did not seem to be the dominant type of cell death, but it was the most intensive after an exposure to LA-12. Moreover, LA-12 caused a persistent accumulation of cells in S-phase of the cell cycle while platinum(II) complexes cisplatin and LA-9 caused only a transient accumulation in S-phase.

Taken together, LA-12 was found to be significantly more efficient than cisplatin and LA-9 and was able to overcome the intrinsic cisplatin resistance.

Supported by the IGA grant No. 1QS500040507 of the Academy of Sciences of the Czech Republic and by the Ministry of Industry and Trade of the Czech Republic, Contract No. PZ-Z2/29, “New Medicines for Cancer Therapy”

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PO 5.3

MULTIDRUG-RESISTANCE ASSOCIATED WITH OVEREXPRESSION OF P-GLYCOPROTEIN IN L1210/VCR AND L1210/DOX CELLS.

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Multidrug resistance (MDR) of neoplastic tissue represents a phenomenon when the neoplastics cells became resistant to the diverse group of cytotoxic agents with unrelated chemical structure and mechanisms of pharmacological action. MDR is often associated with over-expression and increased drug transport activity of integral plasma membrane transporter like P-glycoprotein-Pgp or multidrug resistance protein-Mrp and/or with elevation of content and activities of glutathione S-transferase isoenzymes.

In the present study drug sensitive L1210, multidrug-resistant L1210/VCR and cell line resistant to doxorubicin (L1210/DOX) were used. L1210/DOX cell line was prepared by adaptation of parental sensitive L1210 cells to doxorubicin. Cross-resistance to doxorubicin was observed in L1210/VCR cells and oppositely cross-resistant to vincristine was also found in L1210/DOX cells. Pgp was found to be responsible for depression of intracellular accumulation of Calcein AM and Fluo-3/AM (as fluorescent substrates of Pgp and Mrp) in experiments with FACS and confocal microscope. Verapamil and probenecid were used as inhibitors of Pgp and Mrp. It seems that some equal mechanisms of resistance secured by Pgp over-expression are present in both cell sublines (L1210/VCR and L1210/DOX). L1210 cells start to over-express Pgp but not Mrp in both cases i.e., when vincristine or doxorubicin was used as selection substance.

Supported by Slovak Grant Agency for Science (grant No. 2/3122/23, grant APVT 51-013802).

PO 5.4

EFFECTS OF ACYCLIC NUCLEOSIDE PHOSPHONATE TENOFOVIR DISOPROXIL FUMARATE ON CYP450 METABOLIZING SYSTEM OF PRIMARY RAT HEPATOCYTES AND LIVER MICROSOMES

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The action of antiviral agent tenofovir DF [Bis(POC)-PMPA] the prodrug of the nucleotide reverse transcriptase inhibitor tenofovir (PMPA) on rat hepatocytes and liver microsomal drug metabolizing system was investigated. The rats were injected with Bis(POC)-PMPA (25 mg/kg, i.p.) applied on 8 consecutive days for hepatocytes and 3 respectively 8 consecutive days for liver microsome preparations. The effects of PMPA were analysed on day 9 respectively on day 17 folowing the beginning of its administration for hepatocytes preparation and on day 4 and 9 respectively 11 and 17 following the beginning of administration in case of liver microsomes preparation. No changes in the content of hepatocyte nor in the content of liver microsomal protein were found. Content of total liver microsomal and hepatocytes CYP450 remained unchanged at all time intervals. CYP420 an inactive form of CYP450 was detected only at liver microsomal preparations. In hepatocytes samples there was CYP420 absent. The CYP2E1 - dependent formation of 4-nitrocatechol from p-nitrophenol as a marker of hydroxylase activity was also unchanged. In agreement with all of these observations is the finding of no influence of Bis(POC)-PMPA administration on expression of CYP2E1 mRNA in freshly prepared hepatocytes.

The work was a part of research project of Institute of Experimental Medicine ASCR, AVOZ 50390512 and was supported by grant 305/04/0127 from GACR

PO 5.5

INTERACTION OF PENTOXIFYLLINE IMMOBILIZED ON BEAD CELLULOSE WITH PROTEINS FROM SENSITIVE AND MULTIDRUG RESISTANT CELLS

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Cancer cells treated at chemotherapeutic manner gradually acquire a resistance against used cytostatics. This phenomenon, "multidrug resistance" (MDR), is defined as cross-resistance against a wide scale of structurally different anticancer substances (1) and is accompanied with over-expression of membrane transport system, P-glycoprotein (P-gp). Synthetic derivatives prepared from Pentoxifylline (PTX) are good tolerated by human organism and in the previous work we described the influence of N-substituted PTX derivatives on resistance of L1210/VCR cell line (2). The goals of this work is to describe the interactions of proteins isolated from sensitive L1210 and resistant L1210/VCR cells with PTX immobilized on bead cellulose. We prepared batch-systems and flow-systems with PTX immobilized through aliphatic pentoxy-side chain on amino-activated bead cellulose. The protein extraxts isolated from sensitive and resistant cells were applied and bound proteins were eluted using PTX. The eluted proteins were analysed using SDS-PAG electrophoresis and the results showed significant differences in profile of proteins bound to immobilized PTX when compared L1210 and L1210/VCR cells. The obtained data could after precise analysis of bound proteins help to identify the proteins involved in the MDR reversal action of PTX in L1210/VCR cells.

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PO 5.6

DETERMINATION OF ANTIOXIDANT POTENTIAL OF SOME ANTIHYPERTENSIVES

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Substances that increase blood pressure – angiotensin II, renin, aldosterone, vasopressin, endothelin I – stimulate production of oxygen free radicals through enzymes producing oxygen free radicals, such as NAD(P)H oxidase, monomeric NO synthase, xanthine oxidase, and mitochondrial respiratory chain enzymes. There is increasing evidence that antihypertensives, such as ACE inhibitors, AT1 receptor and aldosterone blockers, diuretics, or NO donors have, along with main effect, also significant antioxidant effects. In this study, we compared antioxidant capacity of selected antihypertensives with their beneficial effect against oxidative damage in spontaneously hypertensive rats.

The highest antioxidant capacities as determined by the TEAC (Trolox equivalent antioxidant capacity) method was found for captopril (C) and apocynin (A). Antioxidant capacity of indapamide (I) was found to be lower than that of Trolox, and no effect was found for hydrochlorothiazide (HCT). TEAC-based antioxidant capacities were as follows: 1.49 (C), 1.17 (A), 0.39 (I), 0.01 (HCT).

Chronic administration of antihypertensives with antioxidant effects were tested on an antioxidant potential. Administration lead to a significant decrease in levels of conjugated dienes only in case of indapamide (I) and combination of indapamide with captopril (I+C). Chronic administration of captopril alone (C), hydrochlorothiazide (HCT), or apocynin (A) did not have significant effect on the level of conjugated dienes in kidneys of experimental animals.

PO 5.7

STUDY ON METABOLISM OF CARCINOGENIC 3-NITROBENZANTHRONE AND 3-AMINOBENZANTHRONE AND THEIR POTENTIAL TO INDUCE BIOTRANSFORMATION ENZYMES

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The suspected human carcinogen 3-nitrobenzanthrone (3-NBA) is one of the most mutagenic nitroaromatic compounds identified in diesel exhaust. A 3-NBA metabolite 3-aminobenzanthrone (3-ABA) was detected in the urine of salt mining workers exposed to diesel emissions. Both 3-NBA and 3-ABA were investigated for their ability to induce biotransformation enzymes activating them to genotoxic species and for mechanism of their metabolism.

3-NBA is effectively metabolized by human NAD(P)H:quinone oxidoreductase (NQO1) to 3-ABA. The kinetics of this reduction was studied. 3-ABA is oxidized by rat hepatic cytochromes P450 in microsomes to 3-NBA and two metabolites with unknown structure. The treatment of rats with 3-NBA or 3-ABA led to 10-fold increase in formation of 3-NBA-derived DNA adducts after incubation of 3-NBA with hepatic cytosolic samples. The increase in such adduct formation correlated with an increase in protein expression and enzymatic activity of NQO1 in livers of rats treated with either compounds. The 11-fold increase in formation of 3-ABA-DNA adducts was observed after incubation of 3-ABA with hepatic microsomes of rats treated with 3-NBA or 3-ABA. This stimulation of DNA adduct formation by both compounds corresponded to their potential to induce protein expression and enzymatic activity of CYP1A1/2. These results demonstrate that by induction of hepatic NQO1 and CYP1A1/2, both 3-NBA and 3-ABA increase their enzymatic activation to reactive DNA adduct forming species, thereby enhancing their own genotoxic potential.

PO 5.8

EFFECTS OF SELECTED CYTOSTATIC DRUGS ON CARBONYL REDUCING ENZYMES IN HUMAN BREAST CARCINOMA MCF-7 CELLS

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Carbonyl reduction is a significant step in the phase I biotransformation of biologically and pharmacologically active xenobiotic carbonyl compounds. The metabolic inactivation of cytostatic drugs by carbonyl reduction could represent supplementary mechanism to multidrug resistance. In the present study, we have employed a model of human breast carcinoma MCF-7 cells to assess the effects of anthracycline antibiotic doxorubicin and potential cytostatic drug oracin on reductive activity in cytosolic and microsomal fractions. The subcellular fractions prepared from drug-treated and control cells were incubated with substrates acenaphthenol (1 mM) and oracin (0.01 – 5 mM). It was found that significantly higher amount of reduced oracin (DHO) was formed in microsomal and cytosolic fractions of drug-treated cells as compared to control groups. Generally, the concentration-dependent reductive activity was higher in the cytosolic fractions, when compared with microsomal oracin reduction in drug-treated as well as control cells. In addition, we investigated the stereospecificity of DHO formation. Oracin reducing enzymes in particular subcellular fractions of drug-treated and control cells exhibited the same stereospecifity and produced (+)- and (-)-DHO enantiomers in a ratio of 80:20 (cytosol) and 69:31 (microsomes). Further study will be necessary to determine the participation of microsomal 11β-hydroxysteroid dehydrogenase type 1, cytosolic carbonyl reductase and aldo-keto reductases (AKR1Cs) in the oracin reduction.

PO 5.9

EFFECT OF CISPLATIN ON L1210 CELLS

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Cisplatin (cis-diamminedichloroplatinum(II), cis-Pt) is one of the most active anticancer drugs and inductor of apoptosis. Cisplatin is known as P-glycoprotein (P-gp– multidrug resistance- MDR efflux pump) un-transportable anticancer drug. To resolve if changes in apoptosis are involved in P-gp mediated MDR we have studied the effect of cisplatin on parental mouse leukemia L1210 cells and on the multidrug resistant sublines L1210/VCR, which was obtained by adaptation of vincristine (VCR). This type of resistance is accompanied with expression of multidrug transporter P-glycoprotein.

We found that resistant cells are more sensitive (IC_{50} value for cisplatin was 10 times lower) as sensitive cells. Vincristine was not able to change cisplatin IC_{50} values for resistant cells. Interestingly, we detected by FACS that L1210/VCR are more tolerant to cis-Pt-induced apoptosis as sensitive cells. Many authors found (for review see Dudley and Lamming, 1996) that multidrug resistant (MDR) cells have been shown to exhibit a resistance to apoptosis induced by chemotherapeutic agents. Therefore, we have monitored the levels of proapoptotic Bax and antiapoptotic Bcl-2 proteins in our cells, which are frequently expressed in neoplastic cells. (Adams and Cory, 1998).

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This work was supported:APVT-51-027404, VEGA- 2/4155 a VEGA 2/080/26.

PO 5.10

NITRIC OXIDE AND HEPATOCYTE APOPTOSIS: EFFECTS OF LIPOPOLYSACCHARIDE AND D-GALACTOSAMINE IN CELL CULTURE

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The aim of the present work was to study the effect of D-GalN on certain apoptotic/necrotic markers in hepatocyte culture with or without lipopolysaccharide (LPS) and the involvement of NO production in hepatocyte apoptosis modulation.

Hepatocyte were treated in culture with D-GalN, D-GalN+LPS (*0-24 h*) or maintained in medium alone for the first 24 h (*time point 24 h*) and then cultivated in fresh medium without or with the respective drugs the next 24 h (*24-48 h*). ALT leakage, mitochondrial function assay (MTT test) and NO production were determined. The evaluation of apoptosis determined chemically by caspase-3 activity and cytosolic cytochrome c content and morphologically using Annexin-V/propidium iodide staining kit and with fluorescent microscopy.

There was a significant modulation of NO production by GalN under various conditions and the role of NO is only partly decisive in the apoptotic/necrotic events under the present experimental conditions. NO inhibition and the consequent apoptosis and/or necrosis should be cautiously interpreted and should be further investigated using genomic and proteomic analyses. The present data conclude that LPS/D-GalN combination added in vitro to hepatocyte is an effective model to shed light on the time course of the involvement of NO in apopto/necrotic effects. NO signaling and sequence on hepatocyte apopto/necrosis is a function of a multiple of variables.

This study was supported the research grants GAČR 305/05/2425 and VZ MSM 0021620807.

PO 5.11

CYTOCHROME b_5 POTENTIATES PARTICIPATION OF CYP1A1 AND 1A2 IN OXIDATION OF ANTICANCER DRUG ELLIPTICINE TO PHARMACOLOGICALLY MORE EFFICIENT METABOLITES

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Ellipticine is a potent antineoplastic agent, whose mode of action is based mainly on DNA intercalation, inhibition of topoisomerase II and cytochrome P450 (CYP)-mediated formation of covalent DNA adducts. The most potent activating human enzyme is CYP3A4, followed by CYP1A1 and 1A2. Many CYP-dependent reactions have been shown to be stimulated by another microsomal protein, cytochrome b_5 (b_5). Here, we investigated kinetics of ellipticine oxidation catalyzed by the CYP-reconstituted system containing CYP1A1/2 and NADPH:CYP reductase in the presence and/or absence of b_5. Five metabolites are generated by CYP1A1/2; 9-hydroxy-, 12-hydroxy-, 13-hydroxy-, 7-hydroxyellipticine and the ellipticine N^2-oxide. To examine the effects of b_5 and NADPH:CYP reductase on ellipticine oxidation, we used these compounds in different molar ratios in the CYP-reconstituted system (100 pmol CYP1A1 or 1A2, 5-150 pmol of NADPH:CYP reductase and 0-1000 pmol of b_5). The patterns and amounts of ellipticine metabolites varied significantly when b_5 was present in the incubations. Under the optimal ratio of CYP : reductase and the presence of b_5, 7-hydroxy- and 9-hydroxyellipticine formation was decreased, while generation of 13-hydroxy-, 12-hydroxyellipticine and ellipticine N^2-oxide, the metabolites responsible for formation of the major DNA adducts found in DNA of several tissues of rats exposed to ellipticine, was increased significantly. The results demonstrate for the first time the modulation of CYP1A1 by b_5 and explain the finding that CYPs of a 1A subfamily were found to be, together with CYP3A, the major enzymes responsible for formation of DNA adducts *in vivo*.

Supported by GACR (grants 303/06/0329 and 303/06/0928) and the MSMT CR (grant MSM0021620808).

PO 5.12

THE INFLUENCE OF SEVERAL PHENOLIC ANTIOXIDANTS ON THE TOXIC EFFECTS OF DOXORUBICIN

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Redox cycling and the production of reactive oxygen species is considered to be the main mechanism of the cardiotoxicity of doxorubicin (DOX) and other anthracycline drugs. DOX can undergo an enzymatic one-electron reduction by several oxidoreductases resulting in the formation of semiquinone free radical. The semiquinone further activates oxygen to form very toxic hydroxyl radicals. The aim of the present study was to determine the effect of several phenolic antioxidants on the DOX – mediated formation of hydroxyl radicals and the destruction of total cytochrome P450 (P450) content and individual P450 activities.

We measured the formation of hydroxyl radicals by ESR after incubation of different concentrations of DOX with NADPH and minipig liver microsomes. In the same system we measured the destruction of total P450 and P450 3A and 2E activities. We tested the influence of three natural (quercetin, myricetin, eriodictyol) and one synthetic antioxidant (4-hydroxy-6-methoxyaurone) on these toxic effects of DOX. We observed the greatest inhibitory effect on the generation of hydroxyl radicals in incubations with the synthetic 4-hydroxy-6-methoxyaurone. Other effects were not significant. Since phenolic antioxidants constitute a large and diverse group of chemical structures it is desirable to continue research in this field with the use of other potentially beneficial substances.

Acknowledgement: This study was supported by grant of Czech Ministry of Education, Youth and Sports, no.: OC119 and European COST Action 861.

PO 5.13

OXIDATIVE DAMAGE OF ISOLATED RAT HEPATOCYTES BY TERT-BUTYLHYDROPEROXIDE

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Oxidative stress is one of the most important mechanisms through which hepatotoxins induce cell death. Tert-butylhydroperoxide (tBHP) has been widely used as a model compound to mimic the effect of oxidative stress in various cell types. The aim of our work was to characterize toxic injury of isolated rat hepatocytes induced by tBHP.

Hepatocytes were isolated from male Wistar rats by two steps collagenase perfusion. A portion of cells was used for measurement of O_2 consumption (Oxygraph Oroboros-2k) and for evaluation of mitochondrial membrane potential, MMP (TPP^+). Remaining hepatocytes were cultivated in collagen coated Petri dishes. To estimate the rate of toxic injury we measured TBARS, LDH, MMP (Rho 123, JC-1), and GSH/GSSH (HPLC).

Tert-butylhydroperoxide increases lipoperoxidation which precedes LDH leakage and decreases activity of respiratory Complex I and Complex II, MMP and GSH/GSSG. Respiratory Complex I activity is much more sensitive to the peroxidative action of tBHP than the activity of Complex II. We also found that the mechanism of the tBHP effect on mitochondrial membrane potential is dependent on respiratory substrates and we can supposed two different mechanisms. One of them is inhibition of respiratory complex I and the second one is mitochondrial permeability transition pore opening.

PO 5.14

MODEL OF ACETAMINOPHEN-INDUCED INJURY OF RAT HEPATOCYTES IN PRIMARY CULTURE

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Acetaminophen (AAP) overdose is the most common cause of drug-induced acute liver failure. While metabolic activation of AAP is an essential step in the development of toxic lesion, precise mechanisms of the action of AAP in hepatotoxicity remain to be elucidated. The aim of our study was to establish a model of AAP-induced toxic injury of rat hepatocytes *in vitro*.

Rat hepatocytes were incubated in supplemented William´s E medium without or with AAP (1 – 10 mM) for 24 h. Toxicity of AAP was assessed at various time intervals up to 24 h by LDH activity in medium and by activity of mitochondrial dehydrogenases. All other measurements were performed after 24 h of incubation with AAP. Production of TBARS served as a marker of lipo-peroxidation. Functional capacity of hepatocytes was evaluated by production of albumin. Contents of GSH and GSSG were assessed by HPLC. Morphological changes of cells were observed using phase contrast microscopy. Mitochondrial membrane potential (MMP) was evaluated using mitochondrial probe JC-1. Production of ROS was monitored by the fluorescence emission of DCFDA.

AAP in dose- and time- dependent manner caused damage to hepatocyte membrane and decrease in mitochondrial dehydrogenases activity. Production of albumin, GSH content in hepatocytes and number of cells resp. mitochondria with high MMP were in reciprocal proportion to the dose of AAP after 24 h. Production of MDA increased with the dose of AAP. ROS production reached the highest level at AAP concentration of 3.75 mM. AAP in the range of 2.5 to 5 mM and 24h incubation are optimal for model injury to hepatocytes *in vitro*.

This work was supported by Grant MSM 0021620820.

PO 5.15

SINGLE ADMINISTRATION OF ALBENDAZOLE – EFFECT ON INTESTINAL AND HEPATIC CYP1A ACTIVITY IN MOUFLON *(OVIS MUSIMON)*

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Albendazole (ABZ) belongs to anthelmintics frequently used in veterinary practice. In mammals, ABZ undergoes a two-step sulphoxidation resulting in formation of biologically active ABZ sulphoxide and inactive ABZ sulphone. The second step – deactivation – is mediated by CYP1A. In several species ABZ cause induction of CYP1A. As the mouflons, food-producing animals, are treated with ABZ often, the effect of ABZ on mouflon biotransformation enzymes was evaluated. In our previous study, repeated administration of ABZ to mouflons led to significant induction of their CYP1A. In our present study, the effect of single administration of ABZ on mouflons´ CYP1A was tested.

Adult mouflon ewes were divided in two groups: first one (5 animals) was treated by single therapeutic dose of ABZ, second group (3 animals) was used as a control. 24 hours after the treatment, all animals were culled. Microsomes were prepared from homogenates of liver and small intestine and CYP1A activities, protein amount and ABZ biotransformation were assayed. Intestinal microsomes from treated animals exhibited approximately 25-fold higher CYP1A activity (EROD) than control microsomes. Significant increase of intestinal as well as hepatic CYP1A proteins was confirmed by Western blotting. Velocity of ABZ sulphone formation was approximately 6.8 fold and 4.4 fold higher in liver and small intestine from ABZ treated animals than in controls. This important increase of ABZ deactivation could contribute to ineffectivity of anthelmintic control.

This project was supported by Grant Agency of Czech Republic (524/03/1361)

PO 5.16

PREDICTION OF PEROXIDASE MEDIATED CARCINOGEN-RADICAL AND DNA-ADDUCTS STRUCTURE

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Peroxidase utilizes peroxides to catalyze the oxidation of many substrates including carcinogens. We focused on two model xenobiotics carcinogenic Sudan I and anticancer drug ellipticine. Both chemicals form radicals during their oxidation by peroxidases. Sudan I is metabolically activated by cytochromes P450 and peroxidases to reactive species binding covalently to nucleic acids and proteins. Ellipticine is an anticancer agent oxidized by peroxidases *via* a radical mechanism and this reaction might participate in enhancing its pharmacological efficiencies. Mechanism of peroxidase dependent protein- and DNA-adduct formation consists of 2 independent parts. First enzymatic part includes peroxide-dependent activation of the enzyme, substrate binding and substrate-radical formation followed by radical release. The second part involves radical reactions, adduct formation, oligomerization and radical scavenging. This part occurs predominantly in a solvent environment so it is not affected by peroxidase itself. Since the radical structure and adduct formation process is not directly affected by the enzyme, our model system could consist of the small molecule part only, namely xenobiotic radical species and nucleo-base part. *Ab-initio* computational approach was employed to propose structure of the major DNA-adduct and nature of the most populated reactive radical species – ultimate carcinogens, released by peroxidase.

Present results are discussed in terms of prediction of carcinogen-DNA adduct structure. Suggested structures are compared to the structural data obtained by mass spectroscopy fragmentation and NMR data.

Supported by GA ČR (203/06/0329) and Ministry of Education of the Czech Republic (MSM 0021620808).

PO 5.17

ARE COPPER(II) COMPLEXES WITH DIFFERENT BIOLOGICALLY ACTIVE LIGANDS MEMBRANOUS EFFECTIVE?

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In our previous previous papers we have studied effects of 19 newly synthetised nitrobenzoatecopper(II) complexes with different bioactive ligands on energy-requiring and energy-producing processes in Ehrlich ascites carcinoma (EAC) cells. The attachment of nicotinamidium molecule to all the nitrobenzoates caused the decrease of the cytotoxic effect. The attachment of copper(II) ions and molecules of water essentially increased the cytotoxicity. On the basis of results of primary biochemical screening we selected one of the most effective substance for detailed biochemical studies, e.i. substance No. 4.4, what is monohydrate of dietanol-tetrakis (4-nitrobenzoate)dicopper(II) complex. This compound interferes with energy-producing processes (glycolysis, respiration) of EAC cells. We supposed that the inhibiting effects on processes examined could be caused by the interaction of these complexes with biological membranes of EAC cells. Therefore we studied membraneous activity of compound No. 4.4. An orientation indicator of biological membrane damage is also the release of cytoplasmic materials absorbing at 260 and 280 nm. Substance 4.4 causes increases the absorbance in the concentration range 12.5 to 100 μmol/L. Another indicator of the biological membrane damage is the monitoring of protein concentrations in both EAC cells and the culture medium. A significant release of protein occurs at concentration higher than 25 μmol/L, which is in agreement with UV-absorbing materials. The direct evidence of affecting the integrity of biological mebrane comes from the measurement of "marker" enzyme activity LDH (EC 1.1.1.27) in the culture medium. Substance 4.4 after 120 min incubation caused increased LDH activity proportionally to the tested concentrations. This work was supported by VEGA No 1/1173/04.

PO 5.18

CHARACTERIZATION OF DEOXYGUANOSINE ADDUCT GENERATED IN DNA BY CARCINOGENIC *o*-ANISIDINE

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2-Methoxyaniline (*o*-anisidine) is important pollutant and potent carcinogen causing the tumors of the urinary bladder in rodents. *o*-Anisidine is oxidized by cytochromes P450 to *N*-(2-methoxyphenyl)hydroxylamine. *N*-(2-methoxyphenyl)hydroxylamine is additionally converted to two metabolites, one of them is nitrenium or carbenium ion. The structure of the second metabolite remains to be characterized. Deoxyguanosine adducts derived from *N*-(2-methoxyphenyl)hydroxylamine were found *in vivo*, in the urinary bladder of rats treated with *o*-anisidine. The major adduct was synthesized from *N*-(2-methoxyphenyl)hydroxylamine and deoxyguanosine, isolated by HPLC and characterized by MS-MS spectroscopy. Using this analysis and the characterization of the chemical properties of this adduct we evaluated its structure. The analyses indicate that the adduct is *N*-(deoxyguanosin-8-yl)-2-methoxyaniline. The results obtained in our study can form the basis for further detailed chemical characterization of the deoxyguanosine adducts generated by *o*-anisidine in DNA.

Supported by the GACR (grant 303/05/2195) and Ministry of Education of the Czech Republic (grant MSM0021620808).

PO 5.19

CYTOCHROMES P450: INTERACTIONS WITH ACYCLIC NUCLEOSIDE PHOSPHONATES

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Cytochromes P450 (abbrev, CYP) are enzymes which, through their influence on drug metabolism, often determine the use of many drugs and food additives. Drug interactions based on CYPs involve induction of one or more of the CYP enzymes (causing often lowering of level of another drug taken concomitantly and metabolized by the same enzyme) or competition for the active site of the particular CYP (in case that both drugs are metabolized by the same form of cytochrome P450). Acyclic nucleoside phosphonates are potent antivirals used to treat infections like HIV (tenofovir) or hepatitis B (adefovir). Surprisingly, little is known about their interactions with human liver microsomal CYPs.

In this study, interactions of these drugs (and their prodrugs, adefovir dipivoxil and tenofovir disoproxil) with liver microsomal CYP enzymes in the isolated microsomal fraction as well as in the membraneous system expressing single CYP (bactosomes) is studied. Results show that activities prototypical for the most important CYP enzymes of human liver are influenced by the presence of adefovir and tenofovir to a different extent. All the compounds studied can affect the activity of CYP2C9; adefovir and adefovir dipivoxil inhibit the activity of CYP3A4. Tenofovir disoproxil was shown to influence the activity of CYP2E1. The character of inhibitions was predominantly competitive and was apparent mostly at higher concentrations (equal or higher than 100 μM). Preliminary experiments on an influence of drugs tested on the expression of individual CYP forms did not reveal significant changes in the amount of a particular CYP enzyme. As the concentrations of the compounds studied in human plasma are generally lower that these exhibiting significant inhibition of microsomal enzymes, the influence of adefovir and tenofovir on metabolism of other drugs seems not to be of clinical importance. This result is in fact favorable for the drugs tested in this study.

Acknowledgments. The authors acknowledge financial support from the Grant Agency of the Czech Republic (305/04/0127) and from the Czech Ministry of Education through the Czech-Slovenian project KONTAKT 24/2005-06.

PO 5.20

PURIFICATION OF NOVEL HUMAN LIVER MICROSOMAL CARBONYL REDUCTASE

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Carbonyl reductases are responsible for biotransformation of many xenobiotic carbonyl compounds and most of them can be grouped into two distinct protein families: the short-chain dehydrogenase/reductase and aldo-keto reductase superfamilies. Some of these enzymes have been already purified and characterized (e.g. 11β-hydroxysteroid dehydrogenase). The isolation of membrane bound protein requires solubilization of the biological membrane containing the protein of interest prior to purification. Solubilized membrane proteins form diverse sources and with different properties can be purified by a combination of standard techniques. It is desirable that the molecules of interest are obtained in their native, biologically active form in high yield and purity.

The purification protocol consists of several steps. Solubilized human liver microsomes were desalted with the help of HiTrap Desalting column, the sample was applied to ion-exchange chromatography column – cation exchange, SP Sepharose HiTrap FF. Adsorbed proteins were eluted with increasing gradient of NaCl in elution buffer. Active fractions were pooled, concentrated and applied to Gel chromatography column. Proteins in active fractions were separated by use of chromatofocusing. All protein purification steps were carried out at 4-8°C. The reductive activity was measured via HPLC quantification of the metabolite dihydrooracin after incubation of each enzyme fraction with oracin and an NADPH regenerating system. SDS-Page was assessed to prove the purity of active fractions.

Using various separation techniques we partly purified an unknown enzyme from human liver microsomal fraction which displays reduction activity against oracin.

PO 5.21

CLONING , EXPRESSION AND PURIFICATION OF AKR1C3

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AKR1C3 is believed to play an important role in breast cancer, so different regulation and inhibition strategies are employed to influent its activity.

The coding sequence of AKR1C3 was amplified from full length clone, IRAUp969D0346D, (RZPD company) by PCR using primers of the following sequence: forward primer, GGAATTCCATATGGATTCCAAACACCAG; reverse primer, CGCGGATCCTTAATATTCATCTGAATATG. PCR was performed with Taq-Polymerase in 30 cycles with an annealing temperature of 45°C. The resulting construct was ligated into the pCR 2.1-Topo (Invitrogen) vector. After using restriction enzymes NdeI and BamH1, the resulting construct was subcloned into the pET-15b vector. For expression of AKR1C3 protein, SG13009 cells (QIAGEN) were transformed with the expression plasmid, and a 200-ml culture containing ampicilin (100 μg/ml) and kanamycin (25 μg/ml) was grown to OD_{600} = 0.6 at 37°C. Expression was induced by supplementing the culture medium with 1 mM IPTG. Cells were harvested after 4 h by centrifugation (6000g, 10 min) and resuspended in 20 ml of Tris buffer before lysation in a French-pressure cell. The resulting suspension was centrifuged for 1 h at 100000g at 4°C, and supernatant was either stored at -80°C or directly used for Ni-affinity chromatography. Final purification of recombinant AKR1C3 was achieved by fast protein liquid chromatography on a 1-ml Ni^{2+} HisTrap-FF column.

This project was supported by Grant Agency of Charles University, Grant No 108/2006/C.

PO 5.22

EFFECTS OF CpG AND/OR 13-*cis* RETINOIC ACID ON EXPRESSION OF VITAMIN D RECEPTOR IN MNU-INDUCED MAMMARY GLAND TUMOURS

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The hormonally active form of vitamin D, $1,25(OH)_2D_3$, is involved in many biological functions throughout body, such as regulation of calcium and phosphate homeostasis, bone remodeling and controlling cell proliferation and differentiation. There are two mechanisms of vitamin D action: at the level of gene transcription through a nuclear receptor (VDR) or at level of the plasma membrane via membrane-associated receptor. VDR can function as a homodimer, but heterodimerization with the retinoid X receptor, and also *all-trans* retinoic acid receptor or thyroid hormone receptor increases its affinity for vitamin D response elements in the promotion of target genes [1]. Vitamin D_3 acting via VDR has significant antitumour activities [2] and together with retinoids has shown some promise as chemopreventive and chemoterapeutic agents against chemically induced mammary gland carcinogenesis in rodents [3]. *In vivo* expression of nuclear all-trans retinoic acid receptors in mouse spleen can be influenced by genetic immunization itself, or in combination with coinjection of immunostimulatory CpG motifs [4]. We have already shown that CpG and 13cRA reduced tumour volume and tumour burden [5] and also nuclear receptors are differently expressed in various types of these tumours. In this experiment we have focused on expression of VDR in MNU-induced tumours of mammary gland of female Sprague-Dawley rats.

This work was supported partly by the VEGA grant No. 2/5017/5.

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PO 5.23

INTER-TISSUE AND INTER-SPECIES VARIABILITY OF BUTYRYLCHOLINESTERASE ACTIVITY BY RAT AND RABBIT IN VITRO

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Abstract: Butyrylcholinesterase (BuChE, EC 3.1.1.8) is a serine esterase that plays important role in toxicology and pharmacology due to its ability to hydrolyze various ester-containing drugs. So far no clear physiological function has been assigned to BuChE. Several authors suggest that BuChE could be involved in the development of nervous system and can participate in pathology of some neurodegenerative diseases.

Catalytic properties of BuChE were studied in the hydrolysis of benzoylcholine and its N-alkyl derivates, N-(2-benzoyloxyethyl) – alkyldimethylammoniumbromides, (BCHn) as substrates. Hydrolysis of (BCHn) was studied using BuChE from different sources rabbit and rat liver microsomal fractions (membrane-anchored enzyme) and serum (soluble form). Hydrolytic activity of both these BuChE forms were compared with rat recombinant BuChE. Hydrolytic product, benzoic acid, formation was recorded as a function of time, and hydrolytic rate was determined. Hydrolytic activity of BuChE with BCHn was depended on tissue, species and the length of N-alkyl chain of benzoylcholines. Hydrolysis rate versus alkyl chain length differed in each studied species.Inter species variability of BuChE activity was particularly observed in case of rat and rabbit microsomal fraction. There were not significant differences in the serum by both of studied species. Inter-species and inter-tissue variation of hydrolysis may be explained by different catalytic properties of BuChE from different species and organs.

PO 5.24

MODULATIONS OF LIPID SIGNAL TRANSDUCTION PATHWAYS ARE INVOLVED IN INHIBITION OF GJIC BY ENVIRONMENTAL AROMATIC CONTAMINANTS

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Inhibition of gap junctional intercellular communication (GJIC) is strongly associated with tumor promotion. Model tumor promoters, such as phorbol esters and epidermal growth factor (EGF), activate protein kinases (PKC, ERK1/2) phosphorylating connexin43, a constitutive protein of GJIC in rat liver epithelial cells, subsequently leading to closure of this type of intercellular communication. We found that the modes of inhibitory action of polycyclic aromatic hydrocarbons, polychlorinated biphenyls and/or other aromatic environmental contaminants on GJIC are clearly different from the effects of phorbol esters or EGF. A pretreatment with specific chemical inhibitors of signal transduction pathways revealed that modulation(s) of enzymes of lipid signaling, and not protein kinases, might be involved in inhibition of GJIC by aromatic xenobiotics. D-609, a specific inhibitor of sphingomyelinase (SMase) and sphingomyelin synthase (phosphatidylcholine-specific phospholipase C), blocked efficiently inhibitory effects of aromatics. We also found that exposure of the WB-F344 cells to ceramide or external bacterial SMase mimicked the effects of aromatic contaminants, such as inhibition of GJIC without hyperphosphorylation of Cx43. Other intracellular events, such as late activation of ERK1/2 and a release of arachidonic acid, were not directly associated with the GJIC inhibition. Finally, determination of SMase and PC-PLC activities confirmed the former findings; however, the exact activation steps remain to be identified. [Supported by the Czech Ministry of Agriculture, grant No. 00002716201, and the Czech Science Foundation, grant No. 524/05/0595.]

PO 5.25

DEREGULATION OF GJIC BY NONDIOXIN-LIKE PCB 153 IN RAT LIVER WB-F344 CELLS

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Polychlorinated biphenyls (PCBs) can be divided into two groups according to their structure and the main toxic modes of action. The coplanar dioxin-like PCBs activate AhR, while the noncoplanar non-dioxin-like PCBs may act through CAR and other mechanisms. Non-dioxin-like 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) disrupts gap junctional intercellular communication (GJIC) in vitro. This might be related to potency of noncoplanar PCBs to promote carcinogenesis. Inhibition of GJIC is detected as early as after 30 min of exposure to PCB 153 and lasts at least up to 48 hours. In this study, we investigated the PCB 153-induced alterations of GJIC by immunocytochemistry and Western blotting using antibodies against connexin 43 (Cx43), a principal transmembrane protein forming gap junctional channels in rat liver oval WBF344cells, and by PCR. PCB 153 reduced size and number of gap junctional plaques, caused their cytoplasm internalization and subsequent degradation of Cx43. Western blot analysis indicated a decrease in P2-form of Cx43 corresponding with the disappearance of gap junctions. This is a different mode of action than that of phorbol esters and EGF, which hyperphosphorylate Cx43. Taken together, PCB 153 increased internalization and degradation of Cx43 protein, which contributed to disruption of intercellular communication; however, the exact mechanism of acute inhibition of GJIC remains to be elucidated. [Supported by Ministry of Agriculture, grant No. 00002716201 and the Czech Science Foundation, grant No. 524/06/0517.]

PO 5.26

RELATION BETWEEN P-GLYCOPROTEIN OVEREXPRESSION AND CALCIUM HOMEOSTASIS IN L1210/VCR CELLS

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L1210/VCR (R) cell line was obtained by adaptation of the L1210 (S) mouse leukemic cells to vincristine and showed an over-expression of P-glycoprotein (P-gp) accompanied with multidrug resistance (MDR). Substances influencing calcium homeostasis could effectively reverse MDR of these cells. Therefore in the present study we have compared the parental L1210 and MDR L1210/VCR cells in respect to their sensitivity to high extracellular calcium concentration. We observed much more expressive influence of increased extracellular Ca^{2+} concentration on viability of R as on S cells. Ca^{2+} entry blockers as flunarazin and verapamil did not exert considerable effect on IC_{50} values for calcium, both calcium entry blockers were found to be more toxic for R as S cells. Enhancement of extracellular calcium induced increase in $^{45}Ca^{2+}$ accumulation and R cells accumulated higher amount of calcium as S cells. Cell localization of Ca^{2+} was found to be different in S and R cells, especially if media with lower Ca^{2+} concentration were used**.** Other sources of calcium are calcium proteins as e.g. calnexin which is chaperone for transmembrane proteins. In S cells we found this chaperon in higher amount as in R cells.

Acknowledgement: This work was supported from grant APVT-51/027404 and Slovak Grant Agency for Science VEGA grant No. 2/4155, 2/6080

PO 5.27

INTERACTIONS OF AHR LIGANDS AND ENDOGENOUS GROWTH REGULATORS IN RAT LIVER "STEM-LIKE" CELLS

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Infectious diseases or toxic compounds induce inflammatory responses in liver, leading to release of pro-inflammatory cytokines, such as TNF-alpha. TNF-alpha has been shown to affect cell proliferation/apoptosis ratio in various liver cell populations, thereby contributing to tumor promotion. However, little is known about its possible interactions with other known liver tumor promoters, such as ligands of the aryl hydrocarbon receptor (AhR), including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or co-planar PCB 126. We have reported that, apart from induction of enzymes involved in metabolic activation of promutagens, such as CYP1 enzymes, these compounds disrupt contact inhibition in rat liver "stem-like" cell line. In this study, we investigated interactions of TNF-alpha and AhR ligands in regulation of cell proliferation and expression of CYP 1A1/1B1 enzymes in WB-F344 cells. TNF-alpha temporarily inhibited AhR-dependent induction of CYP1A1, while the induction of CYP1B1 was enhanced by TNF-alpha at both mRNA and protein levels. TNF-alpha also significantly potentiated AhR-dependent disruption of contact inhibition and expression of cyclin A. Taken together, our results suggest that AhR ligands might be even more effective under inflammatory conditions encountered during various liver disease states. [Supported by grant No. 524/05/0595 from the Czech Science Foundation.]

PO 5.28

EFFECT OF PHENOLIC COMPOUNDS ON GLUCOSE METABOLISM IN RAT HEPATOCYTES

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Diabetes and its complications, including oxidative stress, are major reasons for medical intervention and one of the most frequent causes of death in developped countries. The liver plays an important role in regulation of glucose plasma level and is therefore, beside the pancreas, a target organ for prevention and therapy of disorders in glucose metabolism. We have previously shown extracts containing phenolic compounds from *Smallanthus sonchifolius* leaves to be able to reduce glucose production in suspensions of rat hepatocytes. Moreover, these extracts displayed an insulin-like effect on the expression of cytochrome P450 (CYP) 2B1 and 2E1 mRNA in rat hepatoma Fao cells (Valentova *et al.*, Cell Biol Toxicol 2004). In this contribution, we present the effect of phenolic acids (caffeic, chlorogenic, rosmarinic and ferulic) on glucose production in hepatocytes and on CYP2B1, 2E1, glucokinase, glucose-6-phosphatase and phosphoenol-pyruvate carboxykinase mRNA expression in Fao cells. All the phenolic acids tested at 5 mM and after 1 h incubation reduced glucose production via both gluconeogenesis (10 mM alanine or dihydroxy-cetone as precursors) and glycogenolysis as compared with metformin. Maxi-mum reduction (60.7%) was observed in the case of caffeic acid and alanine. Phenolic acids at 100 μM increased the level of glucokinase mRNA after 24 h in the same way as insulin (10^{-7} M). The effect of phenolics on glucose metabolism seems to be mediated mainly through their impact on glucokinase expression and/or its mRNA stabilisation. The effect of phenolic acids on glucokinase in a transient transfection assay is a subject of our ongoing research.

Supported by MSM6198959216 and by a Barrande (CZ-FR) project 2005-2006.

PO 5.29

CYTOTOXICITY OF ISOQUINOLINE ALKALOIDS IN HL-60 CELLS DURING DIFFERENTIATION INTO NEUTROPHILS

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The presence of 1.25% of dimethyl sulphoxide (DMSO) in the culture medium induces human promyelocytic leukemia HL-60 cells to differentiate down the neutrophil pathway. In this study, we compared cytotoxicity of six isoquinoline alkaloids in nondifferentiated HL-60 cells (nHL-60) and in cells differentiated with DMSO for 3 days to the myelocyte stage (dHL-60). Cells were seeded in 96-well plates at 1×10^5 cells/0.1 ml/well and treated for 24 hours with sanguinarine (SA), dihydrosanguinarine (DHSA), chelerythrine (CHE), dihydrochelerythrine (DHCHE), berberine (BER), or protopine (PRO) at concentrations up to 10 μM. Under given experimental conditions, untreated nHL-60 cells doubled the cell number within 24 hours while dHL-60 cells did not proliferate. The IC_{50} values determined in nHL-60 cells by MTT reduction assay were 0.62 ± 0.04 μM SA, 8.74 ± 1.37 μM DHSA, 1.84 ± 0.19 μM CHE, and 10.11 ± 0.12 μM BER. In dHL-60 cells, we obtained the IC_{50} values 2.64 ± 1.40 μM SA and 5.95 ± 0.08 μM CHE, but the cell viability after treatment with DHSA and BER did not decrease below 90%. Moreover, DHCHE and PRO at concentrations up to 10 μM did not display cytotoxic effect in either nHL-60 cells or dHL-60 cells. We conclude that SA and CHE inhibit both proliferation and viability of nHL-60 cells while DHSA and BER affect the cell proliferation rather than the cell viability.

The work was supported by grants No. MSM 6198959216 and No. 303/06/P193.

PO 5.30

MOLECULAR MECHANISMS OF FLAVONE-INDUCED EFFECTS ON AHR IN RAT LIVER „STEM-LIKE“ CELLS

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Aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor mediating toxic effects of dioxins, co-planar PCBs or polyaromatic hydrocarbons (PAHs). Apart from these prototypical AhR exogenous agonists, a number of structural diverse natural compounds, such as flavones, behave as partial AhR agonists/antagonists. A synthetic 3´-methoxy-4´-nitroflavone (3M4NF) is considered to be almost pure AhR antagonist. In our study, we used 3M4NF, TCDD (a model persistent AhR agonist) and beta-naphthoflavone (BNF) as a model AhR ligands, in order to describe differences between activities of AhR agonists and antagonists at cellular level. All three compounds induced AhR nuclear translocation, but only TCDD and BNF caused binding of AhR to its DNA responsive elements (DRE) in promoter of target gene, *Cyp1a1*. When applied with 3M4NF (but not with BNF), TCDD failed to activate AhR-mediated transcriptional activity, at least at the DNA-binding level. All three ligands strongly potentiated proliferation of contact-inhibited rat liver ´stem-like´ cells WB-F344. However, 3M4NF was the most efficient disruptor of contact-inhibition, inducing high levels of cyclin A expression and decreasing levels of proteins mediating cell-to-cell adhesion, such as cadherins or plakoglobin. [Supported by grant No. B6004407 from the Grant Agency of the Academy of Sciences of the Czech Republic and No. 524/06/0517 from the Czech Science Foundation.]

PO 5.31

REPRODUCTIVE FUNCTION STUDY OF THE WISTAR RATS AFTER PERINATAL TREATMENT WITH CARBENDAZIM

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Carbendazim (MBC) affects male reproductive organs and causes testicular toxicity of adult experimental animals by disruption of microtubular structures.

The aim of this study was to examine the effect of MBC on developmental and reproductive parameters of perinataly treated rats. The female rats were treated with MBC (0, 7.8, 15.6 and 62.5 mg/kg bw per day) by gavage from GD 15 until 3 PND. The pups were observed for viability and weight gain (PND 1, 4, 7, 14 and 21) landmarks of reproductive system and morphological development (Anogenital distance (AGD), vaginal opening, testicular descensus (TD), preputial separation(PPS)), neuromuscular function as surface righting (PND 1 and 7), cliff avoidance test (PND 7) and open field test (PND 21 and 35) as well as functional parameters as estrus cycle (EC) and fertility.

The measurement of the AGD in both female and male pups did not reveal significant differences from the control animals' data. Also there was not a delay in TD and PPS in males from treated groups compared to controls. Changes in behavior were observed in all neuromuscular tests for each treated groups compare to control animals. Differences in the regularity of EC and duration of its phases against control values were not demonstrated for the perinatally treated groups. In the animals from treated groups weren't recorded significant changes in copulation index and fertility index compared to controls.

There are no data for an effect on reproductive system development and fertility of Wistar rats treated perinatally with MBC (7.8, 15.6 to 62.5 mg/kg per day) but perinatal treatment with 62.5 mg/kg/day MBC induced malformations of CNS and behavioral development disturbances.

This study was supported by RTN NUTRICEPTORS, Contract HPRN – CT-2002-00268. P. D. and Z .S. – present address: Institute of Virology, SAS Bratislava Slovakia

Section 6

FREE RADICALS IN BIOLOGY AND MEDICINE

PO 6.1

ROS: CHEMILUMINESCENT DETECTON *VS*. PHOTODYNAMIC EFFECT

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The photodynamic effect involves the combination of light, photosensitizer and molecular oxygen. Upon irradiation, free radicals and singlet oxygen (ROS) are produced. Various chemiluminescent compounds (e.g. luminol) have been studied in order to find a suitable probe for their detection. *Cypridina* luciferin analogues (CLA and MCLA) are thought to emit light when reacting with superoxide anion and singlet oxygen.

The aim of this work was to compare the killing effect of the photosensitizers (e.g. fluorescein, eosin, phthalocyanines) on *Paramecium caudatum* and the chemiluminescent detection of ROS by using CLA and MCLA.

The photodynamic experiments were done in Petri dishes with *Paramecium caudatum* solution (density about 25 individuals in 1 ml). The lifetime means the time when all *Paramecium caudatum* are dead (immovable).

The chemiluminescent measurements were done by luminometr BioOrbit 1250 and Fluoroskan Ascent FL. The concentrations of CLA and MCLA have depended on the sensitivity of luminometr in the range from10^{-5} to 10^{-7} M. The temperature was 25°C and 36°C.

The specific or non-specific quenchers (ascorbic acid, trolox, SOD, NaN_3) were used in order to distinguish Type I and Type II of the photodynamic effect.

This research was supported by the grant from Ministry of Education MSM6198959216

PO 6.2

STUDY OF SYSTEMIC INFLAMMATION IN THE MODEL OF ADJUVANT ARTHRITIS ON DETERMINING LEVELS OF IL-6 AND GAMMA-GLUTAMYL TRANSFERASE ACTIVITY

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In the model of adjuvant arthritis (AA) the clinical parameters (hind paw volume - HPV, body mass, arthrogram) were monitored along with selected biochemical parameters in order to characterize the progress of the disease as well as the effect of the therapy tested. AA was induced by intradermal administration of *Mycobacterium butyricum* in incomplete Freund`s adjuvans to male Lewis rats. To assess the development of systemic inflammatory response and its modification due to the phytotherapy tested (Boswellia serrata extract; arbutine), IL-6 levels were studied by ELISA (professional kit was used – Quantikine R&D Systems) and the activity of gamma-glutamyl transferase (GGT) was measured by spectrophometric assay. GGT was determined in the homogenates of spleen and joints using the modified method of Orlowski and Maister (1970). The experiments included healthy intact animals as reference controls, arthritic animals without any phytotherapy, and arthritic animals with phytotherapy administration in the dose of 50 mg/kg b.w. The treatment involved daily oral administration of the substances tested from day 0, i.e. the day of immunization with MB, to the end of the experiment - day 28. The selected biochemical parameters were in excellent agreement with clinical parameters concerning the characterization of the disease progress. Phytotherapy failed to affect the clinical parameters yet it significantly reduced biochemical parameters studied (VEGA 2/5051/05, APVV-51-017905, APVV-21-055205).

PO 6.3

DETERMINATION OF HYDROXYL RADICALS IN PERFUSED RAT HEARTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH COULOCHEMICAL DETECTION

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Reactive oxygen species (ROS) are generated in biological systems and have been implicated in a variety of pathological states. Between the most important ROS are considered very toxic hydroxyl radicals. They have short half-life and therefore are present at extremely low concentrations in biological system. The aromatic hydroxylation is one of the specific methods for measuring of OH radicals. These radicals attack the phenyl ring of aromatic molecules and form hydroxylated products of those molecules. Salicylic acid as aromatic compound is good trapping agent and its hydroxylated compounds can be detected.

The formation of hydroxyl radicals was assessed by measuring the amount of 2,5-dihydroxybenzoic acid (2,5-DHBA) by HPLC with coulochemic detection. The detection limit and linearity were also determined. Isolated rat hearts were perfused using Langendorff´s technique and 2,5-DHBA was determined in collected effluent. It was proved that hydroxyl radicals were produced in significant amount during ischemia and reperfusion in these experimental conditions.

In conclusion, this method is suitable for determination of hydroxyl radicals in perfused rat hearts and can be applied to assessment the role of OH radicals in myocardial ischemic and reperfusion injury.

The work was supported by the project MSM 0021622402 of the Ministry of Education of the Czech Republic.

PO 6.4

THE CHANGES SOD AND CAT ACTIVITY AFTER POSTCONDITIONING

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In the present study we report changes of endogenous antioxidant enzymes SOD and CAT, specifically a very rapid increase of activity caused by *de novo* synthesis of proteins, which can be observed 5 hours after the second ischemia, much like after the first ischemia.

Searching for mechanisms of protection we measured the activity of endogenous antioxidant enzymes. Ten minutes of ischemia was used as preconditioning and two days after the preconditioning, a second ischemia was induced for 5 minutes as postconditioning in the cortex, hippocampus and striatum. Enzymatic activities of total SOD, CuZn-SOD, Mn-SOD and CAT were measured 5 hours and 24 hours after postconditioning. Significant increase of total SOD activity in all studied regions of the brain was observed 5 hours after postconditioning (5 minutes of ischemia). Twenty-four hours after postconditioning SOD activity was down at control values. Similarly, a large increase in the activity of catalase induced by postconditioning (5 minutes of ischemia) was detected after 5 hours. It is interesting that the biggest changes were established in selectively vulnerable hippocampus and striatum

The results of the present study provide evidence that, if 10 minutes of ischemia are used as preconditioning, followed by 5 minutes of ischemia used as postconditioning, the activities of the antioxidant enzymes SOD and CAT are significantly increased after 5 hours of reperfusion in all studied brain regions. But the higher levels of SOD and CAT activity returned to control values after 24 hours of reperfusion.

Study was supported by VEGA 2/3219/23, VEGA 2/6210/26, APVT 51-021904 grants.

PO 6.5

EFFECT OF ANAESTHETIC ADMINISTRATION OF KETAMINE AND DIETHYLETHER ON LIPID PEROXIDATION IN RAT LIVER HOMOGENATES.

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The aim of our study was to compare the influence of two kinds of anaesthesia to production of lipid peroxidation (LP) products in rat liver homogenates. Animals were divided into three groups: I. control, II. with anaesthesia by intramuscular administration of ketamine (100 mg/kg) with xylasine (15 mg/kg) and III. with anaesthesia by inhalation of diethylether.

Both anaesthesias didn't influence the content of thiobarbituric acid reactive substances (TBARS) in liver homogenates. Significant differences were observed in the TBARS production in conditions of activated LP, depending on different anaesthesias. TBARS production was determined in vitro after 30-min incubation at 37°C in the presence of these LP effectors: 125 μmol/L ascorbate, 5 μmol/L $FeSO_4$ and the activator of LP (125 μmol/L ascorbate + 5 μmol/L $FeSO_4$). In the control group, the effectors significantly increased the TBARS production. Similar results were observed in the group III., when diethylether was used for anaesthesia. Ketamine (100 mg/kg i.m.) with xylasine completely supressed activatory effect of the LP effectors on the TBARS production.

These results indicate, that ketamine seems to act as a radical scavenger and/or disturbs the activatory effect of Fe^{2+}, that is necessary for activation of lipid peroxidation. The different effects of the used anaesthetics on LP could be explained by their different physicochemical properties, as well as various ways of their application that could be connected with their different concentrations in blood or tissues.

PO 6.6

EFFECT OF SOME NATURAL COMPOUNDS ON DNA DAMAGE IN HUMAN LEUKEMIA CELL LINE HL-60

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The aim of the present study was evaluation of influence of some natural compounds with biological effects on DNA damage in cultured human leukemia cell line HL-60.

In one group of experiments, protective effect of flavonoid baicaline against DNA damage caused by oxidative stress (100 uM H_2O_2 for 1 hour) was monitored. The other experiments were designed to investigate DNA damage caused by alkaloids. For this, the cells were cultured for 1 hour in supplemented DMEM medium with either chelerythrine or sanguinarine, both representing main species of benzophenathridine alkaloid group.

Alkaline - neutral modification of the comet assay method was employed for evaluation of DNA damage. Cell suspension in PBS was mixed with low melting point agarose, aliquots were poured on microscopic slides and let to polymerize. Lysis of the cells was carried out for 1 hour in a Tris-borate buffer (TBE), pH 8.4, containing 1mM EDTA and 2.5% SDS. After lysis, the slides were placed into denaturating buffer for 20 min, neutralized in the TBE and laid side by side into electrophoresis chamber filled with cold TBE buffer. Electrophoresis has been performed for 15 min at constant 1 V/cm. The slides were silver stained and evaluated under microscope Leitz Orthoplan (objective 16x, magnification 160x) attached to the camera. Images of the comets were analyzed with the PC software LUCIA (L.I.M. Prague).

We have found, than DNA damage of HL-60 cells caused by H_2O_2 is significantly ameliorated by the presence of 100 uM baicaline. By contrast, incubation of the cells with both tested alkaloids caused concentration dependent DNA damage.

This study was supported by grants FRVŠ 1418/2006 and GAČR 525/04/0017

PO 6.7

PHOTOGENERATION OF SINGLET OXYGEN BY HYPERICIN IN COMPLEX WITH LOW DENSITY LIPOPROTEINS

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Photosensitized generation of singlet oxygen ($^1O_2$) by molecules of photosensitizers (pts) is a crucial process in photodynamic therapy (PDT). Upon administration into blood stream pts associate predominantly with serum proteins (low density lipoproteins (LDL), human serum albumin (HSA)). The interaction of hypericin (Hyp), natural photosensitizing agent occurring in the plants of the genus *Hypericum*, with HSA has been already intensively studied, however only few works about physicochemical properties of Hyp bound to LDL have been published.

In this study we present time–dependent fluorescence and phosphorescence study of Hyp in complex with LDL as well as the evolution of $^1O_2$ formation under illumination of Hyp/LDL complex. We have demonstrated that amount of produced $^1O_2$ increases linearly with Hyp concentration until concentration ratio Hyp/LDL reaches 25:1. Beyond this ratio the formation of $^1O_2$ shows saturation behavior. With respect to this result we assume that the amount of produced $^1O_2$ depends on the quantity of monomeric molecules of Hyp inside the lipidic part of LDL. The dependence of the intensity of Hyp phosphorescence on Hyp/LDL ratio is similar to that for $^1O_2$ formation. The shortening of Hyp fluorescence lifetime starting at Hyp/LDL ratio 25:1 suggests quenching of excited singlet state of monomeric Hyp at higher Hyp/LDL concentration ratios.

Acknowledgement
This work was supported by the Scientific Grant Agency of the Ministry of Education of Slovak Republic under the grant VEGA No. 2265, by the Slovak Science and Technology Assistance Agency under the contract APVT-20-036104 and by the project MSM0021620835 from the ministry of Education of the Czech Republic.

PO 6.8

RELATIONS BETWEEN REDOX STATUS AND BIOCHEMICAL PARAMETERS IN SPONTANEOUSLY HYPERTENSIVE RATS: EFFECTS OF PRAZOSIN AND LOSARTAN.

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Oxidative stress has an important role in hypertension. It is associated with changing of oxidant/antioxidant balance and organs damage. In spontaneously hypertensive rats (SHR) we investigated effects of antihypertensive therapy on lipophilic antioxidant status, lipoperoxidation in heart, liver, kidney and plasma biochemical parameters. SHRs were treated from 4 to 9 weeks of age with prazosin (10 $mg.kg^{-1}.day^{-1}$) or losartan (20 $mg.kg^{-1}.day^{-1}$ b.w.). SHRs had lower concentrations of tocopherols and CoQ_{9ox} in the heart, in the liver moreover decreased CoQ_{9red}, in plasma were lower tocopherols, tChol, TAG and higher activities of ALP, ALT and AST. Increased TBARS were found in the heart. Both prazosin and losartan prevented γ-toc decline in plasma but did not prevent increased myocardial TBARS and moreover induced TBARS formation in the liver and kidney. Losartan prevented decline in liver γ-toc and CoQ_{9red} and decreased tChol, TAG and ALP in plasma. Conclusions: Antihypertensive therapy both with prazosin and losartan had beneficial effects on blood pressure, myocardial hypertrophy, plasma γ-tocopherol, tChol and TAG concentrations. Improved liver redox status associated with anticholesterolemic and antihypertriglyceridemic effects could indicate some antioxidant properties of losartan. Besides the beneficial effects, increased lipoperoxidation should be taken into consideration. Supplementation of antioxidants could be useful in prevention of possible organ damage and improved effectiveness of antihypertensive therapy. (*Supported by grants VEGA 1/3442/06 and 2/6139/26)*

PO 6.9

RETINOIC ACID-INDUCED NEURAL DIFFERENTIATION OF P19 EMBYONAL CARCINOMA CELLS IS EFFECTED BY MODULATION OF INTRACELLULAR REDOX STATE

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Retinoic acid (RA), the derivative of retinol, plays significant roles in the regulation of cell proliferation, differentiation, and apoptosis. In vitro, RA induces the pluripotent embryonal carcinoma (EC) and embryonic stem cells to differentiate into various lineages. It was documented previously that growth in serum-free media itself committed EC cells to neural differentiation and the addition of RA intensified this effect.

Intracellularly produced reactive oxygen species can modulate differentiation of EC cells via alternation of redox sensitive signaling pathways. In our preliminary study, various antioxidants or inhibitors of intracellular ROS production – glutathione, N- acetyl cysteine, ascorbic acid, diphenylene iodonium chloride, and apocynin were tested to modulate P19 cells differentiation to neural like cells. It was shown that all tested antioxidants potentiated P19 cells differentiation as was determined by downregulation of E-cadherin and Oct-4 expressions.

It could be summarized that cell redox status influence the direction of P19 embryonal carcinoma cell differentiation.

PO 6.10

ANTIOXIDANT AND ANTIMUTAGENIC PROPERTIES OF BEER, MALT AND BARLEY

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This work was focused on the study of antimutagenic effects beer and selected brewer's commodities including their active components and intermediates. Biological activity was compared with the content of active components. Analysis of active substances was performed by the RP-HPLC/UV-VIS and by RP-HPLC/MS-ESI. In most of analyzed samples high content of antioxidant substances was found. The highest amount of (-)-catechin was detected in the malt wort and hopped wort samples. In most of samples presence of caempherol, rutin and ascorbic acid was also confirmed. The highest values of total polyphenols and flavonoids as well as TAS were found in malt. Antimutagenic effects of beer, malt and other intermediates were studied using two *in vivo* tests: i) yeast *Saccharomyces cerevisiae* D7 and ii) protozoon *Euglena gracilis*. In general, antimutagenic effect decreased in following line: malt, malt wort, hopped wort. In analyzed beers, the best antimutagenic effect was found in the beer „Červený drak“, further in black beer, beers with original hopped wort extract of 10 to 12 % wt. and at least in non-alcoholic beer. No activity was detected in the hop extract. Although samples with higher content of antioxidants exhibited also higher antimutagenic effect, no correlation between antioxidant content, total antioxidant capacity and antimutagenic activity was found. This fact confirms that other biologically active substances present in beer and malt can influence its biological properties.

ACKNOWLEDGEMENTS: THIS WORK WAS SUPPORTED BY PROJECT MSM 0021630501 AND FRVS 3150/G1/2006 OF CZECH MINISTRY OF EDUCATION.

PO 6.11

INFLUENCE OF SELECTED CHEMICAL AND BIOLOGICAL FACTORS ON SUPEROXIDE DISMUTASE LEVELS IN BARLEY AND MALT

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This work was focused on monitoring of superoxid dismutase (SOD) activity in barley and malt. SOD is antioxidative enzyme responsible for protection of barley and malt before free radicals, lipid peroxidation and other negative changes which can influence malt quality as well as sensory properties of beer. Changes of enzyme activity were evaluated with regard to barely variety, fungal infection, type of fungicide and type of previous agricultural plant. Five varieties of barley were studied – Malz, Yersey, Kompakt, Sebastian and Prestige. During cultivation individual barley varieties were infected by arteficial mould infection using *Fusarium* sp. Three types of fungicides were tested – Mirage, Horizont and Silwer. Barleys were cultivated on experimental fields after rape, corn, maize and sugar-beet. Malt was prepared from selected samples using micro-malting procedure. SOD activity was measured by Ransod kit (Randox). Radical level was monitored by EPR spectroscopy.

SOD activity was slightly dependent on barley variety – higher activity was detected in varieties Kompakt, Jersey and Sebastian. Positive effect on SOD activity exhibited rape as pre-plant. Activity of SOD was higher in all samples of malt when compared with appropriate barley samples. No statistically significant effect on SOD activity was detected after artificial fungal infection as well as after fungicide application.

ACKNOWLEDGEMENTS: THIS WORK WAS SUPPORTED BY PROJECTS MSM 6019369701

PO 6.12

IS OUR HEALTHY POPULATION EXPOSED TO THE RISK OF OXIDATIVE DNA DAMAGE?

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Oxidative damage to DNA, which is a manifestation of oxidative stress, belongs to most frequent damages in aerobic cells. Modified bases and breaks of one or two DNA chains represent significant pool of mutagenesis and cancer risk. The most important products of oxidative damage to bases are 8-oxoG and 8-oxodG, determined in tissue cells or lymphocytes.

The aim of our study has been the investigation of the DNA damage in healthy adult volunteers and children from different regions of Slovakia.

To investigate DNA damage in lymphocytes we employed: single cell gel electrophoresis (comet assay) without enzyme modification according to Collins et al. [1], enzymatically modified comet assay using formamidopyrimidine-DNA glycosylase and HPLC method with coulochemical detection [2].

The median concentration of 8-oxoG in lymphocyte DNA from adult volunteers in Slovakia determined by HPLC was 6,615 per 10^6 guanines and median concentration of FPG-sites, measured by comet assay, 0,29 per 10^6 guanines. The total DNA damage in children from selected regions of Slovakia are different. No statistical significance was found between children (average age 13,06 years) and adults volunteers (average age 19,6 years) in Bratislava and between genders.

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PO 6.13

INFLUENCE OF PYRIDOXALYLAMINOGUANIDINE ON SELECTED PARAMETERS OF OXIDATIVE STRESS IN RATS WITH DIABETES MELLITUS

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Diabetes mellitus is associated with the oxidative stress. Free radicals and oxidative stress can contribute to pathogenesis of diabetes, as well as to its later complications. Different drugs inhibit oxidative stress and formation of advanced glycation end products.

We examined the effect of structural analogue of the antidiabetic compound aminoguanidine, pyridoxalylaminoguanidine (PAG) on the production of selected markers of oxidative stress. We found that administration of PAG to diabetic rats decrease antioxidant potential of plasma and decrease formation of malondialdehyde (MDA). Its administration did not change glucose level. However, on the other hand, PAG increases susceptibility of lipoproteins to oxidation and increases formation of conjugated dienes in both, diabetic as well as in control animal. Therefore it is necessary to search for other structural modifications of aminoguanidine that would combine its higher antidiabetic activity with less toxicity.

PO 6.14

PROTEIN OXIDATION PRODUCTS IN RATS WITH STREPTOZOTOCINE-INDUCED DIABETES MELLITUS

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The oxidative damage provoked by reactive free radicals has been demonstrated to play a significant role in aging, diabetes and several pathological events. The imbalance between production and scavenging of free radicals, due to an increase in oxidative flux or a decrease in the antioxidant ability, is responsible for cellular and tissue damage in a number of acute and chronic illness, including diabetes mellitus. High concentration of free radicals damages cellular components such as lipids, nucleic acids and proteins. The attack by reactive oxygen species against proteins modifies aminoacid residues generating carbonyl moieties, which can be used as a measure of protein damage.

In this study the level of plasma protein carbonyl groups in rats with streptozotocine-induced diabetes mellitus was determined; moreover the antioxidant capacity of plasma was studied and correlated. The relationships between oxidative damage and some metabolic and clinical parameters were also evaluated.

Aknowledgments

This work was supported by NATO collaborative linkage grant LST.CLG.980106.

PO 6.15

CARVEDILOL, PROFESSIONAL PHAGOCYTES AND REACTIVE OXYGEN SPECIES GENERATION

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Superoxide, nitric oxide (NO) (intra- and extracellular messengers that mediate diverse signalling pathways in target cells) and myeloperoxidase (MPO) derived from professional phagocytes seem to be coupled through complex and interdependent pathways. The biological consequences of NO - peroxidase interactions and of superoxide - nitric oxide balance may have broad implications for the regulation of local infectious, inflammatory, and cardiovascular events *in vivo.* The effect of carvedilol [0.1, 1, 10, 100μmol/l], a vasodilating beta-blocker with the potential for cardiovascular organ protection, was studied on stimulated intact neutrophil degranulation and verified by comparing its effect on cell-free system. Phospholipase D (PLD) mediation of superoxide anion production in human neutrophils was investigated by use of wortmannin (inhibitor of 1-phosphatidylinositol 3-kinase) and propranolol (inhibitor of phosphatidic acid phosphohydrolase). Carvedilol [10, 100 μmol/l] significantly decreased OZ and fMLP stimulated superoxide generation and MPO release. There was no effect of carvedilol in the three lower conc. on LPS stimulated iNOS expression and NO production on RAW 264.7. In the 100μmol/l conc., the effect appeard to be rather cytotoxic. The beneficial effects of carvedilol seen in clinical practice seem to be brought about by cumulative effect of antioxidative and scavenging activities, interference with signal transfer transduction and its physicochemical properties.

Supported by grants: APVV–51-029602, GACR 305/04/0896 CR

PO 6.16

INFLUENCE OF ANTI-RADICAL ACTIVE SUBSTANCES OF PYRIDOINDOLE TYPE ON INFLAMMATION AND OXIDATIVE STRESS IN THE MODEL OF ADJUVANT ARTHRITIS

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Chronic activation of the immune system and oxidative stress are involved in rheumatoid arthritis (RA). The aim of this study was to evaluate the prophylactic and therapeutic effects of three pyridoindoles (PI) – stobadine dipalmitate (STB.DP), SMe1.2HCl and SMe1EC2.HCl. Adjuvant arthritis was induced in male Lewis rats by a single intradermal injection of heat-killed *Mycobacterium butyricum*. The experiments included healthy animals as reference controls, healthy animals with PI administration, arthritic animals without any drug administration and arthritic animals with PI administration in the oral daily dose 15 mg/kg b.w. The PI studied ameliorated the arthritic parameters in the order of potency: STB.DP, by SMe1.2HCl and SMe1EC2.HCl. This finding was further supported by reduced γ-glutamyl transferase (GGT) activity (inflammatory parameter) in the spleen and joints and with the effect on TBARS levels (parameter for monitoring oxidative stress–induced lipid damage) in spleen homogenates. The effect observed in plasma was different, with the two new PI derivatives being more effective than STB.DP in influencing TBARS levels. Protein carbonyl group levels (2nd parameter for monitoring oxidative stress) assessed in treated control groups in liver and kidney homogenates showed a similar trend like TBARS. These findings suggest that systemic chronic inflammation can be beneficially affected by decreasing the oxidation stress with the aid of anti-radical active substances (VEGA 2/5051/05, APVV-51-017905).

PO 6.17

ANTIOXIDANT AND PROOXIDANT PROPERTIES OF BOSWELLIA SERRATA EXTRACTS

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B. serrata is a tree from the Burseraceae family commonly found in India, northern Africa and the Middle East. Strips of its bark yield a gummy oleoresin which contains oils, terpenoids and gum. Its active principle consists of 4 pentacyclic triterpenes, β-boswellic acid being the major one. Extracts of this oleoresin have been used as a folk medicine for centuries in Ayurvedic medicine in India. Boswellia extract tablets have been employed for asthma, arthritic conditions and ulcerative colitis and other chronic inflammatory diseases.

In our work, we assessed redox properties of B. serrata commercial extract, Boswellin® and its purified form, that can be responsible for its anti-inflammatory properties. Both extracts showed negligible free radical reducing properties evaluated by the reaction with free stable 1,1`-diphenyl-2-picrylhydrazyl radical. On the other hand, the extracts were shown to be responsible for oxidation of ferrous ion as determined by thiocyanate method followed by accumulation of a reaction product with absorption maximum λ_{max} = 342 nm. The iron oxidizing properties may be attributed to α-keto-β-boswellic acid constituents of B. serrata extracts and may be responsible for some adverse effects of B. serrata preparations.

PO 6.18

ANALYSIS OF OXIDATIVE/ANTIOXIDATIVE BALANCE IN TOXIC LIVER INJURY

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Introduction: Oxidative stress is one of the most important processes playing crucial role in toxic injury of the hepatocytes. Hepatotoxic injury may be caused by both increased levels of reactive oxygen species (ROS), and by decreased activity of antioxidant systems. Therefore, sufficient characterisation of oxidative stress requires use of various methods. These can help to distinguish particular components of the oxidative processes, but also antioxidant system. The aim of our work was to evaluate the oxidative stress and the changes in antioxidant capacity during toxic injury of isolated rat hepatocytes induced by model compound - tert-butylhydroperoxide (tBHP).

Methods: Hepatocytes were isolated from male Wistar rats by two step collagenase perfusion. Isolated hepatocytes were cultivated in collagen coated Petri dishes. Mitochondria were isolated by differential centrifugation. The intracellular ROS probes, DCFDA (in both cell cultures, and isolated mitochondria) were used to measure the relation of different tBHP concentrations and ROS production. Levels of glutathione, as the main intracellular antioxidant, were analyzed together with the activity of glutathione reductase. As the marker of oxidative stress TBARS levels were measured.

Preliminary conclusions: As supposed, we found that the action of tBHP increases oxidative stress through various mechanisms. ROS production has been increased only up to tBHP concentration of 1.5 mM in both cultivated cells, and isolated mitochondria. The GSH/GSSG ratio has been decreased and TBARS levels increased in dependence on the tBHP concentration. This work was supported by grants GAČR 303/03/H065, GAUK 90/06/C, and FG360007.

PO 6.19

MECHANISM OF FREE RADICAL REACTIONS IN ERYTHROCYTE MEMBRANES

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Free radicals initiate the chain reactions of lipid peroxidation in biological membranes. In this work we studied time-course of production of various markers of lipid peroxidation (such as conjugated dienes, TBARs, lipofuscine-like pigments - LFP, and pentane) during in vitro peroxidation of erythrocyte membranes. In vitro peroxidation of erythrocyte membrane was initiated by the addition of ascorbic acid, $FeSO_4$ and EDTA to erythrocyte membranes suspension. Samples were taken immediately at the beginning of peroxidation and then after 2h, 4h, 6h, 24h and 48h of peroxidation. The concentration of conjugated dienes didn´t change until 48h when it decreased significantly. TBARs concentration increased after 4h of peroxidation, and after 6h of peroxidation started to decrease. LFP (excitation 358 nm, emission 408 nm) concentration increased at 4h of peroxidation and stayed increased all the time of peroxidation. Fluorescence spectra didn´t revealed any qualitative changes in LFP composition. The content of pentane increased between 2h and 4h of membrane peroxidation. After 6h of peroxidation pentane wasn´t produced. These results indicate that peroxidation was finished after 6h, most probably due to the exhaustion of the substrate, PUFA. The time course of individual markers of peroxidation imply that for the measurement of oxidative damage in biological systems are suitable LFP because their concentration stay increased after termination of free radical generation.

PO 6.20

LIMITED DEGRADATION OF OXIDATIVELY MODIFIED CALMODULIN BY PROTEASOME: FORMATIONS OF PEPTIDES

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Oxidative stress is known to result in selective oxidative modification of calcium regulatory proteins like calmodulin (CaM), thus modulating signal transduction and cellular energy metabolism. Restoration of cell function may require the degradation of oxidized CaM (oxCaM) and other signaling proteins. The 20S proteasome has been suggested to play a critical role in mediating the degradation of crucial proteins under conditions of oxidative stress.

We studied the effects of various concentrations of H_2O_2 on CaM and its degradability by the purified 20S proteasome. Oxidized fractions of CaM were identified by electrophoretic separation on SDS-polyacrylamide gels. The electrophoretic mobility of oxCaM depended on the degree of oxidation and on the presence or absence of Ca^{2+} ions. Two fragments of oxCaM were distinguished by the immunoblot techniques. The extent of protein carbonyl formation in oxCaM increased concentration-dependently on H_2O_2 and was not dependent on presence or absence of Ca^{2+}. The 20S proteasome degraded concentration- and time-dependently the oxCaM, without affecting the unoxidized CaM. Oxidized CaM was degraded by 20S proteasome to four proteolytic fragments. The sequence of amino acid residues in the above-mentioned fragments was determined by mass-spectrometric sequenation. Our work suggests that CaM oxidized by H_2O_2 was degraded by the 20S proteasome without ubiquitin conjugation and without affecting the unoxidized CaM.

This work was supported by VEGA 2/5012/26, APVV 51017905 and COST B35.

PO 6.21

PROOXIDANT AND ANTIOXIDANT EFFECT OF SELENIUM IN ANIMAL TISSUES

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Selenium is an essential nutritional trace element incorporated into proteins to make selenoproteins. Some of them are important antioxidant enzymes. Although selenium is required for health of humans and animals, excessive dietary selenium may be toxic. Many organic and inorganic selenium compounds have been investigeted as selenium supplements. Their safety and efficacy differs because of their differential metabolic processing by the body.

The effects of feed supplementation with inorganic (sodium selenite) and organic (Se-yeast) forms of selenium on the activities of antioxidant enzymes (GSHPx, CAT and SOD) were examined in the liver and heart of lambs. Extra dietary Se resulted in significant increase of Se content in the liver and heart tissues in both supplemented groups. There were no correlation between the selenium tissue distribution and glutathione peroxidase activity (GSHPx). Significantly lower GSHPx activity was found in Se-yeast supplemented group when compared to both control and selenite fed groups in the liver. In the heart, GSHPx activity increased in both experimental groups. The similar effect of supplementation was obtained for catalase activity. The activity of superoxide dismutase (SOD) increased only in the heart after selenium supplementation. In addition, differences in SOD isoenzyme patterns in both supplemented groups indicate possible differential metabolic processing of extra dietary selenium in the heart. Two more intensive Cu, Zn-SOD isoenzymes appeared in Se-yeast group were probably generated as a result of the oxidative stress, which has also been manifested by significantly increased TBARS values.

The presented results showed that the response of the organs to chemical form of extra dietary selenium is different and possible prooxidant or antioxidant effect of extra dietary selenium depends on chemical form of selenium and could be connected to distinct metabolism of selenium in the individual organs.

PO 6.22

LIPID PROXIDES AND 8-OXOdG IN DIABETIC RATS

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Reactive intermediates are implicated in pathogenesis of various disorders, including diabetes mellitus (DM). DM is mediated by an inflammatory process in which the primary cause of islet destruction is due to the hydroxyl radical [1] ($^{\cdot}$OH) that causes damage to lipids as well as to DNA. It is supposed that methylnicotine amide (MNA) may sequester $^{\cdot}$OH and therefore, it is able to protect pancreatic cells from oxidative stress.

The purpose of this study was to investigate the effect of MNA administration on the level of lipid peroxides in plasma as well as 8-oxodG in tissues of rats with streptozotocine-induced diabetes.

Male white rats were used for this study. Diabetes was induced in experimental animals by streptozotocine (45 mg per kg body weight).

Lipid peroxides were determined spectrophotometrically and for determination of 8-oxodG HPLC-ECD was used.

According to our running results MNA had no effect on plasma level of lipid peroxides. Further results concerning the effect of MNA administration on 8-oxodG in tissues will be presented at the conference.

Reference

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Acknowledgement

This study was supported by VEGA grant of Ministry of Education of SR 1/1157/04, and Mind&Health-civil association.

PO 6.23

EFFECT OF AGING ON HEART MITOCHONDRIA

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The exact cellular and molecular mechanisms of the aging process are unclear, but there is growing evidence that age-related changes are consequences of oxidative stress. In the present study we examined the effect of 4-hydroxynonenal (HNE) on cytochrome c oxidase (COX) activity, oxidative modifications of mitochondrial lipids and proteins in hearts from aging Wistar rats (6-, 15- and 26-month old). The activity of COX was 44.6±1.6% ($p<0.001$, vs. 6-month old rats) loss in hearts from 26-month old rats, whereas the COX protein level, detected by Western immunoblotting, was unchanged. Lipid peroxidation (LPO) of mitochondrial membranes, measured by conjugated diene formation, increased progressively with age. Cytochrome c oxidase activity was inhibited, with concomitant increase in endogenous HNE level in mitochondria. The activity of COX was also inhibited following incubation of mitochondria with 5-100 microM HNE. To assess possible structural modifications of proteins we measured sulfhydryl group content, fluorescence of dityrosines and lysine conjugates with LPO end-products. The total sulfhydryl group content was approximately 14.6±1.0% ($p<0.001$), lower in 26-month old rats. Compared to 6-month old rats there was also significant increase in protein dityrosines and lysine conjugates with LPO end-products. These results suggest that loss of COX activity during aging may be due in part to oxidative modifications of mitochondrial proteins and/or lipids. We propose that mitochondrial dysfunction increases in age-dependent manner and is mediated, in part, by modification of specific mitochondrial proteins by the lipid peroxidation product HNE.

This work was supported by grants VEGA 1/2263/05, APVT-51-027404 from the Ministry of Education and Science of the Slovak Republic.

Section 7

PATHOBIOCHEMISTRY

PO 7.1

GLYCATION OF ASPARTATE AMINOTRANSFERASE: COMPARISON of 3 *in vitro* MODELS.

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Non-enzymatic glycation is a process in which reducing sugars react spontaneously with amino groups in proteins to form advanced glycation end-products (AGEs). This process causes impairment of protein functions during diabetes mellitus and age-related diseases. Several oxidative steps are included in the non-enzymatic glycation, which are supported by transition metal ions. Fructose (Frc) and its metabolites, such as methylglyoxal (MGO), may participate in the intracellular glycation process at much faster rate than glucose.

Three models of the glycation of aspartate aminotransferase (AST, E.C. 2.6.1.1) were developed in our laboratory. In the first model, AST was incubated with D-fructose in 50 mM concentration at 37°C for up to 21 days. AST was incubated with 50 mM Frc and 10 μM Cu^{2+} ions in the second model. In the third model, AST was incubated with 0.5 mM MGO at 37°C for up to 4 hours for enzyme activity measurements and 7 days for fluorescence measurements, respectively. Decreases in enzyme activity (kinetic UV method) and generation of fluorescent AGEs ($\lambda_{ex}/\lambda_{em}$ 370/440 nm for total AGEs and $\lambda_{ex}/\lambda_{em}$ 335/385 nm for pentosidine) were taken as the criteria of the course of glycation.

Methylglyoxal is more potent glycating agent than D-fructose. It caused 65% decrease of the AST activity after 4 hours of incubation. Comparable results were obtained after 15 days in the first model and after 8 days in the model with Frc and Cu^{2+}. Also generation of both total AGEs and pentosidine was faster in the incubation mixtures containing MGO. Presence of Cu^{2+} ions led to the increased formation of fluorescent AGEs in the models with fructose.

Acknowledgements: Supported by the Charles University Grant 128/2005.

PO 7.2

ASYMMETRIC DIMETHYLARGININE (ADMA) AS A NOVEL INDEPENDENT RISK FACTOR FOR CARDIOVASCULAR DISEASE IN HEMODIALYSIS PATIENTS

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ADMA is an endogenous inhibitor of nitric oxide synthase and is regarded as a novel risk factor for cardiovascular disease. ADMA concentrations are increased in hemodialysis (HD) patients and may contribute to endothelial dysfunction in them. We tested a relation of ADMA to other cardiovascular risk factors. We analyzed ADMA levels by ELISA method, total and HDL cholesterol, apolipoproteins AI (apoA) and B (apoB), triglycerides (TG), oxidized LDL (oxLDL), C-reactive protein measured by ultrasensitive method (uCRP), lipoprotein (a), thiobarbituric acid reactive substances (TBARS) and homocysteine (hcy) in serum of 176 HD patients and 73 healthy men which served as controls. Relations among these risk factors were evaluated using Spearman correlation and multiple regression analysis. Average ADMA concentration of HD patients was significantly higher than control values (1.13 [0.22] vs. 0.75 [0.15] μmol/l, $p<0.001$). There were interesting correlations between BMI and oxLDL ($r=0.33$, $p<0.001$), apoA and uCRP ($r=-0.28$, $p<0.001$), apoB and oxLDL ($r=0.75$, $p<0.001$) and finally TG and oxLDL ($r=0.45$, $p<0.001$). No correlation was found between ADMA and hcy ($r=-0.07$, $p=0.39$). Multiple regression analysis revealed that ADMA is independent from BMI, age and all other measured parameters. ADMA is independent of all other measured cardiovascular risk factors including hcy. The study was supported by GAUK Grant 78/2005/C/LFP.

PO 7.3

EFFECT OF FRENCH MARITIME PINE BARK EXTRACT, PYCNOGENOL®, ON THE HORMONE STATUS IN CHILDREN SUFFERING FROM ATTENTION DEFICIT HYPERACTIVITY DISORDER (ADHD)

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The main symptoms of Attention Deficit Hyperactivity Disorder (ADHD), a neurodevelopmental disorder, are impulsivity, hyperactivity and inattention. Children have imbalance in catecholamine's metabolism, what can modify attention, senses, the way of thinking and reactivity of second messengers.

Pycnogenol® (Pyc), a standardized extract from the French pine bark (*Pinus Pinaster*), consists of procyanidins, catechin, taxifoline and phenolic acids. Among other things, it acts as strong scavenger of free radicals in vitro and it stimulates activities of antioxidant enzyme.

The aim of our randomized, double-blind, placebo controlled study was to determine the hormone status (catecholamines, thyreotropine (TSH) and growth (GH) hormone) of ADHD children (6-15 years) after Pyc/placebo administration, and to compare levels of catecholamines in ADHD and healthy children.

We have found increased levels of adrenaline, noradrenaline and dopamine in the urine of children with ADHD in comparison to the group of healthy children. After Pyc administration we found a trend of reduction of adrenaline and noradrenaline and significant decrease of dopamine.

One month after Pyc administration the level of GH increased, but not significantly. The level of TSH didn't change after Pyc administration.

PO 7.4

FUNCTIONAL ADAPTATION OF CARDIAC MITOCHONDRIA TO ACUTE DIABETES: ROLE OF MEMBRANE FLUIDITY AND MEMBRANE POTENTIAL.

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Remodeling of subcellular membrane systems represent a part of endogenous protective mechanisms (EPM) acting in the diabetic (DIA) myocardium. Present study is devoted to elucidation of the role of changes in mitochondrial (MIT) membrane fluidity (MF) and transmembrane potential (MP) in remodeling of the MIT, associated with EPM and leading to adaptation of the heart to DIA. *Experimental:* DIA was induced by a single dose of streptozotocin (STZ, 65 mg/kg i.p.) to male wistar rats ($220^{\pm}20$). Hearts were investigated on 8^{th} day after STZ administration when the animals exhibited 300-330% increase in blood glucose. MF of isolated MIT was assessed by measuring fluorecence anisotropy of 1,6-diphenyl-1,3,5-hexatriene. MP of the MIT was monitored by confocal microscopy using JC-1 as a fluorescent indicator. Mg-dependent and 2,4-DNP-stimulated ATPase was assessed by estimation of P_i. Conjugated dienes (CD) in MIT membrane lipids were estimated spectrophotometrically at 230 nm. *Results and Discussion:* DIA heart MIT exhibited decreased oxygen consumption, lowered RCI, rate of phosphorylation (all $p<0.01$) and the MP, probably due to free radicals induced damage (a ~17% increase in oxidized form of Q_{10} ($p < 0.05$). On the other hand, MF, the total MIT ATPase and the formation of membrane transition pores all increased ($p<0.05$), without any considerable elevation of CD formation. Regression analysis revealed a significant association between the increase of MF and decrease in MP 46% of variability of cases, with 5% confidence interval. *Conclusion:* Remodeling of function and physical properties of MIT membranes is an essential part of EPM that are yielding in adaptation of the myocardium to DIA. Grants: 2/5110/25, APVT: 51-027404, 51-013802, 51-017902; SP51/0280901, SP51/0280902

PO 7.5

UREA AND URIC ACID UROLITHIASIS

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The main disadvantage of uric acid as a waste product in humans is its relatively low solubility at pH below 5.5 which oftenly leads to uric acid stone formation. The frequency of uric acid stones in our population is around 0.1 %. Because human urine is always supersaturated with uric acid, so urinary pH is the key factor for precipitation of uric acid.

Since 1932 is known the enhancing effect of urea on uric acid solubility (Medes, 1932). However, by our knowledge, the effect was not tested in the patients with uric acid urolithiasis. In the paper we present our results of in vitro and in vivo study of urea and uric acid relationship. The in vitro study consisted of solubility determination of uric acid in isotonic solutions at 37 °C without and with urea in relationship to pH, and gel chromatography of the same system. The in vivo study on more than 70 patients with uric acid urolithiasis showed significant relationship ($p < 0.001$) between urea and uric acid levels in the first morning urine. The effect of different load of urea (3 g and 30 g) on urea and uric acid levels in the first morning urine was also studied in 11 volunteers.

The authors discuss potential significance of urea in relationship to uric acid urolithiasis, its treatment and prevention.

The partial financial support by IG4 13/2002 is appreciated.

Ref.: Medes, G.: Proc. Soc. Exp. Biol. Med. 1932, 30, 281-284

PO 7.6

EVALUTION OF DRUGS CHEMORESISTENCE IN VITRO IN CARCINOMAS OF LUNG, COLON AND RECTUM

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Cancer continues to cause significant mortality and the use of standard cytotoxic chemotherapy has reached a therapeutical plateau. The improvement of treatment results is expected from ex vivo drug sensitivity testing which may allow to choose the most effective drugs for individual patient and to exclude agents to which the tumor cells exert resistance.

We introduced method for in vitro testing of sensitivity/resistance of tumor cells to cytostatics. The principle of the assay is 3-day cultivation of tumor cells with different concentrations of cytostatics. Finally, the concentration of drugs killing 50% of tumor cells is evaluated. We examine 68 carcinomas of lung, colon and rectum.

Primary chemoresistance could be observed in lung carcinomas in 28 % for cisplatin, 35% for paclitaxel, 55% for etoposide, 57% for vinorelbine, 62% for topotecan, 77% for gemcitabin and 85% for dacarbazine. Cisplatin was the most efficient drugs in vitro also in colorectal carcinoma. The chemoresistance to 5-fluorouracil approaching to 71% corresponds to some 20-30% effectivity of this drug observed in clinical studies with standard 5-FU regiments. Two of twenty-seven (7%) colorectal carcinoma showed chemoresistance to all tested drugs.

Not only more experience in needed with this individualized approach to cancer chemotherapy, but also contribution of clinicians on interpretation of testing and correlation studies of tailored chemotherapy.

This work was supported by grants MVTS BIL/ČR/SR/UK/06 and AV 4/0013/05 from the Ministry of Education and Science of the Slovak Republic.

PO 7.7

PROTEIN DETERMINANTS OF COELIAC DISEASE

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The specific part of the human population is permanently intolerant to specific cereal gluten protein included in diet. The toxicity of gluten protein results in numerous metabolic derangements leading to the health condition known as coeliac disease or glutensensitive enteropathy. About 3000-5000 coeliac patients is supposed to live in Slovakia. Therefore, the resolution of causality of protein intolerance and research of substitutional plant food resources for gluten-free diet are of high importance. Several properties of cereal storage proteins affect the initiation of coeliac disease. The main factor is the presence and frequency of immunodominant peptide epitopes that are recognized by T-lymphocytes. The most reactive fragments have following amino-acid sequences: PFPQPQLPY, PQPQLPYPQ and PYPQPQLPY. The high content of glutamine (50%) and proline (15%) residues in the gliadin molecules determines the high frequency of immunodominant peptide epitopes. The reactivity of gliadin fragments is dependent also on the secondary structure of protein.In addition to exogenous factors, the sensitivity of a human organism, particularly the susceptibility of mucosal cells in small intestine and response of the immune system, plays important role in the initiation and pathogenesis of coeliac disease.

The objectives of present study are analysis of the fractional composition of grain protein complex in the wide spectrum of cereals and pseudocereals, estimation of the frequency of amino acid residues, testing of different kits designed for the estimation of gluten protein content and proposition of the pseudocereal food resources for gluten-free diet.

Supported by project 2003SP 270280E010280E01.

PO 7.8

SERUM MATRIX METALLOPROTEINASE (MMP-2) AND ITS TISSUE INHIBITOR (TIMP-1) IN RELATION TO PRO- AND ANTI-FIBROTIC FACTORS IN PATIENTS WITH HEART FAILURE

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Matrix metalloproteinase MMP-2 and its endogenous inhibitor TIMP-1 are associated with extracellular matrix remodeling, which may lead to interstitial fibrosis and thus contribute to heart failure. Besides, pro- and anti-fibrotic factors such as endothelin-1 and nitric oxide, respectively, regulate fibrosis by modulating collagen synthesis and also MMP/TIMP activity. Nowadays, there is thought that progressive remodeling accompanied with changes in the extracellular matrix may lead to severe heart failure. The aim of this study was to find a relationship between MMP-2/TIMP-1 and other above mentioned parameters in patients with heart failure.

Forty patients (52 ± 10 years, 32 males, 8 females) with chronic heart failure (NYHA II–IV with ejection fraction of left ventricle less than 40 %), ICHS 15, DKMP 25 were enrolled in the study. Peptides and nitric oxide were measured by commercially available ELISA or assay kits.

There were found significantly increased values of measured parameters in comparison with the physiological values and some statistically significant correlations between these parameters.

The work was supported by the project MSM 0021622402 of the Ministry of Education of the Czech Republic.

PO 7.9

BIOCHEMICAL CHANGES OF CNS CELLS AFTER GLOBAL ISCHEMIA AND REPERFUSION

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Simvastatin is cholesterol lowering agent and also a modulator of cytokine in the nervous system. The functional significance and neuroprotectiove mechanism of simvastatin in ischemic brain injury is not yet solved. The purpose of study is to evaluate the effect of simvastatin on ischemic brain injury. A variety of endoplasmic reticulum (ER) stresses trigger the unfolded protein response (UPR), a compensatory response whose most proximal sensors are the ER membrane bound proteins IRE1 and ATF6. We were simultaneously examined the activation of ATF6, IRE1, Bip and Xbp-1 at mRNA levels after 15 minutes 4-VO ischemia and different times of reperfusion (1, 3 and 24h). The results in state without neuroprotection were compared with results using simvastatin. Levels of ATF6 mRNA was not significantly affected by simvastatin. To determine whether the alterations seen in IRE1 in previous studies correlated with its endoribonuclease activity, we assessed the processing of endogenous *xbp-1* mRNA. Active IRE1 cleaves a 26-nt sequence from *xbp-1* mRNA resulting in processed *xbp-1*. Although inactive IRE1 was maximally decreased by 90-minute reperfusion, there was no evidence that its substrate *xbp-1* messenger RNA had been processed by removal of a 26-nt sequence. Similarly, there was only small signifficant changes in expression of the 55-kd XBP-1 with and without simvastatin. The extreme differences we observed in mRNA and protein levels of Bip (Grp78). These data indicate that statins, in addition to their preventive effect on cerebral ischemia, exert a neuroprotective role in the attenuation of brain damage after acute stroke.

This work was supported by VEGA 3380/06, MVTS 39 grants and UK grants 254/06 and 250/06.

PO 7.10

THE ROLE OF METALLOTHIONEIN IN PROCESS OF THE RESISTANCE AGAINST PLATINE CYTOSTATICS FORMATION

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From plenty of clinic studies there is known that cytostatics resistance is a serious complication of the malignant tumor treatment. The resistance of tumor cells against cytostatics consists from various mechanisms and all the process is secured at the cell level multifactorial. Especially in the case of multidrug resistance (MDR) is a tumor disease therapy complicated. One of the tumor cells resistance causes is lowered cytostatics concentration in the target place of treatment. Within general known mechanisms the MDR resistance formation can be linked with increased expression of low-molecular protein metallothionein (MT). The biological function of MTs is to bind heavy metals in organisms and to keep their homeostasis. Exprimed MT binds the administered anticancer drug immediately. The resulting effect is a rapid depression of drug concentration under effective level.

In our project we study the role of MT in process MDR resistance against platinum cytostatics formation. We used neuroblastoma cell lines resistant and sensitive to *cis*-platinum. Our results show that in *cis*-platinum resistant lines is the MT content multiple higher than at sensitive lines.

This work was supported by grant GACR 525/04/P132.

References. 1.R. Prusa et al.: Clin. Chem. **51** (Suppl. 6), A56 (2005); 2. R. Prusa et al.: Clin. Chem. **50** (Suppl. 6), A28 (2004); 3.R. Kizek et.al.: Anal. Chem. **73** (20), 4801 (2001); 4. M. Strouhal et. al.: Bioelectrochemistry 60 (2003) 29;

PO 7.11

THE EFFECT OF CHRONIC NOS INHIBITION ON REGULATORY PROTEINS IN RAT HEARTS

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We found previously that hearts from rats with chronic NO deficiency (NOD) showed better recovery of contractile function after ischemia/reperfusion. Aim of the present study was to characterize the effect of chronic nitric oxide synthases (NOS) inhibition on NOS, Akt kinase and matrix metalloproteinases (MMPs). NOD was induced by L-NAME (40/mg daily, 4 weeks). Isolated hearts were Langendorff-perfused and subjected to global ischemia. Tissue samples were taken from the left ventricles. Activities of MMPs were analyzed by gelatine zymography. Levels of specific proteins were determined using specific antibodies. We found that L-NAME treatment decreased the levels of iNOS. In hearts from rats with NOD we found also inhibition of specific eNOS phosphorylation. The NOS inhibition and ischemia did not influence levels of Akt. However, the development of NOD was connected with decreased activation of Akt kinase and also with reduction of tissue activities of MMP-2. Inhibition of MMP-2 activities we observed also after ischemia. The protein levels of MMP-2 were not changed by L-NAME treatment and ischemia. After ischemia we found increased levels of TIMP-2 and that in both control and L-NAME-treated hearts. The results point to the role Akt kinase, NOS and MMP-2 in responses of myocardium to chronic NOD and in adaptive responses of these hearts to ischemic stress.

Supported by grants: VEGA SR 2/6170/26, APVT 51-027404

PO 7.12

STUDY OF HYPOXIA-INDUCED CARBONIC ANHYDRASE IX IN THE MOUSE MODEL USING NEW MONOCLONAL ANTIBODIES

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Carbonic anhydrase IX (CA IX) is a cancer-related enzyme involved in regulation of pH homeostasis, cell proliferation and adhesion. CA IX is normally present in the gastrointestinal tract, with the highest level observed in the human gastric mucosa. However, overexpression of CA IX was found in a number of human malignancies derived from CA IX-negative tissues. CA IX level is strongly increased in response to hypoxia via a direct transcriptional activation of *CA9* gene by hypoxia inducible factor-1 (HIF-1).

So far, only a polyclonal rabbit serum directed to mouse CA IX (mCA IX) has been available. We have produced new monoclonal antibodies (MAbs) in CA IX-deficient mice immunized by recombinant GST-mCA IX protein. Out of 2400 hybridomas, five produced mCA IX-specific MAbs, offering us an opportunity to initiate a study of CA IX in the mouse model.

Until recently, the expression of CA IX in the mouse was considered quite similar to that of the rat and human tissues. Using new MAbs in WB and IHC, we detected strong membrane reaction in the glandular stomach epithelium and moderate reactions in the intestine and colon. No staining for mCA IX was found in the heart and brain. Surprisingly, IHC staining of the kidney revealed positive signal in the basolateral membrane of proximal tubules suggesting an important physiological role for CA IX in this tissue.

Supported by EUROXY (LSHC-CT-2003-502932).

PO 7.13

LIPOPEROXIDATION IN ELDERLY HUMANS WITH HYPERHOMOCYSTEINEMIA IN THE COURSE OF SELENIUM SUPPLEMENTATION

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Increased oxidative stress plays an important role in the aging processes. Besides, elevated plasma homocysteine, particularly evident in elderly, has been recognized as a risk factor for many pathological states including oxidative stress. Selenium as a component of antioxidative enzymes is associated with the protection against oxidative stress. The aim of this study was to assess plasma malondialdehyde as a marker of lipid peroxidation in relationship to selenium in hyperhomocysteinemic elderly subjects in the course of a long-term selenium supplementation.

A cohort of 50 elderly subjects (74 ± 5 years) with hyperhomocysteinemia has been divided into 3 groups according to the amount of selenium supplementation, which was carried out over a period of 3 years. The analysis of thiols and malondialdehyde was performed by HPLC/FD and selenium was measured using neutron activation analysis.

We found a temporal statistically significant fluctuation of malondialdehyde and homocysteine, but without relation to the selenium supplementation. Moreover, we found no evidence that plasma total homocysteine was associated with lipid peroxidation. Therefore, we do not consider selenium alone as a potential factor for the lower of lipid peroxidation in elderly hyperhomocysteinemic humans.

The work was supported by grant NR/7820-3 of the Ministry of Health of the Czech Republic.

PO 7.14

ZINC, COPPER AND SELENIUM PLASMA CONCENTRATIONS IN CHILDREN WITH ATTENTION DEFICIT HYPERACTIVITY DISORDER TREATED WITH PYCNOGENOL

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Attention Deficit Hyperactivity Disorder (ADHD) is a neurodevelopment disorder of children. The exact etiology is unknown, genetics play a role, but major etiologic contributors also are included. It is assumed that free radicals play a role in the pathogenesis of ADHD.

The aim of present work was to examine the effect of Pycnogenol® (PYC) (1 mg/kg of body weight/day) on the plasma levels of Zn, Cu, Se and some parameters of oxidative stress related to ADHD in a randomized, double – blind and placebo – controlled study. Children with ADHD were examined at start of the trial, one month after PYC or placebo administration and one month after termination of treatment. The concentration of Zn, Cu and Se in the plasma of children with ADHD and in the healthy controls were determined by atomic absorption spectrometry (AAS).

At the beginning of the study, the plasma Zn concentration was lower in children with ADHD in comparison to healthy controls, just opposite to Cu levels that were the same. After one - month of PYC administration degreased plasma level of Cu was found. The level of Se was in physiological range before the study and was not changed after PYC administration. Although PYC did not influented significantly the level of Se, Zn and Cu, clinical symptoms of ADHD were improved.

Section 8

BIOTECHNOLOGY

PO 8.1

GENETIC MARKERS OF TECHNOLOGICAL QUALITY OF WHEAT

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The main goal of our work was to determine the technological quality of 33 new wheat genotypes and wheat genotypes (*Triticum aestivum.* L) on the base of the protein markers. HMW-glutenin subunits (HMW-GS) and gliadins were separated by SDS-PAGE and A-PAGE. The collection of bread-wheat with HMW-GS composition 0, 7+9, 5+10 and Glu-score 7 was the most frequent. The highest value of technological quality (Glu-score 9) achieved genotypes Magvas, Favorit with HMW-GS composition 1, 7+9, 5+10 and Astela with HMW-GS composition 2*, 7+9, 5+10. HMW-GS 0, 7+8. Secalin block Gld 1B3 was detected in wheat gliadin spectra what has negative effect of the whole technological quality and Glu-score is decreased by two points.

In the present DNA markers have now higher application in the detection of genes controlling important wheat properties for breeding process. The aim of our work was to analyze the same set of wheat genotypes as with protein markers. Wheat cultivars were analyzed for the presence of major HMW alleles at A, B and D genome loci with multiplexed PCR assay. Three primer pairs were used in a single PCR reaction and agarose gel assay. Multiplex PCR analysis were compared with protein analysis done by SDS-PAGE. PCR analysis for gene controlling bread-making quality confirmed that primers used in the present study are specific for the studied alleles and can be used for the detection of the wheat technological quality, for the introgression positive HMW genes that improve wheat technological quality and DNA-based identification of wheat genotypes.

PO 8.2

ESSENTIAL AMINO ACIDS ENHANCEMENT IN CROP PLANTS THROUGH GENETIC ENGINEERING.

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Plants are the primary source of dietary proteins for humans and livestock. In comparison with meat, plant proteins are less expensive to produce. However, with reference to the needs of humans as well as monogastric domestic animals and poultry, the amino acid composition of most legumes and cereals is unbalanced in terms of defficiency of certain essential amino acids (EAAs), such as methionine (M), cysteine (C) and lysine (K).

There are several transgenic approaches to increase the content of a specific essential amino acid in target plants, including heterologous expression of a high EAA containing proteins, introduction of a synthetic protein into plants, modification of protein sequence, and metabolic engineering of the free amino acid pool.

We used two approaches to improve the EAA content in two plant species: one legume, alfalfa (*Medicago sativa* L.), and one cereal, barley (*Hordeum vulgare* L.), i.e. i) expression of a desirable heterologous high EAA containing storage protein, and ii) modification of a biochemical pathway of particular amino acid synthesis.

The first approach was used to improve the M+C content in a forage legume alfalfa, using the *Agrobacterium tumefaciens*-mediated genetic transformation with a gene *Ov* coding for an ovalbumine from Japanese quail (Coturnix coturnix). Of the 32 transgenic lines evaluated, three lines contained 188-223% M+C comparing to non-transformed control (0,228 % DW).

The second approach was used in the effort to improve the K kontent in a cereal crop barley, using the particle bombardment-mediated introduction of the *dap*A gene from *E.coli* coding for a feedback-insensitive enzyme dihydrodipicolinate synthase (DHDPS) into barley genome. The expression of this gene can eventually increase the free-lysine content in barley grains.

PO 8.3

VERIFICATION OF BARLEY GENOTYPES HOMOGENITY BY HORDEIN GENETIC MARKERS

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Considering the effect of variety on malting properties in the malting and brewing industries, there is a need for quick and accurate methods of varietal verification. Prolamins have been recently used to study genetic diversity in barley. The analysis of prolamins by PAGE, was therefore internationally recommended by the International Seed Testing Association (ISTA) for the verification of species and cultivars.

The polymorphism of hordein storage protein was studied in seed samples of spring barley. For identification and characterization of barley varieties the standard reference method ISTA was used. The dendrogram based on hierarchical cluster analysis - Ward´s methods was prepared and Jaccard coeficient was calculated.

Vertical PAGE was used to analyze the hordein polypeptide patterns of Slovak barley varieties (*Hordeum vulgare* L.). Forty different polypeptide bands with molecular weights ranging from 28 to 96 kDa were found in the seeds of the 25 species studied. Twenty of the 25 varieties examined showed intravarietal polymorphism. Number of bands ranged from 10 to 17, depend on the variety and from 3 to 13 among individual seeds, with a total of 26 bands in *Hordeum vulgare*. Phenograms using each seed as an operational taxonomic unit showed that the seeds from most varieties did not from distinct clusters.

Acknowledgement
This work was supported by a VEGA project No. 1/3474/06.

PO 8.4

REAL-TIME PCR: A TOOL FOR RAPID DETECTION AND QUANTIFICATION OF CEREAL PATHOGENS

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Conventional-PCR has emerged as a major tool for the diagnosis and study of phytopathogenic fungi and has contributed to the alleviation of some of the problems associated with the detection, control and containment of plant pathogens. Therefore, it is desirable to develop a reliable, rapid and sensitive diagnostic method for quantification of different pathogens.

Real-time PCR (RT-PCR) differs from classical PCR by the measurement of the amplified PCR product at each cycle throughout the PCR reaction. Compared with classical PCR, one of the main advantages of RT-PCR is its rapidity to provide reliable data. Typically, the time of a whole RT-PCR run ranges from 20 min to 2 h. In the simplest and most economical format, the reporter is the double-strand DNA-specific dye SYBR® Green (Molecular Probes). The advantages of SYBR Green are that it's inexpensive, easy to use, and sensitive. The two most popular alternatives to SYBR Green are TaqMan® and molecular beacons, both of which are hybridization probes relying on fluorescence resonance energy transfer (FRET) for quantitation.

Several reviews on the use of real-time PCR in fields other than plant pathology have been published recently. In plant pathology, reviews of interest have focused on crop biosecurity and on the detection of seed-borne fungal pathogens and phytopathogenic bacteria. However, real-time PCR detection systems are available for viruses, nematodes, bacteria and fungi. In our work SYBR® Green was used for detection and quantitation of the major barley and wheat pathogens.

PO 8.5

ANTIMICROBIAL PROPERTIES OF ENVIRONMENTAL ENTEROCOCCI ISOLATES

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The properties of 166 environmental enterococci isolated mainly from surface- and waste-waters were studied. The strains were characterized by detection of enterocins, virulence factors and antibiotic resistance. The presence of different enterocin genes (*ent*A, *ent*B, *ent*P, *ent*31, *ent*L50AB) was frequently observed in strains, 109 strains contained at least one enterocin gene. The distribution of enterocin genes varied according to the species, the genes were present mainly in *E. hirae and E. faecium*. In the enterocin spot assay, 10 isolates showed inhibition against listeria. Eleven strains were detected to contain virulence genes (*cyl*A, *gel*E and *esp*). The resistance of environmental strains to eleven antimicrobial substances (Penicillin, Ampicillin, Vancomycin, Erythromycin, Tetracycline, Ciprofloxacin, Rifampin, Chloramphenicol, Gentamicin, Nitrofurantoin, Amoxicillin / clavulanic acid) was tested and we found that 74 strains over 166 isolates were resistant to some antibiotic. Only two strains showed resistance to five or more antibiotics. By these results is possible to consider the environmental enterococci as GRAS (generally recognized as safe).

PO 8.6

CHANGES OF KINETIC PARAMETERS IN BACTERIAL OXIDATION OF ELEMENTAL SULFUR

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Biooxidation of elemental sulfur plays an important role in biohydrometallurgy and spontaneous biogeochemical processes in sulfide deposits (sulfide wastes, abandoned ore mines). The purpose of this study was to investigate a correlation among growth conditions, physiological factors and selected kinetic constants of elemental sulfur oxidation by *Acidithiobacillus ferrooxidans*. The values of maximum sulfur oxidation rate (v) and growth yield on sulfur (Y) were determined from data obtained in 73 batch cultures. The changes of the apparent Michaelis constant for sulfur (K_m) were investigated in resting cells.

Both the v and Y demonstrated very different values which exceeded one order. However, no trend changes of the v and Y within the same culture occurred. The changes in the Y values are related to the varying metabolic utilization of elemental sulfur for growth or energy formation. Although the results showed a high Y variability, the reason for the significant Y changes among different cultures of the same strain remains unclear. No significant correlation between the v and Y values was observed for most of data. Changes in the K_m were demonstrated in relation to different physiological conditions.

This work was supported by grants No. 525/04/1309 from the Czech Science Foundation and MSM0021622413 from the Czech Ministry of Education.

PO 8.7

REGULATION OF MICROBIAL PRODUCTION OF CAROTENOIDS BY EXOGENOUS STRESS FACTORS AND BY CHEMICAL MUTAGENESIS – A COMPARATIVE STUDY

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Under stress, various specific compounds are overproduced. In this work, carotenoid profiles in subcellular fractions of selected carotenogenic yeasts and bacteria were analyzed. Yeast *Rhodotorula glutinis* and bacteria *Erwinia carotovora* were cultivated in optimal conditions, in presence of chemical mutagens and under exogenous osmotic, UV and oxidative stress. First, processes of microbial cell lysis were optimized using several enzymes and detergents. Further, efficiacy of several processes of carotenoid extraction from microbial cells were compared. Carotenoid pigments (torulen, lutein, lycopene, alpha- and beta-carotene) were extracted from bacterial and/or yeast cell membranes using saponification and SPE and analyzed by RP-HPLC/MS. Optimized extraction technique described above could be applicable namely for extraction of non-polar carotenoids. Under all types of stress most of tested carotenoids were overproduced. While in bacterial cells only slight increase of lutein production was observed in presence of hydrogen peroxide, production of carotenoids by yeast cells was about 5-6x higher under oxidative stress. Some bacterial and yeast mutants were able to produce 5-10x higher amount of beta-carotene and different carotenoid profiles than natural producers. Production of carotenoids in yeasts grown under exogenous stress changed simultaneously with ergosterol production.

Acknowledgements: This work was supported by projects MSM 0021630501 of the Czech Ministry of Education, Youth and Sport and IAA400310506 of Grant Agency of the Academy of Sciences of the Czech Republic

PO 8.8

HETEROLOGOUS EXPRESSION OF ENTEROLYSIN A, HEAT-LABILE BACTERIOCIN FROM *ENTEROCOCCUS FAECALIS*, IN *E. COLI*

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Bacteriocins are antimicrobial peptides and proteins that are synthesized by bacteria. Many bacteriocins from Gram-positive bacteria have fairly broad inhibitory spectra, and have a great potential as antimicrobial agents in food and feed production however, isolation and purification of bacteriocins from native hosts is time consuming and laborious process. The cloning and expression of enterolysin A (EnlA), heat-labile bacteriocin from *Enterococcus faecalis* II/1 with anti-listerial activity, was studied in *Escherichia coli* SG13009. The PCR amplified products of enterolysin A structural gene, N-terminal domain of EnlA possessing endopeptidase activity and C-terminal domain of EnlA similar to a lysis gene of bacteriophage, were cloned in prelinearized pQE-30UA expression vector. The constructs were subsequently cloned into *E. coli* cells. The expression of EnlA structural gene led to the synthesis and secretion of functional active His-tagged enterolysin A protein, which was purified to homogeneity using His-Select^{TM} Cartridge and was shown to be fully active against the indicator strain. The expression of N-terminal and C-terminal domains of EnlA led to the synthesis of biologically non-active proteins. This is the first example of heterologous expression of large bacteriocin.

PO 8.9

TOTAL POLYPHENOL CONTENTS IN DIFFERENT TISSUE CULTURES OF HOP (*HUMULUS LUPULUS* L.)

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The evolving commercial importance of the secondary metabolites has in recent years resulted in a great interest in secondary metabolism, and particularly in the possibility to alter the production of bioactive plant metabolites by means of tissue culture technology. Hop (*Humulus lupulus* L.), a traditional brewery crop, contains medically interesting flavonoid compounds with a broad range of biological activities. The aim of our study was to establish a convenient *in vitro* system, based on the induction of callogenesis and establishment of cell suspension culture in hop for chemical analyses of constituents of *in vitro* cultures and for potential production of interesting flavonoids in *in vitro* culture systems. We studied the effect of growth regulators (BAP + NAA or 2,4-D), culture conditions (continual dark *vs*. photoperiod of 16 h light/8 h dark), explant type (internodal segments *vs.* leaf segments) and genotype (K-31/3/7, K-70/4/1 and Lučan4/3) on callus culture of hops. The results suggested that the callus induction rate was independent of explant type and it was higher on MS+2,4-D media in photoperiod and on MS+NAA in dark conditions. For maintenance of calli, culture in dark was more favourable due to a higher frequency of necrosis of calli in conditions of photoperiod. Cell suspension cultures were established from stabilized callus cultures in liquid MS media containing 1.0 mg/l BAP with combination of 1.0 mg/l NAA or 1.0 mg/l 2,4-D. Cell suspension cultures derived from both the types of explants showed higher biomass accumulation (FW and %DW) in conditions of photoperiod. Cell proliferation was higher in both culture conditions in cell suspension cultures derived from internode-calli. Higher biomass accumulation was observed on media with NAA in comparison with media with 2,4-D. The viability of cells (assessed as % of TTC-positive cells) depended on the concentration of pectinase added to liquid media to liberate cells from cell clumps and ranged from 60.9-90.6 % in media without pectinase to 36.2-65.4 % in media with 1000 µl pectinase/g tissue FW. Content of total polyphenols depended on the type of *in vitro* culture and ranged 60.5-137.1 mg/l of gallic acid equivalent (GAE) in cell suspension cultures and 76,6-158,5 mg/l GAE in callus cultures in comparison to 121,4 mg/l GAE in shoot cultures of hops.

PO 8.10

BACTERIAL ALKALINE PEPTIDASE AND HER PRACTICAL UTILIZATION

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In our work we studied in vitro hydrolysis of plant proteins. Three commercially available enzymes – Trypsin, Subtilizin A, Subtilizin BPN´ and an enzyme „Nitrazym“ prepared at Dept. Biochem. Biotechnol. were applied. Protein fractions of concentration 0.1% (w/v) and 1% (w/v) were used as substrates, prepared by extraction from wheat groats, were hydrolysed by 0.5% (w/v) and 1% (w/v) enzymes. Enzymatic treatment was carried out at the temperature 37°C, pH 8,0 and during 15 - 360 minutes. For determination of relative molecular weight of hydrolysis products was used SDS-PAGE.

From results it follows that the most suitable substrate is bovine serum albumin and less suitable were wheat prolamins grain. The optimum of hydrolysis time was 30 minutes. Molecular weights of hydrolyzates prepared by Trypsin were about 24 kDa and 45 kDa – 66 kDa, by Subtilizin A 28 kDa, by Subtilizin BPN´ 45 kDa – 66 kDa and by „Nitrazym“ were 14 kDa – 27 kDa and 5 – 6 kDa.

Complete hydrolysis of proteins to aminoacids decreases solubility and emulgation capability of peptides. Products of protein enzyme hydrolysis which are small peptides qualitatively improve food characteristics and make it possible to prepare a wide assortment of hypoallergenic and dietetic foodstuffs. Hydrolysates of plant proteins have a wide range of application as a substitute for proteins of animal origin.

PO 8.11

EXPRESSION OF TRUNCATED FORM OF CP4 5-ENOPYRUVYL-SHIKIMATE-3-PHOSPHATE SYNTHASE (CP4 EPSPS) FROM GM PLANT IN *ESCHERICHIA COLI* AND COMPARISON OF ITS PROPERTIES WITH THE FULL LENGTH ONE.

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During the study of horizontal gene transfer of the *aro*A CP4 gene encoding CP4 EPSPS from genetically modified feed through gastrointestinal tract to bacteria living in animal gut we have observed beside full length gene also functional truncated one present in bacteria. We have used two different *E. coli* expression systems (with *tac* promoter and T7 promoter) to compare enzymatic properties and functional activities of both forms of CP4 EPSPS protein.

For the protein expression we have prepared pKK1 and pKK2 plasmids - *tac* expression vectors derived from pKK233-2 plasmid, and pMD2 and pMD72, T7 expression vectors derived from pET28a plasmid. Gene *aro*A CP4 (full leght as well as truncated one) from its ATG codon was amplified by PCR from GM plant and cloned into NcoI and HindIII sites of both expression vectors. We have compared phenotypic properties of full lenght and truncated CP4 EPSPS forms by growth on solid M9 medium. We have also investigated growth curves of *E. coli* Δ*aro*A strains containing either pKK1 or pKK2 plasmid on liquid M9 medium without aromatic compounds. Suitable expression levels of both proteins with T7 expression system was received for further partial protein purification based on Ni^{2+} His tag procedure and comparative enzymatic assay.

PO 8.12

METAL CHELATE ADSORPTION OF PROTEINS ON MAGNETIC COPPER ALGINATE AGGREGATES

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Ústav systémové biologie a ekologie AVČR České Budějovice

Purification of proteins from crude extracts and homogenates is one of the most important procedures in biochemistry and biotechnology. Different chromatography procedures are available to fulfill this goal. Among them, Immobilized Metal Ion Affinity Chromatography (IMAC) is currently one of the most popular chromatography procedures for both group protein separation and highly selective purification of individual target proteins. Many commercially available chromatography matrices are commercially available.

It has been shown recently that inexpensive zinc alginate microbeads can be used efficiently for IMAC of specific proteins. Such a type of materials could find interesting applications especially in biotechnology processes.

Magnetic adsorbents have been used many times for the separation of proteins and peptides. In our work we have developed a very simple procedure for rapid preparation of magnetic copper alginate aggregates. The average diameter of the particles is ca 0.1 mm. This adsorbent exhibits specificity to copper binding proteins, such as bovine hemoglobin. The protein adsorption could be described by the Langmuir adsorption model. It has been shown that under appropriate conditions the maximum adsorption capacity is ca 15 – 20 mg of hemoglobin per 1 ml of the adsorbent.

PO 8.13

HEN EGG WHITE LYSOZYME SEPARATION USING MAGNETIC ION EXCHANGERS

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Hen eggs belong to cheap and easily available materials containing around 10% of proteins in white and about 15% proteins and more then 30 % lipids in yolk. Both egg white and yolk are viscous materials and therefore the separation of individual proteins may be difficult. For biochemical separations, usually standard procedures are used, such as gel, ionex, hydrophobic and affinity chromatography, electrophoresis, ultrafiltration, centrifugation, salting out, biocompatible two-phase systems etc.

Magnetic separation techniques represent a relatively new strategy for the isolation and purification of biologically active compounds. A complex of diamagnetic molecules (e.g. protein) and magnetic particles is formed and subsequently separated in an external magnetic field.

In our experiments different types of strongly and weakly acidic cation exchangers were mangetized by water based magnetic fluids and used for the isolation of lysozym from egg white. Lysozyme of purity 80 - 90% was separated on postmagnetized Sephadex CM C-25. Similar results were obtained with commercial magnetic cellulose cation exchanger Iontosorb MG CM. Lysozyme of similar purity (ca 87 %) was isolated on immobilized reactive textile dye Ostazin oliv HB, although the yield of lysozyme was very low. Commercial preparation of lysozyme (purity ca 65%) was highly purified (purity over 90%) on magnetic derivative of lignocelulose material.

PO 8.14

MAGNETICALLY MODIFIED *BACILLUS CIRCULANS* CELLS FOR CYCLODEXTRIN GLUCANOTRANSFERASE PRODUCTION

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Cyclodextrin glucanotransferase (CGTase, EC 2.4.1.19) is a unique enzyme converting starch into cyclodextrins. *Bacillus circulans* ATCC 21783 cells, alkalophilic producer of CGTase were magnetically modified and the effect of different types of magnetic particles on the enzyme production and thermostability was studied. The magnetic immobilization of cells offers several advantages, such as their easy manipulation using an external magnetic field.
Silanized magnetite and ionic ferrofluids (pH 7.9 and 13.5) were used for the magnetization. Magnetite particles altogether with cells were also entrapped into agar beads. A significantly enhanced enzyme yield and specific CGTase activities were achieved by batch and semicontinuous cultivation of the magnetically modified cells compared to free cells. The highest CGTase production was achieved after 96 h semicontinuous process when the increase of the CGTase level was 1.4 - 2.2-fold than those of free cells. In the case of cells immobilized on silanized magnetite, the specific enzyme activities were 4.14-8.40-fold higher during 144 h repeated batch cultivation. CGTase produced by magnetic *Bacillus circulans* cells was 13-14% more thermostable in comparison to free cells under identical heat treatment conditions.

PO 8.15

DETERMINATION OF TOKAJ WINE QUALITY FACTORS.

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The aim was to analyse must and wine made from Tokay variety of vine. The vine bunchs were pick up on October 2005 and several types of Tokay wine (Muškát žltý, Tokajská Lipovina and special Tokay wine „Tokajský výber 4-putňový“) were prepared at Galafruit, s.r.o. Malá Tŕňa from October 2005 to February 2006. The highest content of N-substances was in Furmint must, but the most concentration of proteins was in Muscatel must. For determination of qualitative factors of wine enzymatic reactions (Megazyme Int. Ireland Ltd.) were used. The concentrations of glucose and fructose were determined by effect of hexokinase, glucose–6-phosphate dehydrogenase and phosphoglukose isomerase; the amount of malic acid was determined by the reaction catalysed with malate dehydrogenase and glutamate-oxaloacetate aminotransferase; determination of succinic acid utilized the activity of succinyl-CoA synthetase, pyruvate kinase and lactate dehydrogenase and the concentration of ethanol was determined by alcohol dehydrogenase and acetaldehyd dehydrogenase. For determination of activity of proteolytic and amylolytic enzymes were used chromolytic substrates.Both, Furmint and Muscatel must didn´t contain malic acid. The highest level of succinic acid and glucose had Muscatel must. Analysis of wine shown that the highest content of glucose and fructose was in „Tokay výber 4-putňový“ made with „cibéby“ in 2005. Tokay Lipovina wine had the mist concentration of malic acid, on the other hand the content of succinic acid in wines was under detection limit. The concentration of ethanol was the highest in Tokay Lipovina wine and the least one was in the oldest „Tokay výber-4 putňový“.

Supported by project APVT -20-026604.

PO 8.16

USING OF BIOAUTOGRAFY TO DETERMINE OF THE ANTIBACTERIAL SUBSTANCES FROM PLANT MATERIAL

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Bioautografy is method of identification the substances on the TLC separation natural samples in combination with proof of antibacterial activity. This method enable tested biology activity of substances after separation without necessity their isolation from TLC. Limit of detection is in nanogramms of tested substance. This method was primary used for identification of antibiotics; its perspective using is in screening of antibacterial activity of substances from samples of plant material with antimicrobial substances contents.

Our task is focused on the matter with antibacterial effect occurred in the apple peels. Antibacterial effect of these substances against *Acidovorax avenae, Erwinia mallotivora* and *Xanthomonas sp.* was comparable with standard antibiocide TMTD (tetra-methyl-ethyl-uram-disulfide). The apple peels shown the high concentration substances with antibacterial effect. By the autobiography we identified polyphenolic character of active substances. This matter was present in different species of apple. Chemical preservation of apple tree by industrial biocides caused depression of production antibacterial matters in apple coat.

Bioatografy, as combination TLC separation with antibacterial testing is perspective in testing of chemical individual substances with protective effect from plants.

PO 8.17

APPLICATION OF MOLECULAR METHODS FOR IDENTIFICATION AND GENOTYPING OF ENTEROCCI ISOLATED FROM FOOD SAMPLES

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Bacteria belonging to the genus *Enterococcus* are widespread in nature and are frequently present in dairy and other fermented food products. Some of these microorganisms are used as probiotics because of their possible positive health effects, but enterococci have been also identified as human pathogens and many enterococcal strains can possess virulence factors contributing to the disease. These phenotypic features are strain-dependent, so it is important to reveal genetic variability of the enterococcal strains occurred in food.

In the present study, enterococcal isolates collected from various cheese products were characterised by two molecular methods. PCR amplification of the intergenic spacer between the 16S and 23S rRNA gene (ITS-PCR) was used for species identification; the most of the strains were classified into three species: *E. faecalis*, *E. faecium* and *E. casseliflavus*. Fluorescence-based amplified fragment length polymorphism (fbAFLP) was used for genotyping of strain variability. The fbAFLP assay is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA. By fbAFLP, phylogenetically informative characters of isolated strains were obtained, which were used for cluster analysis and assessment of strain relativeness. The results indicated the diversity of bacterial populations belonging to the same species, strains clustered mainly according to their origins.

Section 9

GLYCOBIOCHEMISTRY

PO 9.1

A NEW XYLOGLUCAN ENDOTRANSGLYCOSYLASE ENZYME FROM NASTURTIUM (*TROPAEOLUM MAJUS*) SEEDS

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XTHs are wall proteins that exhibit two distinct enzymatic activities: xyloglucan endotransglycosylase (XET) and/or xyloglucan endohydrolase. Mechanism by which XTH degrades or disproportionates molecules of xyloglucan is primarily transglycosylation (Smith and Fry, 1991). There is no evident correlation between the structure of the *XTH* genes and their function in plants (Malinowski *et al*., 2004) and precise roles of XTHs in cell growth are still under debate. In this work, by combining different chromatographies, preparative IEF, and IEF two new XET isoforms were isolated from nasturtium (*Tropaeolum majus*) seeds. These isolated isoforms exhibited hetero-transglycosylating activity (Ait-Mohand and Farkaš, 2006). Interaction with Con A. and absence of reaction with N-glycosidase indicated that they were *O*-glycosylated, probably via serine and/or threonine. Tryptic digests of these XET isoforms gave a very similar mass spectra with little differences, thus we assume that both isoforms are from the same protein. When the mass spectra were compared with the known XTH proteins in data base (www.prospector.ucsf.edu), these later didn't match any spectrum already stored in the data base which means that the protein isolated in our work is a new one, different from the other one, isolated previously by others from the same source (de Silva *et al.*, 1993).

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PO 9.2

FURTHER STUDIES ON MYCOBACTERIAL GALACTOSYL TRANSFERASE Rv3782

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Members of the *Mycobacterium* genus are still responsible for major health problems in mankind, such as tuberculosis, leprosy and opportunistic mycobacterioses in immunocompromised individuals. One of the factors that significantly contribute to the success of mycobacteria as pathogens is their unique, extremely hydrophobic, and largely impermeable cell wall, with backbone consisting of covalently linked mycolic acids, arabinogalactan (AG) and peptidoglycan. Within this, the D-galactofuran, composed of about 30 alternating 5- and 6- linked β-D-galactofuranose (Gal*f*) units is attached to the peptidoglycan by a linker unit, α-L-rhamnopyranosyl-(1→3)-*N*-acetyl-α-D-glucosaminyl-phosphate [Rha-(1→3)-Glc*N*Ac-P].

Previously we have shown that the synthesis of AG begins with the transfer of GlcNAc-1-P from UDP-GlcNAc to the decaprenyl-P (C_{50}-P), followed by the addition of Rha from dTDP-Rha, and attachment of galactofuranose residues from UDP-Gal*f*. Recently, we have identified galactosyl transferase Rv3782 as an enzyme catalyzing addition of first and/or second galactose on lipid-linked linker unit. In this report we suggest that the enzyme is, indeed, bifunctional, based on experimental evidence with recombinant enzyme expressed in heterologous host.

Acknowledgements: This work is supported by EC 6th Framework Programme, Contract No. LSHP-CT-2005-018923; NIH, Fogarty International Center; R03 TW 006487; Slovenská grantová agentúra VEGA, grant 1/2324/05.

PO 9.3

COMBINATION OF TANDEM MASS SPECTROMETRY (MS/MS) TECHNIQUES WITH HPLC FOR IDENTIFICATION OF PARTICULAR CARBOHYDRATES IN COMPLEX MIXTURES

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The application of MS techniques on carbohydrate evaluation in the woody research is not so widespread like in the food and beverage chemistry.
This study is focused on identification of "low" molar mass carbohydrates (up to four monosaccharide units) isolated from needles of Norway spruce by means of mass spectrometry (MS) techniques. Due to the complexity of samples either on-line or off-line connection with liquid chromatography (LC) prior to MS determination was implemented. Fractionated extracts were analyzed using two sensitive soft ionization MS techniques: i) electrospray ionization (ESI); and ii) matrix assisted laser desorption/ionization (MALDI) time-of-flight MS. Particularly, MS/MS experiments achieved in both ion modes showed its potential of identification of low mass compounds and was successfully applied to reliable identification of carbohydrate structure. Two types of sample pretreatment were used for signal enhancement (addition of inorganic salts or easy volatile cosolvent to the samples). No derivatization of the samples was required.
The developed procedure involves modern analytical techniques to fulfill current requirements in the area of biochemical and ecological research.

Work was supported by the grant No. 526/03/1182, Grant Agency of the Czech Republic and Institutional research plan AVO Z40310501.

PO 9.4

BIOCHEMICAL CHARACTERISATION OF MYCOBACTERIAL MANNOSYL TRANSFERASE PimA

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Phosphatidylinositol (PI) and phosphatidylinositol mannosides (PIM) are the important and the most abundant phospholipids of mycobacteria. They also provide the basic structure from which lipomannan (LM) and lipoarabinomannan (LAM) - mycobacterial lipoglycans, which play an important role in pathogenesis of tuberculosis and leprosy, are derived.

Biosynthesis of phosphatidylinositol mannosides proceeds *via* stepwise mannosylation of phosphatidylinositol. The donor of the mannose residues in these reactions is GDP-mannose, PIM_1 and later higher PIMs (PIM_3-PIM_6) are the products. The first mannosylation step is catalyzed by the mannosyl tranferase encoded by *pimA* (Rv2610c), essential gene for growth of mycobacteria (Korduláková et al., 2002).

Purpose of this study was to determine selected biochemical properties of PimA. In our previous work, recombinant PimA protein was produced in *E. coli* BL21(DE3)pLysS. In the *in vitro* system employing purified protein, PI and GDP-[^{14}C]Man as a donor of mannose residues, we have studied activity of the enzyme in the presence of different ions, we have determined the pH optimum, temperature optimum and kinetic parameters of the reaction. This information will be used for further development of the spectrophotometric assay for high throughput screening of inhibitors against PimA.

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Acknowledgements: This work is supported by EC 6th Framework Programme, Contract No. LSHP-CT-2005-018923; Slovenská grantová agentúra VEGA, grant 1/2324/05.

PO 9.5

ROLE OF COWPER GLANDS SECRETION IN BULL

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The Cowper gland secretion (CGS) seems to support the aggregation state of the bull seminal plasma and to increase its viscosity. Three groups of CGS proteins were identified to be responsible for bull seminal plasma binding. The possible biological importance of their influence over the bull seminal plasma was studied. First, we examined the rate of sperm releasing from seminal plasma in accordance with its composition, then compared the influence of CGS on the formation of the sperm surface protein layer, and last we studied whether the CGS affects sperm capacitation.

This work was supported by grant No. 303/06/0895 of the Grant Agency of the Czech Republic.

PO 9.6

VARIATION IN β-D-GLUCAN CONTENT IN CEREALS AND PSEUDOCEREALS WHOLE GRAINS

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(1→3)(1→4)-β-D-glucan, also called β-D-glucan, is a linear molecule of partially water-soluble polysaccharide consisting of glucose linked by both beta-(1→3) and beta-(1→4)-linkages [1]. Grass species use β-D-glucans as structural elements of the walls of growing cells and as an endosperm storage material that is hydrolyzed during germination to provide an extra source of carbon during early seedling establishment [2]. β-D-glucan has a broad spectrum of health-promoting impacts on the human organism. Soluble β-D-glucan is a potent inductor of humoral and cell-mediated immunity, and their regular daily consumption significantly increases immunological activity [3]. This type of soluble dietary fiber has also a moderating effect on post-prandial blood glucose and insulin response and reduces elevated blood cholesterol level [4, 5].

In total, using Mixed-linkage beta-glucan assay kit (Megazyme, Ireland) we analyzed 122 genotypes of spring barley, 79 genotypes of oat, and 14 genotypes of spring wheat for soluble β-D-glucan content. We also monitored the value of soluble β-D-glucan in buckwheat (14 genotypes), millet, and amaranth (both 10 genotypes).

This study was supported by project of the Ministry of Agriculture of the Slovak Republic (No. 2003 SP27/028OE02/028OE02).

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PO 9.7

OPTIMALIZATION OF FLUORIMETRIC 'PAPER DOT-BLOT' ASSAY FOR ACTIVITY ASSESSMENT OF XTHS ACTING IN XET MODE

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Xyloglucan, the major hemicellulose matrix polysaccharide in the primary plant cell walls and xyloglucan endotransglycosylase/hydrolase (XTH/XET EC 2.4.1.207) are thought to play an important role in the expansion and/or assembly of plant cell walls. Xyloglucan is capable of forming tight non-covalent bonds via hydrogen-bonds with cellulose. XTHs acting in XET mode cleave and rejoin xyloglucan chains or suitable xyloglucan derived oligosaccharides (XGOs).

At least five useful assay methods for determaning XET activity are currently described. Its transglycosylation activity can be followed radiometrically, colorimetrically or fluorimetrically, using appropriately labeled substrates or colored complexes. This work is focusing on further optimalization of non-radioactive high-throughput assay method for XET. The assay is using the transfer of (non-labelled) xyloglucan polysaccharide fragments onto the fluorescently labelled XGOs acceptor substrate, to its non-reducing end. The product of the glycosyl transfer is a fluorescently labelled xyloglucan which exhibits strong hydrogen-binding to cellulose fibres of Whatman paper dot-blot plate. The unreacted fluorescently tagged XGOs are washed out by using combination of polar solvent solutions. After drying, the paper is fixed onto solid plate and scanned in a multi-detection microplate reader.

ACKNOWLEDGEMENT

This work is supported by The European Commission 6th Framework Programme with contract number MRTN-CT-2004-512265.

PO 9.8

EVALUATION OF HEALTH-PROMOTING STARCH IN SEVERAL PLANT CROPS

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Resistant starch (RS) is considered a dietary fibre and prebiotic provided by functional foods. It has many proven health-promoting benefits, particularly in the prevention of cardiovascular and oncological diseases.

The commercial functional foods contain usually chemically-modified type RS_4 or RS_2 from genetically-modified lines with high amylose content. The aim of this study was to screen for the natural RS_3 type sources within cereals, pseudocereals and legumes.

The RS_3 level was determined after hydrothermal treating by Resistant Starch Assay Kit (Megazyme Int., Ireland) based on the method accepted by AACC and AOAC. There were evaluated 248 genotypes of 18 different plant crops.

There were found significant differences in resistant and total starch content within crops as well as within their genotypes.

The wrinkle peas were detected as the crop with the highest RS_3 level. Wrinkle natural mutants are homozygous for recessive allele *r* which causes a wrinkling of the pea seeds and high amylose level from which RS_3 resulted. Mutation at the *R* locus causes a loss of enzyme activity of the starch branching isoenzyme SBEIIb and decrease in amylopectin and starch content.

Very suitable natural RS_3 sources were also triticale (genotype Kendo, Pinokio, Presto, Tricolor), rye (Dankowskie Nove, Esprit), buckwheat (Pyra), chickpea (Alfa), lentil (Renka) and faba bean (Omar).

Key words: resistant starch, dietary fibre, functional foods, cereals, pseudocereals, legumes

PO 9.9

INTEGRIN CD11B/CD18 – RECEPTOR FOR ADENYLATE CYCLASE TOXIN

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Adenylate cyclase toxin (ACT or CyaA) is a key virulence factor of *Bordetella pertussis*, the causative agent of whooping cough (pertussis). CyaA is a bi-functional toxin belonging to the RTX (Repeat in ToXin) family of bacterial cytolysins capable to permeabilize cellular membranes by forming cation selective channels. The major activity of CyaA, however, is to deliver its adenylate cyclase domain into target cells where, upon activation by endogenous calmodulin, it catalyzes uncontrolled conversion of ATP to cAMP, a key signalling molecule subverting cell functions (1). It has recently been demonstrated that CyaA uses the CD11b/CD18 integrin (alphaMbeta2) as a specific cellular receptor (2). CD11b/CD18 is a rather promiscuous cell surface receptor, playing an important role in several biological functions of myeloid phagocytic cells, among which are bactericidal functions, such as chemotaxis, phagocytosis, degranulation and superoxid generation (3). Inhibition of those functions by CyaA action then appears to play an important role in *Bordetella* virulence.

PO 9.10

STRUCTURAL ANALYSIS OF NEUTRAL STARCH OLIGOSACCHARIDES BY MASS SPECTROMETRY

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The need to fully cover the structure of oligosaccharide and distinguish between them has been established as a one of the important tasks in biopolymer analysis. In recent years, electrospray ionization mass spectrometry (ESI-MS) has demonstrated its potential for correct and precise description of saccharide sequence.

This study dealt with detailed characterization of the structure of model starch oligosaccharides in negative ion mode of ESI-MS. Anion adducts of saccharides were produced by use of the appropriate salt as additives. Relationship between amounts of ionic species added to samples and sensitivity of the technique as well as the exploitation of the adduct ions formation for MS/MS experiments was investigated. The MS/MS spectra have been searched for characteristic ions or neutral loses associated with the specific structural feature. The obtained fragmentation data was examined and compared with the results obtained for both deprotonated molecules and adducts with sodium cation.

It seems that the described approach based on anion attachment find wide application in the field of structure analysis and metabolism of saccharides.

Work was supported by the project Research Centre for Study of Extract Compounds of Barley and Hop No. 1M6215648902.

PO 9.11

GLUCURONOYL ESTERASE - NOVEL CARBOHYDRATE ESTERASE PRODUCED BY *SCHIZOPHYLLUM COMMUNE*

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Cross-linking between hemicellulose and lignin in plant cell walls involves ester linkages between 4-*O*-methyl-D-glucuronic acid residues of xylan and OH-groups of lignin alcohol building blocks [1, 2]. This is the first report on existence of a microbial enzyme hydrolyzing such linkages. The enzyme is called glucuronoyl esterase. During growth of the wood-rotting fungus *Schizopyllum commune* ATCC 38548 on 1,5 % cellulose, the fungus produced wide variety of extracellular proteins. In addition to cellulases, xylanases, acetylxylan esterase and feruloyl esterases, the growth medium contained an esterase hydrolyzing various alkyl and aryl esters of 4-*O*-methyl-D-glucuronic acid. None of these compounds was attacked by other known carbohydrate esterases, such as acetylxylan esterases, feruloyl esterases and pectin methylesterases. Fractionation of extracellular proteins of the fungus *Schizophyllum commune* resulted in purification of a glucuronoyl esterase that allowed determination of its internal amino acid sequence. Based on the sequence, a homologous gene in *Phanerochaete chrysosporium* was found. The gene sequence of the enzyme from *Phanerochaete chrysosporium* did not show any similarity to gene sequences of known enzymes, supporting the fact that we have discovered a novel type of carbohydrate esterase. The biotechnological potential of the new esterase will be investigated.

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PO 9.12

TENTATIVE PRIMARY STRUCTURE OF LIPID A FROM *RICKETTSIA TYPHI* LIPOPOLYSACCHARIDE

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Rickettsia typhi, the causative agent of endemic typhus, is a small obligate intracellular parasite requiring host eukariotic cells for replication. It is a Gram-negative bacterium maintained in rodents and transmitted to humans by the rat flea *Xenopsylla cheopsis.* Endemic (murine) typhus is a relatively mild, acute febrile illness characterized by headache and macular rash. It is known that *R. typhi* contains a lipopolysaccharide (LPS), which is thought to display a noticeable endotoxic activity. This depends mainly on its lipid A structure which has been unknown thus far. Lipid A isolated from the *R. typhi* LPS was investigated by the latest MS techniques. Its considerable microheterogeneity indicated the presence of hexa- penta-, and tetraacyl lipid A moieties. It appears that hexaacyl lipid A represents a major molecular species that shares the classical backbone of diphosphorylated D-GlcN disaccharide in which both GlcN I and GlcN II have amide-linked 3-OH-C14:0. In GlcN I , two ester linked C16:0 are present. One is located at C-3 of the sugar residue while the other is linked to 3-OH-C14:0. Location of ester linked C16:0 and C18:0 in GlcN II could not be established with certainty thus far. Other, less abundant hexaacyl molecular species are closely related to the major one and differ one from another by the mass difference of ± 28 (CH_2) in dependence on the fatty acid bound. The data show that both composition and structure of major molecular species of *R. typhi* lipid A resemble to those of classical form of enterobacterial lipid A with high a endotoxic activity.

PO 9.13

GLYCOSYLATION IN LYME BORRELIOSIS SPIROCHETES

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Introduction

The spirochete *Borrelia burgdorferi* s.l. is the causative agent of Lyme borreliosis (LB). LB is a world-wide tick-borne infection. Glycosylation of some proteins and cell-structures has been reported. Specifically, OspA, OspB, and FlaA have been reported to be glycoproteins.

Results and discussion

Several proteins were labeled by lectins in Western blotting experiments in samples from three LB spirochete species and subsequently identified by mass spectroscopy. Glycoconjugates were also localized on the surface of LB spirochetes by electron microscopy. The specificity of the labeling was verified by deglycosylation of samples with glycosidases.

The surfaces of spirochetes were labeled by anti-BSA and rabbit antiserum. Glycoproteins originating in the growth medium appear to associate with the surface of borrelia cells. These findings suggest specific binding of medium-based glycoproteins by LB.

In contrast to previously reported results, OspA/B, and FlaA protein was not labeled by lectins or stained by a periodic acid-Schiff staining.

Supported within a joint research project of the NRC Canada and ASCR S&T Cooperation, 2003-2006.

PO 9.14

COMPOSITION AND TENTATIVE STRUCTURE OF A LIPOPOLYSACCHARIDE FROM *PISCIRICKETTSIA SALMONIS*, THE ETIOLOGICAL AGENT OF SALMONID RICKETTSIAL SEPTICEMIA

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Piscirickettsia salmonis is the causative agent of salmonid rickettsial septicemia and is an economically significant pathogen of salmonids that is responsible for extensive mortalities in the South American aqua-culture industry. *P. salmonis* is a gram-negative obligate intracellular bacterium that ranges in diameter from 0.5 to 1.5 μm and is found within cytoplasmatic vacuoles of the cells from infected tissues. The major carbohydrate antigen of the bacterium is presumed to be a lipopolysaccharide (LPS) but both its composition and structure are unknown. Using a hot phenol-water method, an LPS was isolated that was shown to be of R (rough) type on SDS-PAGE. Compositional analyses revealed the presence of D-mannose (Man), D-glucose (Glc), D-galactose (Gal), L,D-heptose (Hep), and D-glucosamine (GlcN) in a molar ratio 3.0:4.8:3.0:1.0:0.4, respectively. Methylation-linkage analysis uncovered mainly terminal Man and Glc, 6-linked Man and Gal, 4-linked Man and Glc(Gal) in a minor abundance, and 2,3-disubstituted Hep. It appears that some hexose residues are heavily phosphorylated. More detailed structural studies are in progress.

PO 9.15

STRUCTURAL STUDIES OF LIGAND BINDING OF NATURAL KILLER CELL RECEPTOR, PROTEIN CD69

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CD69 protein [1], an early activation antigen of human lymphocytes, is one of the most studied surface receptor molecules involved in tumour recognition by natural killer cells. Crystal structures of carbohydrate recognition domain (CRD) of CD69 molecule have been already published [2,3], but crystallization conditions used did not favour ligand binding. In this work we present our structural approach to describe CD69 binding properties. After recombinant production and optimalization of *in vitro* refolding of soluble CRD domain of CD69 protein, we analyzed its homogeneity by FT-ICR mass spectrometry and its secondary structure was determined by drop coating deposition Raman spectroscopy method [4]. Further, ligand binding was examined both by Raman spectroscopy and by protein crystallization. We tested several types of proposed CD69 ligands, from single monosaccharide ligand *N*-acetyl-D-glucosamine, to more complex structures, such as antenary oligosaccharides isolated from hen egg white protein ovomucoid, synthetic peptidomimetic ligands based on calixarene core, or heptapeptide ligand from mycobacterial heat shock protein hsp60, which all binds with high affinity to CRD domain of CD69 receptor. Comparison between computational docking model of calcium binding and observed results by crystallography is discussed.

This work was supported by Ministry of Education of the Czech Republic No. MSM 0021620808, by Institutional Research Concept No. AVOZ 50200510, by Grant Agency of the Czech Republic No. 301/05/P567, and by Grant Agency of the Academyof Sciences of the Czech Republic No. A5020403.

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Section 10

TEACHING BIOCHEMISTRY AND MOLECULAR BIOLOGY

PO 10.1

PRINCIPLE OF METHODS POLYMERASE CHAIN REACTION (PCR) AND REAL-TIME PCR; TEACHING PROGRAM FOR PRE- AND POST-GRADUATE STUDIES

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Polymerase chain reaction (PCR) represents one of the biggest discoveries in molecular biology. In was developed in 1983 by K. B. Mullis who was awarded for it by Nobel Prize in Chemistry in 1993. PCR amplifies DNAs by repeated rounds of DNA replication in vitro. It has extremely wide applications that were even extended by the procedure known as Real-Time PCR, which is a method of simultaneous DNA quantification and amplification through the application of the polymerase chain reaction technique.

We have prepared a tutorial on CD ROM, which in 6 Chapters covers the most important aspects of Real-Time PCR: 1) Introduction into the subject of PCR, including the history of its discovery; 2) Principles of PCR and Real-Time PCR; advantage of Real-Time PCR over the simple PCR; 3) Animation of PCR reaction; 4) Detailed description of Real-Time PCR with the description of the instruments used for this method; 5) Sample protocol of the experiment of Real-Time PCR, description of used reagents, methods for quantification of results and the detection of impurities; 6) The use of Real-Time in biomedicine, both as diagnostic tool for many diseases and as a very efficient research tool for the study of gene-related regulatory processes. All chapters are written in a simple way, which is suitable even for pre-graduate students.

Section 11

NEW METHODS

PO 11.1

MULTIPLEX PCR: RAPID AND RELIABLE METHOD FOR INDIVIDUAL IDENTIFICATION OF ENDANGERED SPECIES

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Numbers of flora and fauna species worldwide becoming seriously endangered with survive depended on long-term, managed protection. Habitat degradation due to excessive land use and/or pollution is obvious and most frequent cause. However, direct persecution of many species through shooting, trapping, and poisoning, together with collecting and illegal trading with species occurs widely. Illegal collecting and trading with endangered species represents special type of crime requiring international collaboration in forensic analysis and legislation. DNA analysis of polymorphic loci facilitates unambiguous individual identification providing effective tool in forensic analysis.

Aim of the presented study was to design and optimize powerful and reliable DNA based method applicable to individual identification and parentage testing of endangered raptor bird species living in Slovakia. Simultaneous, multiplex PCR amplification of up to 13 STR polymorphic loci with subsequent fluorescent capillary electrophoresis detection was tested in golden eagle (*Aquila chrysaetos*), and peregrine (*Falco peregrinus*) and saker (*Falco peregrinus*) falcon species. Genoptyping of selected markers in sample of approximately 50 individuals of each species provided information about genetic variability of these loci in studied species populations. Calculated probabilities of identity P_{ID} 3×10^{-8}, 2×10^{-5} and 5×10^{-4} for golden eagle, peregrine and saker falcons respectively, proved satisfactory exclusionary power of the method, further improvements will be tested, however.

PO 11.2

DEVELOPMENT OF A NUCLEIC ACID LATERAL FLOW DEVICE (NALFD) FOR SIMULTANEOUS DETECTION OF *LISTERIA* SPP. AND *LISTERIA MONOCYTOGENES*

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The genus *Listeria* consists of a group of gram-positive, facultative anaerobic rodshaped bacteria. All of the six recognised *Listeria* species can be isolated from a diversity of environmental sources, including soil, water, effluents, a large variety of foods, and from faeces of humans and animals. Although occurrence of *Listeria* strains in food may indicate errors in good hygienic and manufacturing practice, only *L. monocytogenes* is a significant human and animal pathogen responsible for the serious illness listeriosis. Conventional microbiological methods for *L. monocytogenes* detection are laborious and take several days to achieve a confirmed identification.

The aim of this study was to develop a rapid and sensitive one-step nucleic acid lateral flow device (NALFD) for simultaneous detection of *L. monocytogenes* in particular and the genus *Listeria* in general. Genomic DNA was isolated from cultured bacteria and was used as a template for duplex PCR where one of each set of primers was 5'-labelled with biotin and the other 5'-labelled with digoxigenin or fluorescein, respectively. A small volume of the final PCR solution was directly added to a one-step assay device. The device comprised a nitrocellulose membrane with immobilized polyclonal antibodies against the digoxigenin- or fluorescein-tags and colloidal carbon particles with surface adsorbed neutravidin. Double-labelled amplicons were sandwiched between the immobilised antibodies and the carbon-neutravidin conjugate. The appearance and the position of a grey/black line was indicative of the presence of the specific *L. monocytogenes* and *Listeria* spp. amplicons, respectively.

PO 11.3

CONFORMATION OF TAXONOMIC CLASSIFICATION OF ISOLATES FROM POLLUTED ENVIRONMENTS TO THE SPECIES ASPERGILLUS NIGER BY PCR METHODS.

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Aspergillus represents a huge genus of ecologically as well economically important fungi. Members of *Aspergillus* section *Nigri* are a common source of extracellular enzymes and organic acid to be used in food processing. In spite of their importance, the taxonomy of black aspergilli (*Aspergillus* section *Nigri*) is not clear because it is primarily based on morphological criteria and in some cases the differences between the species are very subtle.

Morphological anomalies of *A. niger* isolates as a response to environmental stress still complicate the problem of taxonomic identification. In recent years, a variety of DNA – based methods has been developed to identify fungi. To miss the difficulties with the taxonomy, for the conformation of identity of *A. niger* species, PCR method was used. Method uses small diferences in chromosomal DNA for 5.8S rRNA of two strains of *A. niger* and *A. tubingensis*, major species in the *A. niger* aggregate. A target for endonuclease RsaI that exists in the rRNA gene ITS1 of *A. niger* but does not exist in the sequence of *A. tubingensis*, was used for identification of isolates. Method is favourable because enables the possibility of working with specific fragments of the ribosomal RNA genes without working with Southern blot DNA hybridization techniques. We confirmed morphological identification of our isolates to *A. niger* species with this PCR method. It appears, that it could be usufel tool in the identification of isolates of the *A. niger* aggregate, for instance from polluted environmets.

Our research was supported by Grant agencies APVT- 51-024804 and VEGA 2/5069/25.

PO 11.4

RAPID SCREENING OF BACTERIAL GENES CODING HISTIDINE AND TYROSINE DECARBOXYLASE USING PCR

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Biogenic amines (BA) are the toxic low-molecular organic bases which are formed due to the amino acid decarboxylase activities of microorganisms in fermented foods. The formation of histamine or tyramine in foods depends on the presence of free histamine and/or tyramine in samples and the presence of microorganisms having histidine or tyrosine decarboxylase activity.

The aim of this work was to use recently published PCR procedure (Coton et al., 2005) for the identification of microorganisms with tyrosine and histidine decarboxylase genes. The microorganisms isolated from cheese (3 strains), lactic acid bacteria (LAB) of genus *Lactobacillus* obtained from Czech Collection of Microorganisms (CCM, Brno) (19 strains), Culture Collection of Dairy Microorganisms (CCDM, Tabor, Czech Republic) (7 strains) and newly isolated from human faecal samples (20 strains) were used for the analysis. It was shown that PCR products of specific lenght were amplified using DNA of 3 strains isolated from cheese, 1 strain collected in CCM, 1 strain collected in CCDM and 1 strain of human origin. The results presented in this report show that PCR is suitable and quickly method for the rapid screening of bacterial genes coding histidine and tyrosine decarboxylase.

Finantial supports by IGA Grant of MUAF in Brno IG460271 and long-term research program MSM 151100002 of the Ministry of Education of the Czech Republic.

PO 11.5

COMPARISON OF ASYMMETRIC DIMETHYLARGININE DETECTION - HPLC AND ELISA METHODS

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Asymmetric dimethylarginine (ADMA) is often discussed in connection with hyperhomocysteinemia and its toxic effect on vessel wall. ADMA concentration is usually measured by HPLC after previous derivatization. The aim of the study was to compare HPLC and ELISA methods for ADMA determination. For HPLC determination we used quipments from Thermo separation product (Florida, USA). After solid-phase extraction on polymer cation-exchange column and the following derivatization with o-phthaldialdehyde the samples were separated using C18 column (mobile phase 8.7% acetonitrile, 50 mmol/l phosphate buffer, pH 6.5) and a fluorescence detector. N^G-monomethyl-L-arginine was used as an internal standard. ADMA® ELISA kit, based on a competitive principle, was obtained from DLD Diagnostika, Hamburg, Germany. ADMA was measured in EDTA plasma of 40 healthy blood donors and 40 hemodialysis patients with hyperhomocysteinemia. In spite of different principles both methods showed a very good correlation ($r = 0.944$, $p<0.0001$). ELISA method reproducibility calculated from 40 duplicate measurements of hyperhomocysteinemic samples and expressed as a coefficient of variation, was 4.75%. These results show that time consuming HPLC method of ADMA determination can be replaced by ELISA which gives comparable results and has an excellent reproducibility.

This study was supported by the Grant Agency of Charles University in Prague No 78/2005/C/LFP.

PO 11.6

ELECTROPHORETICALLY MEDIATED MICROANALYSIS AS A TOOL FOR KINETIC STUDIES OF ENZYMES

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Fourteen years ago a new application for the evaluation of enzymatic reactions in capillary electrophoresis (CE) was proposed and developed, electrophoretically mediated microanalysis (EMMA). In this methodology, the capillary is used not only as a separation medium but also as a reaction chamber. Substrate(s) and enzyme are introduced in the capillary as distinct plugs, the first analyte injected being the one with the lower electrophoretic mobility. Upon the application of an electric field, these two zones interpenetrate due the differences in their electrophoretic mobilities. Enzymatic reaction takes place and the resultant reaction product(s) and the unreacted substrate(s) are electrophoretically transported towards detector, where they are individually detected.

In this communication the EMMA methodology was applied to complex kinetic study of haloalkane dehalogenase from *Sphingomonas paucimobilis UT26*. The Michaelis constants for different substrates, the inhibition constant for inhibitor 1,2-dichloroethane, substrate inhibition of 1,2-dibromoethane and the effect of temperature on enzymatic reaction were evaluated.

This work was supported by grant No. 203/06/0047 from Grant Agency of Czech Republic and by research project No. MSM0021622413 and research centre No. LC06023 both from Czech Ministry of Education.

PO 11.7

MICRO-POSITRON EMISSION TOMOGRAPHY – NON INVASIVE FUNCTIONAL IMAGING METHOD

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Positron emission tomography (PET) is currently the most useful imaging technique using radioactive markers for non-invasive regional functional measurement of tissue metabolism and drug pharmacokinetics. The most significant benefit of the microPET technique compared to other traditional functional imaging methods such as autoradiography and direct tissue counting (biodistribution) is that animal can be scanned *in vivo.* This technique enables repeated monitoring of disease progression or a state of metabolic processes. Here we report our first experiences with three different radiotracers in experiments in the rat.

Distribution of 2-[^{18}F] fluoro-2-deoxy-D-glucoze (FDG) in the whole body was used to see metabolic activity of mammary gland tumors. Size and number of tumors were evaluated. FDG was also used to see glucose physiological accumulation in brain and heart. 3-deoxy-3[^{18}F]Fluorothymidine (FLT) is a parameter reflecting cell division. MicroPET technique revealed that only some parts of sarcoma tumors showed proliferation.^{18}F – fluoride(F^-) was used for scanning of the whole rat skeleton. It was shown that this approach might be sensitive for study of bone changes.

MicroPET offers new possibilities for acute and longitudinal *in vivo* experimental animal studies.

Supported by European Social Fund JPD3 2004/4-072, 13120200067

PO 11.8

DETERMINATION OF RUTIN CONTENT IN BUCKWHEAT BY HPLC AND SPECTROPHOTOMETRIC METHODS

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The flavonoid rutin is a flavonol glycoside comprised of the flavonol quercetin and the disaccharide rutinose. Rutin is found in many plants, especially the buckwheat (*Fagopyrum esculentum* Moench). Buckwheat has recently been found to be effective in reducing high blood pressure and slowing the aging process. Other rich dietary sources of rutin include black tea and apple peels. The level of rutin in buckwheat herb is aproximately 5-8% and it has been shown to absorb UV radiation and scavenge free radicals such as the superoxide anion, the hydroxyl radicals, and peroxy radicals. Rutin is widely present in plants but is relatively rare in their edible parts. Different cultivars of buckwheat may have different contents of rutin. Different parts of plants contain different concentrations of rutin. Most rutin is accumulated in the inflorescence (up to 12% d.w.b. – dry weight basis), in upper leaves (8-10% d.w.b.) and in stalks (0.4-1% d.w.b.) /1/.

The aim of this work is to compare the rutin content in different buckwheat species and in different parts of plants by several analytical methods (two spectrophotometric and HPLC methods). HPLC-RP was performed with gradient elution of methanol (A) and 2.0% acetic acid in water (B) (20-60% A in 0-6min) and detection at 355 nm. The Lichrospher 100RP-18 (5um) column (250x4mm) was used. The spectrophotometric analyses were performed by measuring of the absorbancies at 420 nm in methanol extracts with and without the addition of $AlCl_3$.

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Results were obtained with support of the project No. 0002700602 of the Ministry of Agriculture of the Czech Republic.

PO 11.9

UTILIZATION OF CHIP ELECTROPHORESIS FOR CHARACTERIZATION OF CEREAL VARIETIES

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For identification of cereal varieties we used new electrophoretic system. The Experion automated electrophoresis system applies a combination of microfluidic separation technology and sensitive fluorescent sample detection to perform rapid and automated analysis of protein. It automatically performs all steps of gel-based electrophoresis (sample separation, staining, destaining, imaging, band detection, and even some data analysis) to deliver reproducible separation and quantitation results in just 30 minutes.

The Experion system includes three instruments that automate and expedite the electrophoresis process – the electrophoresis station; the priming station (it prepares microfluidic chips for electrophoresis) and vortex station. Protein analysis is performed using the Experion Pro260 analysis kit, which contains the reagents, microfluidic chips, and other supplies required for the separation and analysis of 10-260 kDa proteins. Each chip holds up to ten samples. The other advantages over traditional gel electrophoresis are lower sample and reagent volume requirements, specialized protein standards for accurate and reproducible sizing and quantitation, and decreased exposure to hazardous chemicals (SDS, etc.). The Experion software displays separation results in both electrophorerogram (peak) and simulated gel views. The software automatically calculates protein component size, concentration, and percent of total sample. Results for each peak appear in the Results table.

In our laboratory we used this system for analysis of protein of wheat (LMW and HMW glutenin subunits) and barley (hordeins). This approach was compared with the classic electrophoresis these proteins in polyacrylamide gel and sodium dodecyl sulphate. Results were characterized by considerable reproducibility and especially protein patterns of wheat obtained these techniques were very comparable.

1. Zhu, K., Nguyen, M., Strong, W.: Bio-Rad Laboratories, Inc.

Results were obtained with support of the project No. 0002700602 of the Ministry of Agriculture of the Czech Republic.

PO 11.10

OPTIMIZATION OF PARAMETERS OF MELTING CURVE ANALYSIS FOR THE EVALUATION OF AMPLIFICATION OF SPECIFIC PCR PRODUCTS

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One of the most important inventions among quantitative methods of molecular biology was the design of real-time PCR which uses fluorescence techniques to monitor amplification as it occurs. In addition, real-time PCR instruments offer the possibility to acquire DNA melting curves. Each dsDNA fragment has a unique melting temperature, which depends mostly on the length and GC ratio of the fragment.

In our study we proposed a method for the evaluation of PCR reactions (performed in standard PCR cycler) by melting curve analysis (MCA) on a real-time PCR instrument. This process provides necessary advantages for laboratories where larger number of classic PCR reactions are evaluated by gel electrophoresis. MCA eliminates the need of standard electrophoresis, which is laborious and time consuming. It can be performed in 96-well format in less than 30 minutes. For the confirmation of evaluation of quality we performed MCA of 100 samples. In all cases, the genotyping gave same results as evaluation according to gel electrophoresis, which provided further evidence about the reliability of the method. We also proved the marked effect of denaturing agents on the melting temperature of PCR products. We constructed a calibration curve according to our results of the degree of decrease in Tm values by the increasing of the concentration of denaturing agent (DMSO). In the future we want to start to design PCR primers specifically for use with MCA.

PO 11.11

A NOVEL SELF-PROCESSING MODULE DERIVED FROM THE FRPC PROTEIN OF *NEISSERIA MENINGITIDIS* FOR A SINGLE-STEP PURIFICATION OF FREE RECOMBINANT PROTEINS

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Purification of recombinant proteins to homogeneity is often a challenging process and typically requires several chromatographic steps that must be individually optimized for each protein of interest. To overcome this difficulty, a system that enables purification of free recombinant proteins in a single affinity chromatographic step has been developed. The system is based on a 250 amino acid residues long self-processing module of the FrpC protein of *Neisseria meningitidis* that is genetically fused at its C-terminus to an affinity tag enabling simple one-step purification and at its N-terminus to a protein of interest. Upon binding of the fusion protein to an affinity matrix and washing out of contaminating proteins, specific cleavage between amino acid residues Asp and Pro of the self-processing module is induced by calcium ions. This results in release of the free protein of interest, having only one extra amino acid residue (Asp) at its C-terminus. The self-processing module - affinity tag fusion partner remains trapped on the affinity matrix. This system has been successfully tested with several proteins of interest (adenylate cyclase, chloramphenicol acetyltransferase, β-galactosidase, maltose-binding protein, or glutathione-S-transferase) and two different affinity tags (chitin-binding domain, or poly-His).

PO 11.12

DETECTION OF *CLOSTRIDIUM TYROBUTYRICUM* IN SEMI-HARD CHEESES USING MICROBIOLOGICAL AND PCR METHODS

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Butyric acid fermentation caused by the outgrowth of clostridial spores present in raw milk can create considerable loss of product, especially in the production of semihard cheeses. This defect is called the late blowing defect in cheese. Modern studies show, that only one bacterium of the genus *Clostridium* is able to provoke late blowing - *Clostridium tyrobutyricum*.

Semihard cheeses with visible clostridial defect were used for analysis. In this work, microbiological detection of butyric acid producing clostridia in semihard cheeses was optimalized. Also biochemical analysis (using API tests) of mentioned bacteria was performed. Further experiments were focused on optimalization of rapid PCR metod for *Clostridium tyrobutyricum* identification. The results show, that this PCR metod is suitable for rapid screening of the presence of *Clostridium tyrobutyricum* in late-blowing defect cheeses.

PO 11.13

DETECTION HIS 1069GLN MUTATION IN PATIENTS WITH WILSON DISEASE USING BIDIRECTIONAL PCR AMPLIFICATION OF SPECIFIC ALLELES (BI-PASA) TEST

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Wilsonn disease (WD) is an autosomal recessive disorder of hepatic copper metabolism caused by mutations in a gene encoding a copper-transporting P-type ATPase. The majority of known mutations affecting this gene are rare, except for the His 1069Gln mutation often found in patients of Northern or Eastern European origin. In our previous work we examined the frequency of the His 1069Gln mutation in Slovak patients with Wilson disease using two different DNA-based methods: ACRS (amplification created restriction site) for Alw21I in combination with nested PCR, and ARMS (amplification refractory mutation system) test. Although, both mentioned methods are reproducible and precise, ACRS requires seminested PCR and digestion of PCR product and in ARMS test two PCR reaction must be performed to detect the His 1069Gln mutation. In the present work we describe a method based on bidirectional PCR amplification of specific alleles (Bi-PASA) which identifies His 1069Gln both in homozygotes and heterozygotes in one PCR reaction. Comparing results of BI-PASA test with ACRS and ARMS tests on 27 WD patients homozygous and heterozygous for His 1069Gln mutation and on 120 random DNA samples genotyped showed 100% concordance. In conclusion, BI-PASA is more simple and rapid method for detecting the of His 1069Gln mutation when compared to both ACRS and ARMS method.

Keyword: Wilson disease, BI-PASA test, ARMS test

Section 12

BIOINFORMATICS

PO 12.1

COMPUTER MODELLING OF PROTEASOME ACTIVITY

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At the moment three proteasome cleavage prediction methods are publicly available on the internet: PAProC (www.paproc.de) developed at Tuebingen University[1,2], MAPPP (www.mpiib-berlin.mpg.de/MAPPP/) developed at the Max-Planck Institute in Berlin[3,4] and the NetChop (www.cbs.dtu.dk/services/NetChop/) developed at the Center for Biological Sequence analysis at the Technical University of Denmark [5].

In our contribution, an alternative method for the proteasome cleavage pattern prediction is presented, together with results. Our method, named KAGA aims to make use of novel approach to cleavage prediction. KAGA utilizes regular expression patterns to match cleaved fragments and the regular expressions itself are developed using genetics algorithms [1]. The method leads naturally to efficient distributed computing implementation.

Acknowledgement. This work was supported by the VVGS grant No. 214/2005 and VEGA grant 1/1018/04.

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PO 12.2

THE USE OF TRIPEPTIDE CONFORMATION FAMILIES IN PROTEIN MODEL BUILDING

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Conformation family is a useful concept for classification of proteins and nucleic acid structures. This classification is used in methods for prediction of protein structures, various model building programs, and as a tool for protein structure verification. There is no clear definition of conformation family in the literature. Clusters of similar structures usually represent conformation family.

Conformation families for tripeptides were determined by a method of conformation-family search in multidimensional torsion-angle space [1]. The families were classified according to positions in the Ramachandran map and studied for the purpose of automatic protein model building. The model building method was tested on a green fluorescent protein which is frequently used as a reporter protein [2]. The crystal structure with PDB code 1EMB was selected for the testing. The structure of the protein was build up automatically with a program NUT [3] and the structure building was analyzed in details with respect to sequence, secondary structures, loops and cis peptide bonds. The results are compared with model building techniques based on monopeptides and dipeptides. The novel method is a promising tool for building low resolution crystal structures.

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PO 12.3

*phi*SITE – A DATABASE OF GENE REGULATORY NETWORKS IN BACTERIOPHAGES

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We built *phi*SITE - a database system collecting data describing regulation of gene expression in phage genomes. The database collects information about experimentally confirmed and predicted regulatory sequences in bacteriophages. These are represented primarily by signal sequence motifs for transcription regulation (promoters, operators, terminators). This information is accessed basically from two main sources. The first is represented by a group of sequence databases of known primary structures of DNA and proteins (EMBL and UNIPROT). The second (with greater coverage at the time being) is searching in bibliography related to research of gene regulation in bacteriophages. The database is build on the SQL standard and therefore very robust, reliable and scalable. Collected data represent a database system of DNA motifs together with information about source bacteriophage, its host bacteria and their taxonomy, bibliographic references, protein(s) involved in regulatory pathway and genes regulated by this pathway. Information (if possible) is linked to other relevant databases (EMBL, SWISS-PROT, MEDLINE and others) and represented also in a graphical way. To date, the database contains several hundreds of described DNA regulatory motifs. Further development will lead to a system enabling to search potential regulatory motifs in phage genomes with unknown regulatory network.

PO 12.4

MMASS DATA MINER - AN OPEN SOURCE ALTERNATIVE FOR MASS SPECTROMETRIC DATA ANALYSIS

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Current development of the new tools for mass spectrometric data analysis tends to higher level of automation and high throughput analysis. On the other hand, there are still many tasks where the automation is not possible and interpretation of mass data is far more dependent on detailed analysis and researcher's experience. Program mMass presents an open source package of simple proteomic tools, usable in many mass spectrometric data analysis. It consists of several modules and tools for spectrum processing and interpretation with focus on common proteomic tasks.

The main part of the program is a spectrum viewer/analyser where peaks can be labelled. Using the protein digest tool, any given protein or peptide sequence previously defined in internal sequence editor can be virtually digested by a specified enzyme or chemical reagent; masses of generated peptides can be matched to current peak list within specified tolerance. Peptide fragmentation tool works in the same way to generate a list of all common peptide fragments. There are several additional tools for protein/peptide sequence analysis, checking differences between all the peaks in peak list, comparison of unlimited number of peak lists and many more.

Although this program was primarily developed for MALDI-TOF MS, it can be used for other mass spectrometry techniques as well. As the program is written in Python language and is released under GNU General Public License, it is portable to many computer platforms and has a good potential to be easily modified or extended by module of specific need. Program can be downloaded from the internet address http://mmass.biographics.cz/.

Section 13

VARIA

PO 13.1

GENE EXPRESSION OF RAS COMPONENTS, LEPTIN, ADIPONECTIN, GLUT4, PPAR (GAMMA) IN VARIOUS FAT DEPOTS.

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The renin-angiotensin system (RAS) has long been recognized as an important regulator of systemic blood pressure and renal electrolyte homeostasis[1]. Over the last decade, several components of the RAS have been detected in a variety of tissue, for example, adrenal gland, kidney, brain, heart, blood vessels and adipose tissue[2-6].

Obesity is associated with insulin resistance, hyperglycemia, dyslipidemia, hypertension, and prothrombic and proinflammatory states[7].

We have not found significant difference in basal levels of mRNA for angiotensinogen, angiotensin-converting enzyme (ACE), angiotensin II type 1 receptor (AT1 receptor), leptin, adiponectin and GLUT4 between retroperitoneal and epididymal fat depots. Furthermore, we observed significantly lower gene expression of angiotensinogen, ACE, AT1 receptor, leptin, adiponectin and GLUT4 in mezenteric adipose tissue in contrast to retroperitoneal and epididymal adipose tissue. In turn, level of mRNA for PPARγ, was lower in retroperitoneal and mezenteric adipose tissue compared to epididymal fat depot. Our results confirm, at molecular level, differences in metabolic activity of mezenteric adipose tissue on the one hand and retroperitoneal and epididymal adipose tissue on the other hand.

This work is supported by grant VEGA 2/5090/26.

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PO 13.2

THE EFFECT OF STATINS ON RAS LOCALIZATION AND CELLULAR PROLIFERATION.

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Statins are specific inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase). They are used as the hypolipidemics. In recent years these compounds were observed to exhibit also cancerostatic activity. The inhibition of HMG-CoA reductase results in inhibition of Ras protein farnesylation, which regulates cell proliferation and differentiation.

At first the effects of commercialy available statins on a proliferation of human pancreatic cancer cell lines CAPAN-2 and MiaPaCa-2 bearing activating mutation in K-Ras protooncogene and on survival of nude mice xenotransplanted with CAPAN-2 cells were studied.

Then the impact of statins on the localization of Ras proteins was investigated. Both oncogenes K-ras and N-ras were isolated using RT-PCR from total HeLa cells RNA and inserted into pEGFP-CI vector enabling the expression of these gene products in N-terminal fusion with GFP. Fluorescent microscopy revealed that both N- and K-ras are accumulated at cytoplasmic membrane in different manner and that this accumulation was affected by statins.

PO 13.3

THE INFLUENCE OF PURIFICATION TECHNIQUE OF ΔProCANC PROTEIN OF MASON-PFIZER MONKEY VIRUS ON THE MORPHOLOGY OF IN VITRO ASSEMBLED PARTICLES

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Retroviral structural polyprotein precursor Gag directs the assembly of the immature retroviral capsid. To study the assembly of immature particles we used minimized Gag domain comprised of fusion capsid and nucleocapsid protein (ΔProCANC) of Mason-Pfizer monkey virus. The bacterially expressed ΔProCANC proteins were purified by two different purification techniques. Proteins were obtained with or without minimal amount of contaminating nucleic acids. To investigate the effect of protein purification method on the assembly of capsids we used various types of nucleic acids (RNA, ssDNA, dsDNA) and their substitutions (heparin). The assembly reactions of protein and appropriate nucleic acid were performed by dialysis. Formed structures were analysed by transmission electron microscopy of negatively stained material.

We found that proteins purified by two distinct techniques differ in their ability to form capsids. This dissimilarity could be caused by a missing cofactor (or some cellular component) that is removed by high salt concentration during purification. This cofactor may facilitate protein folding into the correct conformation, which allows the more efficient assembly of regularly-shaped spherical particles.

These results might contribute to understanding of capsid assembly mechanism and to a development of inhibitors of capsid assembly. Moreover the *in vitro* incorporation of specific genes represents a promising system for construction of gene therapy vectors.

PO 13.4

DETERMINATION OF NIFEDIPINE AS A PROBE SUBSTRATE OF CYTOCHROME P450 3A1/2 ACTIVITY IN RATS

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Nifedipine is a dihydropyridine calcium channel blocker used in the treatment of hypertension, acute coronary syndrom, stable angina pectoris and congestive heart failure. Oxidation of nifedipine to its pyridine metabolite is used as a valuable tool for the measurement of metabolic activity of the rat 3A1/2 or human 3A4/5 isoform of cytochrome P450. Due to high similarity in amino acid sequence between rat CYP3A1/2 and human CYP3A4/5 it is possible to predict the metabolic activity of human CYP3A4/5 by the measurement of rat CYP3A1/2 activity.

Here we describe a new, very simple, rapid, sensitive and reproducible HPLC method for the determination of nifedipine and its pyridine metabolite in perfuse medium after rat liver perfusion. The samples were analyzed on reverse-phase column using UV detection. The mobile phase consisted of acetate buffer, pH 4.7 and acetonitrile, the flow rate was 0.8 ml/min. The retention times of nifedipine and its pyridine metabolite were up to 10 minutes.

This method is applicable for modeling and description of possible pharmacological interactions on rat CYP3A1/2 or human CYP3A4/5, respectively.

This work supported by Ministry of Education (projects no. MSM 0021622404 and LC06023) and by the Grant Agency of Czech Republic (grant 203/06/0047).

PO 13.5

SPECTROSCOPIC AND CYTOTOXIC STUDIES OF NEW DNA INTERCALATORS POSSESSING AN ANTRACENE SKELETON

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Search for new low molecular weight ligands that could specifically bind to particular DNA sequences has been carried out for years in the hope that new therapeutics could be found. Several anthracene derivatives, such as pseudourea, for example, show antitumor activity and their interaction with DNA was thought to be necessary for biological activity. The planar, anthracene ring system is suitable for intercalation into the DNA helix and many antryl probes have been suggested to prefer intercalation.

The binding properties of four 9,10-bis-substituted anthracene derivatives - with calf thymus DNA were studied. Binding of the probes to the DNA was examined by spectroscopic and melting studies. From spectroflorometric titrations the binding constants for the DNA-drug complexes were estimated ($K = 2.6 – 9.2 \times 10^5\ M^{-1}$).

Cytotoxicity of derivatives of anthracene against the cancer cell lines HeLa and L1210 cells was measured by the MTT assay and by the cell morphology monitoring. Under the long-term influence of 9,10-bis{[-2-(morfoline-4-yl)ethyl] aminomethyl}anthracene and 9,10-bis{[-2-(piperazine-1-yl)ethyl] aminomethyl} antracene, the values of IC_{50} were 21 and 18 μM, respectively, for the Hela cells.

ACKNOWLEDGEMENTS: This work was supported by the Slovak Grant Agency VEGA, grants No. 1/0432/03, 1/1274/04, 1/1173/04 and 1/3254/06.

PO 13.6

A NOVEL 'CLIP-AND-LINK' ACTIVITY OF RTX PROTEINS FROM GRAM-NEGATIVE PATHOGENS: COVALENT PROTEIN CROSS-LINKING BY AN ASP-LYS ISOPEPTIDE BOND UPON CALCIUM-DEPENDENT PROCESSING AT AN ASP-PRO BOND

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Clinical isolates of *Neisseria meningitidis* produce an RTX protein, FrpC, of unknown biological activity. Here we show that physiological concentrations of calcium ions induce a novel type of autocatalytic cleavage of the peptide bond between residues Asp^{414} and Pro^{415} of FrpC that is insensitive to inhibitors of serine, cysteine, aspartate and metalloproteases. Moreover, as a result of processing, the newly generated N-terminal fragment of FrpC can be covalently linked to another protein molecule by a novel type of Asp-Lys isopeptide bond that forms between the carboxyl group of its C-terminal Asp^{414} residue and the epsilon-amino group of an internal lysine of another FrpC molecule. Point substitutions of negatively charged residues possibly involved in calcium binding dramatically reduced the self-processing activity of FrpC. The segment necessary and sufficient for FrpC processing was localized by deletion mutagenesis within residues 400 to 657 and sequences homologous to this segment were identified in several other RTX proteins. The same type of calcium-dependent processing and cross-linking activity could, indeed, be observed also for the purified ApxIVA protein of *Actinobacillus pleuropneumoniae*. These results define a protein cleavage and cross-linking module of a new class of RTX proteins of Gram-negative pathogens of man, animals and plants. In the calcium-rich environments colonized by these bacteria this novel activity is likely to be of biological importance.

PO 13.7

ALTERNATIVE DNA STRUCTURAL MOTIFS AFFECTED BY INTERCALATING AGENTS

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Despite the existence of a wealth of structural and theoretical data relating to repetitive sequences in the genome, the mechanism of formation of alternative structural elements in DNA at the presence of intercalators in miscellaneous biological processes is still poorly understood. Generally, DNA intercalators influence the DNA superhelicity, which plays a key role in the cruciform formation in DNA molecules. The potential of DNA intercalating ligands on the stabilization/destabilization of cruciform in DNA is discussed. Herein, the indirect impact of wide scale of intercalators is analyzed for the first time. Ror example the anthracycline modifies the helical properties of DNA and the overall DNA structure, and then alters any cruciform-dependent processes, mainly DNA replication and transcription.

Acknowledgements

This study was supported by grants from the Slovak Grant Agency (1/1274/04 and 1/3254/06), and the Science and Technology Assistance Agency (APVT-20-006604).

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PO 13.8

STUDY OF INTERACTION OF POLYCYCLIC LIGANDS WITH DNA AND THEIR CYTOTOXIC ACTIVITY.

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The interaction of ligand with double-stranded DNA is fundamental for many intracellular processes. Small binding ligands with reduced or no sequence specificity are often able to interfere with those processes because they are capable of changing mechanical properties of the DNA strands and are, therefore, frequently used in cancer therapy.

The binding ability of new dithiazolidinone hydrochlorides with DNA were investigated by spectroscopic techniques including UV-vis, fluorescence and CD spectroscopy. The effects of studied derivatives on thermal denaturation profiles of calf thymus DNA were analyzed. From spectrophotometric titrations the binding constants for the DNA-drug complexes were estimated (K = 3.9 – 6.2 $\times 10^4$ M^{-1}). DNA binding constants indicate that the length of alkyl chain was a key impact factor for the DNA binding affinity and biological activity. *In vitro* cytotoxic activity of investigated compounds towards murine leukemia cell line L1210 and human uterus carcinoma HeLa cells were determined. 3',3''-dipentyl-2',2''-[(acridin-3,6-diyl)diimino]-1,3-dithiazolidin-4-one hydrochloride showed the highest activity against L1210 and HeLa cells line with IC_{50} values of 6.3 μM and 12.9 μM (72 h). These new drugs afforded interesting possibility to develop novel DNA-targeted anticancer agents.

ACKNOWLEDGEMENTS: This work was supported by the Slovak Grant Agency VEGA, grants No. 1/3254/06, 1/1274/04, 1/1173/04 and 1/0432/03.

PO 13.9

MONOCLONAL ANTIBODIES SPECIFIC TO THE HA2 GP OF INFLUENZA A VIRUS REDUCING ITS REPLICATION, CROSS-REACT WITH INFLUENZA VIRUSES OF HUMAN AND AVIAN ORIGIN.

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The HA2 gp of influenza A virus is responsible for the fusion of virus and cell membranes during its endocytosis. It represents conserved part of the main surface antigen haemagglutinin (HA), as it is evident from the sequential analysis of HA. It induces strong antibody response after the natural infection. Recently it was shown that monoclonal antibodies (MAbs) specific to the HA2 gp inhibit the fusion activity of influenza HA and reduce the replication of virus. Therefore in this study we followed the effect of HA2-specific MAbs on the *in vivo* influenza infection and their cross-reactivity with human and avian influenza A viruses. BALB/c mice were infected intranasally with 1 LD_{50} of influenza A virus of H3 subtype. It was shown that two of the four MAbs specific to the HA2 gp of the same virus improved the survival of mice to 100% MAbs (CF2, FE1) and one MAb (IIF4) to 87,5% in comparison to the control group in which 50% mice survived. These results indicated that HA2-specific MAbs protected mice against the lethal influenza A infection with the homologous virus. Due to their protection ability we examined their cross-reactivity with influenza A viruses of various subtypes of HA (H3, H4 and H7). All four HA2-specific MAbs recognizing different antigenic sites on HA2 gp reacted with all tested human viruses of H3 subtype in a Rapid culture assay and in ELISA. Two from them MAbs (CF2 and IIF4) reacted positively with avian viruses of H4 subtype and one MAb (CF2) even with influenza A virus of H7 subtype HA. These results require further *in vivo* studies to show if the intersubtype cross-reactive HA2-specific MAbs are able to influence the course of infection of mice also by an influenza A virus of avian origin.

PO 13.10

THE EFFECT OF HYDROGEN PEROXIDE ON STABILITY AND STRUCTURE OF FERRICYTOCHROME C

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Cytohrome *c*, located in intermembrane space in mitochondria, is a potential target for reactive oxygen species. Cytochrome c, like all hemeproteins, is inactivated in the presence of catalytic concentrations of hydrogen peroxide (Valderama et al., 2002). This inactivation process is especially important in the absence of reducing substrate and its mechanism has not been fully elucidated. The molecular mechanism underlying the hydrogen peroxide-mediated inactivation of hemeproteins presents an extraordinary complexity arising from the fact that a multitude of reactions may occur subsequent to the hydroperoxide reaction with the heme (Valderama et al., 2002). In our work we focused on effect of hydrogen peroxide on structure and stability of ferricytochrome c using the methods of spectrophotometry, fluorescence and circular dichroism. The effect of hydrogen peroxide on cytochrome c depends on concentration of H_2O_2 and time of its incubation with protein. The loss of the sixth axial methionine ligand to the heme is widely considered to be the first step of the unfolding process. Using the internal probes of cytochrome c, heme and Trp59 allowed us to study the sensitivity of the component parts of this protein.

Acknowledgements. This work was supported by the research grants from the Slovak Grant Agency VEGA No. 1/1272/04.

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PO 13.11

ADIPOSE TISSUE HYPERTROPHY IS ASSOCIATED WITH ELEVATED AT_1 RECEPTOR PROTEIN

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Angiotensin II (Ang II), the biologically active component of renin-angiotensin system (RAS), is demonstrable generated locally in numerous tissues where it has been shown to be involved in cell proliferation, hypertrophy and extracellular matrix production[1,2]. Studies have revealed the presence of a local RAS also in adipose tissue[3,4]. Ang II was proposed as a trophic factor in white adipose tissue growth and development[5] and studies on RAS inhibition with AT_1 receptor blockers indicate that these compounds are useful in the treatment of obesity and diabetes[6].

The aim of this study was to characterize the AT_1 receptor protein in hypertrophied adipose tissue using rats with monosodium glutamate (MSG) – induced obesity and ageing rats as a model of spontaneous obesity.

AT_1 receptor protein in epididymal fat tissue plasma membranes was studied using immunoblot. MSG-treated rats displayed approximately 4-times more AT_1 receptor immunoreactive protein content in fat tissue cell membranes than did the controls (MSG: 10232 ± 1035 OD/mm^2 *vs* Controls: 2583 ± 982 OD/mm^2, $p<0,01$). Similarly, old 26-weeked fat rats displayed significantly increased amount of AT_1 receptor protein (2118 ± 538 OD/mm^2) in contrast to young 9-weeked lean rats (178 ± 25 OD/mm^2), $p<0,05$.

The obtained data are in support of a role of Ang II and AT_1 receptors in the pathogenesis of obesity.

This work was supported by grant VEGA 2/5090/25

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PO 13.12

BIOCHEMICAL CHANGES ACCOMPANYING AGEING OF *TRICHODERMA VIRIDE* VEGETATIVE MYCELIA

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The problem of ageing is more pertinent to and treated in studies of organisms belonging to animal kingdom, however phenomena related to ageing have been found and studied also with filamentous fungi. Here we present our observations made with deuteromycete *Trichoderma viride* as a model.

Identical masses of submerged *T. viride* mycelia of various ages (1 to 30 d) were used as inoculum for the second submerged cultivation lasting for 24 h. It was found that the growth yield of this culture was dependent on the age of inoculum. The growth yields increased if the age of primary culture was less than 3 d while older cultures lost their growth competence. In order to elucidate underlying biochemical processes developmental changes of major metabolic parameters and of specific activities of organellar marker enzymes in the mitochondrial/vacuolar and microsomal fractions of mycelia were measured. These parameters and activities changed during the growth of mycelia in a biphasic manner and their time courses were remarkably similar. The results indicate that the prolonged submerged cultivation triggers coordinated series of biochemical events which leads to the loss of growth competence.

This work was supported by Science and Technology Assistance Agency under the contract No. APVT-20-003904.

PO 13.13

DETECTION OF ALTERNATIVE DNA STRUCTURAL MOTIFS

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Alternative DNA structural motifs frequently occur in DNA repetitions. Some genetic neurodegenerative diseases and three fragile sites have been associated with the expansion of (CTG)n·(CAG)n, (CGG)n·(CCG)n, or (GAA)n·(TTC)n repeat tracts. Palindromic repetitions are frequently located in promoter regions of a specific operon and origin of replication. The propensity to form slipped strand DNA, cruciform, triplex DNA and Z- form is proportional to the length and homogeneity of the repeat tract. Temperature gradient gel electrophoresis can be used to distinguish among various supercoiled DNA topoisomers and to ascertain whether or not the alternative structural motif has been extruded. This technique is implemented for the first time to address the role of temperature in cruciform extrusion from plasmids.

Acknowledgements

This study was supported by grants from the Slovak Grant Agency (1/1274/04 and 1/3254/06), and the Science and Technology Assistance Agency (APVT-20-006604).

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PO 13.14

THE ASSEMBLY OF MASON-PFIZER MONKEY VIRUS AND HUMAN IMMUNODEFICIENCY VIRUS PARTICLES *IN VITRO* AND IN *ESCHERICHIA COLI*

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The retroviral capsid assembly has been extensively studied during the last years and several models were predicted but the exact mechanism of this process is still not clear. All retroviruses form a structural precursor polyprotein Gag that initiates and directs the assembly of immature retroviral capsid. We have focused on assembly of Mason-Pfizer monkey virus (M-PMV) and human immunodeficiency virus type 1 (HIV-1) particles made of deletion mutants of polyprotein Gag. We used fusion capsid-nucleocapsid (CA-NC) protein and its truncated form (ΔProCA-NC), where N-terminal proline is deleted. For comparative assembly studies we have used two systems, *in vitro* and in bacteria.

Gradient ultracentrifugation was followed by SDS-PAGE analysis and electron microscopic comparison of structures assembled from those proteins *in vitro* and in *E. coli*. Differences in efficiency of the assembly and in morphology of formed capsids were observed. ΔProCA-NC M-PMV formed *in vitro* only spherical particles while in bacterial system this protein formed a mixture of spheres and tubes. ΔProCA-NC HIV-1 formed *in vitro* spherical, tubular and conical structures. However, in *E. coli* only tubes were formed from the same protein. Both the *in vitro* and in *E. coli* formed particles were studied by other techniques as atomic force microscopy. The molecular organization of particles and factors affecting their assembly will be discussed.

LIST OF PARTICIPANTS

A

Adam Jan	L 9.1			
Adam Vojtěch	L 7.12	PO 7.10		
Adamčík Jozef	L 1.5			
Adámková Šárka	PO 4.18			
Aimová Dagmar	L 5.16			
Alois Kozubík	PO 4.12			
Amerongen Aart van	PO 11.2			
Andráško Adam	CP 1			
Andrysík Zdeněk	L 5.17	PO 5.1	PO 5.25	PO 5.27
Anzenbacher Pavel	L 5.14	PO 5.4	PO 5.19	
Anzenbacherová Eva	L 5.14	PO 5.4	PO 5.19	
Arlt Volker M.	PO 5.7			
Atanasova Nikolina	PO 8.14			

B

Babincová Melánia	L 9.9			
Babušíková Eva	L 6.5	L 7.4	PO 6.23	
Babjaková Lucia	CP 1			
Baculíková Miroslava	PO 13.1			
Bačiak Ladislav	L 7.13			
Bajdichová Mária	PO 13.5			
Bajzová Magda	L 11.3			
Balážová Andrea	PO 2.17	PO 2.18		
Baldovič Marián	PO 11.13			
Baldus Stephan	L 6.7			
Balgavý Pavol	L 6.4			
Banáš Pavel	L 2.13	L 11.4	PO 2.31	
Bancířová Martina	PO 6.1			
Barančík Miroslav	L 5.2	PO 5.5	PO 5.9	PO 7.11
Baráthová Monika	PL 2	L 1.4	PO 7.12	
Bártová Iveta	L 4.11			
Basler Marek	PL 5	L 3.4	PO 1.7	PO 3.6
Bast Aalt	L 5.6			
Bauerová Katarína	PO 6.2	PO 6.16		
Beláňová Martina	L 9.8	PO 9.2		
Belvončíková Petra	PO 1.1	PO 1.18		
Benková Kamila	PO 4.13			
Benkovičová Vladislava	L 3.7			
Beregházyová Eva	PO 2.7			

Bergamini Christian	PO 3.4					
Bernátová Soňa	CP 1					
Bezáková Lýdia	PO 2.17	PO 2.18				
Bezouška Karel	L 9.2	L 9.3	L 2.4	L 2.5		
Bieliková Marcela	PO 11.1					
Biely Peter	L 9.5	L 9.6	L 9.10	PO 9.11		
Bilka František	PO 2.18					
Bilková Andrea	PO 2.18					
Binková Hana	L 7.12					
Biskupičová Ida	PO 12.3					
Bistaková Jana	L 4.5					
Bíliková Katarína	PO 2.14					
Bílková Zuzana	PO 2.8					
Bílý Jan	L 2.5					
Blahovec Ján	PO 2.15	PO 2.16				
Blanáriková Víťazoslava	PO 2.17					
Blanářová Olga	PO 5.2					
Blaškovičová Hana	L 2.3					
Blaškovičová Jana	PO 1.2	PO 4.21				
Blaštík Ondřej	L 7.12	PO 7.10				
Blatný Radek	L 1.2					
Blažíček Pavol	PO 7.3	PO 7.14				
Blažková Martina	PO 11.2					
Bobáľová Janette	PO 2.2					
Boháčová Viera - UMB	PO 2.29	PO 11.3				
Boháčová Viera - UMFG	PO 5.3	PO 5.5	L 5.2			
Bohmer František	PO 7.14					
Bohušová Tatiana	PO 1.4					
Bochořáková Hana	L 7.1	PO 6.3				
Bojňanská Tatiana	PO 8.15					
Boor Peter	L 7.8					
Bordevik Marianne	PO 9.14					
Borošová Gabriela	L 8.3					
Bořilová Šárka	PO 8.6					
Boušová Iva	PO 7.1					
Božek Peter	L 7.8	PO 6.8				
Bradley Allan	L 1.2					
Bradová Jana	PO 11.9					
Brázda Václav	PO 4.1	PO 4.2				
Brázdová - Jagelská Eva	PO 4.1	PO 4.2				
Brechtlová Marta	L 10.4	PO 6.5				
Breier Albert	L 5.1	L 5.2	L 5.3	PO 5.5	PO 5.6	PO 5.9
	PO 5.26					

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D

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E

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G

H

Hatok Jozef	L 6.5	L 7.11	PO 7.6		
Haubová Šárka	PO 13.3	PO 13.14			
Havlíček Vladimír	L 9.2	L 2.4	PO 13.6		
Havlová Pavla	PO 6.11				
Havrlentová Michaela	PO 9.6				
Hájnická Valéria	PO 1.1				
Hála Jan	PO 6.7				
Hejnová Jindra	L 11.3				
Helia Otto	PO 5.23				
Hensler Michal	PO 3.14				
Hernychová Lenka	L 2.1				
Hladíková Jana	PO 5.8				
Hlinková Elena	L 10.6				
Hoblík Ján	PO 7.7				
Hodek Petr	L 5.15				
Hofbauerová Kateřina	L 2.5				
Hofmann Wilma A.	L 4.6				
Hofmanová Jiřina	L 5.4	PO 4.10	PO 4.12	PO 4.17	PO 5.2
Högger Petra	L 6.2				
Holasová Šárka	L 5.9				
Holková Ivana	PO 2.17	PO 2.18			
Holomáňová Dagmar	PO 6.15				
Holotňáková Tereza	PL 2	L 6.6	PO 4.4		
Holovská Katarína	PO 6.21				
Holý Antonín	PO 4.9	PO 4.14	PO 5.4	PO 5.19	
Horáková Ľubica	L 6.4	PO 6.20			
Horáková Zuzana	L 7.12				
Horváth Anton	L 3.7	L 10.6	PO 3.3		
Horváth Ondřej	L 2.4				
Horváth Viktor	L 5.4	PO 4.17	PO 5.2		
Hostinová Eva	L 8.2				
Hoyerová Klára	L 4.8				
Hozák Pavel	L 4.6				
Hraběta Jan	L 5.18	PO 7.10			
Hrabovská Anna	PO 5.23				
Hradecká Dana	PO 2.25				
Hrkal Zbyněk	PO 2.12	PO 2.13			
Hrnčiarová Marta	PO 6.14				
Hronská Lucia	L 3.1	PO 3.13			
Hrubá Eva	L 5.17				
Hubáčková Miluše	L 1.3				
Hudcovicová Martina	PO 8.4				
Hudeček Jiří	L 5.15				

CH

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Kočí Radka	PO 8.7					
Kočišová Alica	PO 2.15					
Kodet Roman	L 1.3					
Kodíček Milan	L 10.1	PO 12.4				
Koets Marjo	PO 11.2					
Kofronová Oľga	L 4.10					
Kogan Grigorij	L 9.9					
Koháryová Michaela	PO 2.27					
Kojšová Stanislava	PO 5.6					
Kolarov Jordan	PO 4.20					
Kolenová Katarína	L 9.5					
Kollárová Marta	L 10.6	PO 2.27	PO 12.2			
Komínková Viera	PO 3.15					
Komprda Tomáš	PO 11.4					
Kondrashov Alexey	L 6.10					
Kondrová Eliška	PO 5.12					
Konopásek Ivo	L 3.4	PL 5				
Konopka Roman	PO 6.9					
Kopáček Juraj	PL 2	L 1.4	L 7.3	L 4.1	L 7.10	PO 1.2
	PO 2.10	PO 4.5	PO 4.21	PO 7.12		
Kopál Martin	PO 9.2					
Kopecký Jan	L 8.1	PO 3.14				
Kopecký Vladimír Jr.	L 2.5	PO 9.15				
Koprdová Ria	PO 3.9	PO 6.8				
Korduláková Jana	L 9.8	PO 9.4				
Kormanec Ján	L 4.5	L 4.7	PO 4.11			
Korytár Peter	PO 6.12					
Kosinová Eva	PO 1.8					
Kosík Ondřej	PO 9.7					
Kostecká Zuzana	PO 2.15	PO 2.16				
Kostolanský František	PO 13.9					
Košata Bedřich	PO 12.4					
Košinová Pavlína	L 11.4					
Košťálová Daniela	PO 6.17					
Kotašková Jana	PL 1					
Kotrbová Věra	PO 5.11					
Kotrla Rostislav	L 6.3					
Kotyza Jaromír	PO 2.11					
Koubková Zuzana	PO 4.12					
Kováč Peter	PO 11.7					
Kováčiková Michaela	L 11.3					
Kováčová Elena	PO 1.17					
Kováčová Zuzana	L 11.3					

Kovárová Annamária	L 5.3					
Kozubík Alois	L 5.4	L 5.17	PO 4.10	PO 4.17	PO 5.1	PO 5.2
	PO 5.27	PO 5.30				
Kožurková Mária	PO 13.5	PO 13.8				
Kraic Ján	PO 8.2	PO 9.6	PO 9.8			
Králíková Michaela	PO 7.13					
Královcová Dita	PO 1.6					
Královský Josef	PO 2.8					
Krčmář Pavel	L 5.9	L 5.17	PO 5.25	PO 5.27	PO 5.30	
Krejnusová Ingrid	L 2.3					
Kretová Miroslava	PO 1.5					
Krist Pavel	L 9.2					
Kristek František	PO 3.9	PO 6.8				
Krivoš Vladimír	PO 1.19					
Križanová Oľga	L 7.2	L 7.3	L 7.7			
Krížková Jana	PO 4.14					
Kron Ivan	PO 7.5					
Kroutil Aleš	PO 5.2					
Krůšek Jan	L 3.4					
Křen Vladimir	L 9.2	PO 9.15				
Křepela Evžen	PO 4.13					
Křiváková Pavla	PO 5.13	PO 5.14	PO 6.18			
Kříž Zdeněk	L 4.11					
Křížková Jana	PO 5.4					
Křížková Soňa	PO 7.10					
Křížová Veronika	L 5.5	PO 5.15				
Kubala Lukáš	L 6.7	PO 6.9				
Kubeš Miroslav	PO 4.5					
Kubešová Jitka	PO 8.7					
Kubovčáková Lucia	L 7.3					
Kučera Ladislav	PO 1.10					
Kučera Otto	PO 5.13	PO 5.14	PO 6.18			
Kucharská Jarmila	PO 3.9	PO 6.8				
Kukačka Jiří	L 7.12					
Kusenda Branislav	PL 1					
Kušnír Jaroslav	L 11.1	PO 3.12				
Kutejová Eva	L 3.8					
Kutinová Canová Nikolina	PO 5.10					
Kuznetsov Yurii	PO 13.14					
Kuželová Kateřina	PO 2.12	PO 2.13				
Kúdelová Marcela	PO 1.1	PO 1.18	PO 1.19			
Kůs Vladimír	PO 3.14					
Kvasničková Eva	L 5.6					

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M

Majerník Alan	L 3.3	L 3.6	PO 3.1	PO 3.2
Majerníková Katarína	PO 4.6			
Majtán Viktor	L 8.5			
Malbeck Jiří	PO 4.19			
Malčíková Jitka	PL 1			
Maliar Tibor	PO 8.16			
Man Petr	L 9.2	L 2.4	PO 3.3	
Maňásková Pavla	PO 9.5	L 9.7	L 2.8	L 2.9
Mandl Martin	L 2.10	PO 8.6		
Marčeková Zuzana	PO 9.9	PO 1.8		
Marchetti Stefano	PO 8.2			
Marinov Iuri	PO 2.12			
Markovič Oskar	L 2.11			
Maršala Jozef	L 7.9			
Martinec Jan	PO 4.23			
Martínek Jindřich	PO 5.10			
Martínek Václav	PO 5.16			
Marvanová Soňa	L 5.17			
Maser Edmund	PO 5.21			
Mašanová Viera	PO 7.14			
Mašín Jiří	PL 5	L 3.4	PO 3.6	PO 9.9
Matáková Tatiana	L 7.11	PO 1.12	PO 7.6	
Mateašik Anton	L 6.9			
Matejovičová M.	PO 6.6			
Matějová Eva	PO 11.8	PO 11.9		
Matis Ján	PO 1.18	PO 1.19		
Maurizot Jean-Claude	L 6.9			
Mayer Jiří	PL 1			
Mazanec Karel	L 2.6	PO 2.1		
Mazuráková Vladislava	L 4.7			
Márová Ivana	L 6.3	PO 6.10	PO 8.7	
Máleková Ľubica	PO 3.15			
McPherson Alex	PO 13.14			
Melichar František	PO 11.7			
Melkusová Petra	PO 4.14			
Melník Milan	PO 5.17			
Mentel Marek	PO 4.20			
Miadoková Eva	L 9.9			
Mieslerová Barbora	PO 4.18			
Mihalová Danica	PO 6.2	PO 6.16		
Mihálik Daniel	PO 1.9			
Mihók Ľuboslav	L 2.5			
Michalík Ivan	PO 2.30	PO 7.7	PO 8.10	

N

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P

Parkhomenko Natalya	L 3.8					
Parkkila Seppo	PL 2					
Pastorek Jaromír	PL 2	L 1.4	L 4.1	L 6.6	L 7.10	PO 1.15
	PO 2.9	PO 2.10	PO 4.3	PO 4.4	PO 4.5	PO 4.21
	PO 4.22	PO 4.25	PO 7.12			
Pastoreková Silvia	PL 2	L 1.4	L 7.3	L 4.1	L 6.6	L 7.10
	PO 1.15	PO 2.9	PO 2.10	PO 4.3	PO 4.4	PO 4.5
	PO 4.21	PO 4.22	PO 4.25	PO 7.12		
Pauliková Ingrid	PO 5.23					
Paulíková Helena	PO 13.8					
Paulová Hana	L 7.1	PO 6.3	PO 7.8			
Paulovičová Ema	PO 6.2					
Pavelčík František	PO 12.2					
Pavlíková Martina	L 7.4	L 7.5				
Pavlíková Martina	PO 7.9					
Peč Pavel	PO 2.22	PO 2.23	PO 4.18			
Péč Martin	PO 1.4					
Pečinka Petr	PO 4.2					
Pečivová Jana	PO 6.15					
Pecháňová Oľga	PO 5.6	PO 6.5	PO 7.11			
Peličic Vladimír	PO 1.7					
Peregrinová Andrea	PO 13.7	PO 13.13				
Perjési Pal	PO 3.12					
Perret Stephanie	L 9.1					
Pešek Miloš	PO 2.11					
Petráčková Denisa	PO 2.5	PO 3.10				
Petřek Martin	L 11.4					
Petřivalský Marek	PO 2.22	PO 2.23	PO 2.24	PO 4.18		
Pěnčíková Kateřina	PO 5.24	PO 5.25				
Philimonenko Vlada V.	L 4.6					
Phillips David H.	PO 5.7					
Piknová Mária	PO 8.8					
Plíhal Ondřej	L 9.2					
Plíšková Martina	L 5.9					
Pluskalová Michaela	PO 2.12	PO 2.13				
Podhradský Dušan	PO 13.5	PO 13.8				
Podhradský Ján	L 10.4					
Podlipná Radka	L 8.4	PO 2.3				
Pokorná Blanka	L 2.10	PO 8.6				
Pokorná Martina	L 9.1					
Polák Ján	L 11.3					
Poláková Helena	PO 11.13					
Polakovičová Viktória	PO 2.28					

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S

Slunská Z.	PO 6.6					
Smolková Katarína	PO 4.24					
Sobeková Anna	PO 6.21					
Somolová Tereza	L 5.16					
Souček Karel	L 5.4	PO 4.17	PO 5.2	L 1.3	PO 5.12	
Sova Petr	L 5.4	PO 5.2				
Spížek Jaroslav	L 8.1					
Spustová Viera	PO 4.16					
Srbová Martina	PO 6.19					
Stano Matej	PO 12.3					
Staszková Ludmila	PO 2.25					
Stiborová Marie	L 5.15	L 5.16	L 5.18	PO 5.7	PO 5.11	PO 5.16
	PO 5.18					
Stöcklein Walter	L 9.11					
Stojnev Tatjana	PO 2.4					
Stopka Pavel	PO 5.12					
Stratilová Eva	PO 2.20	PO 2.21				
Strelka Ľubomír	PO 7.6					
Strnad Petr	L 1.2					
Strohalm Martin	PO 12.4					
Stropkovská Andrea	PO 13.9					
Stříbrná Jana	L 1.3					
Stuchlík Stanislav	PO 8.11					
Sulová Zdenka	L 5.2	L 5.3	L 3.5	PO 5.9	PO 5.26	
Sulová Zuzana	PO 5.31					
Sumbalová Zuzana	PO 3.9	PO 6.8				
Sumegová Katarína	PO 6.12	PO 6.22				
Supuran Claudin	PO 2.10	PL 2				
Svetlíková Zuzana	L 9.8	PO 9.4				
Svoboda Marek	PO 1.13					
Svoboda Michal	L 7.12	PO 7.10				
Svoboda Miroslav	PO 1.13					
Svoboda Petr	PO 2.5					
Svobodová Jaroslava	PO 2.5	PO 3.10				
Svobodová Martina	PO 5.7					
Sýkorová Světlana	PO 11.8					
Szabová Eva	PO 2.30	PO 8.10				
Szabová Ľudmila	PO 13.11					
Szemes Tomáš	L 8.5	PO 1.14	PO 8.17			
Szotáková Barbora	L 5.5	L 5.9	PO 5.15			

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Šabatková Zdeňka	PO 8.12	PO 8.13

Šafařík Ivo	PO 8.12	PO 8.13	PO 8.14			
Šafaříková Mirka	PO 8.12	PO 8.13	PO 8.14			
Šajgalík Michal	PO 1.9					
Šalplachta Jiří	PO 2.2					
Šantorová Jitka	PO 4.24					
Šantrůček Jiří	PO 12.4					
Šavlík Michal	PO 5.15					
Šebeková Katarína	L 7.8					
Šebela Marek	PO 2.24					
Šebo Peter	PL 5	L 3.4	L 4.10	PO 1.7	PO 1.8	PO 3.6
	PO 9.9	PO 11.11	PO 13.6			
Šemberová Lenka	PO 2.5	PO 3.10				
Šereš Mário	PO 5.26					
Ševčíková Beatrica	L 4.7					
Ševčovičová Andrea	L 10.7					
Šída Pavel	PO 4.8	PO 10.1				
Šimkovič Martin	PO 3.7	PO 13.12				
Šimončíková Petra	PO 7.11					
Šimšová Marcela	PL 5					
Šimůnek Tomáš	L 5.6					
Šimúth Jozef	PO 2.14	PO 8.2				
Široká Romana	PO 7.2	PO 11.5				
Škodáček Igor	PO 7.3	PO 7.14				
Škrlantová Miroslava	PO 2.27					
Škultéty Ľudovít	L 2.1	PO 2.7	PO 2.9	PO 9.12	PO 9.14	
Šlais Karel	L 2.6					
Šmigáň Peter	L 3.3	L 3.6	PO 3.1	PO 3.2		
Šnirc Vladimír	PO 6.16					
Šobrová Pavlína	L 7.12					
Špániková Anna	PO 7.11					
Špániková Silvia	PO 9.11					
Španová Alena	PO 11.4					
Špinar Jindřich	PO 7.8					
Špinarová Lenka	PO 7.8					
Špuláková Veronika	L 3.4					
Šramková Zuzana	PO 8.9					
Štanclová Andrea	PO 1.4					
Štefaňáková Štefánia	PO 6.17					
Štefánik Peter	L 7.3					
Štefek Milan	PO 6.17					
Štěrba Ján	PO 9.13					
Štich Vladimír	L 11.3					
Štípek Stanislav	L 6.8	PO 4.8				

T

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SPONZORI PODUJATIA

XX. Biochemický zjazd podporili:

Hlavní sponzori:

Fisher Slovakia, s.r.o.

Immunotech a.s.

KRD, s.r.o.

K-TRADE

Lambda Life a.s.

Medesa , s.r.o.

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ĎAKUJEME !

Fisher
LABORATÓRNA TECHNIKA

Spoločnosť Fisher Slovakia spol. s.r.o. je dodávateľom širokého sortimentu laboratórnych pomôcok, prístrojov, chemikálií, a nábytku. Zákazníkom z laboratórií v školách, zdravotníctve, lekárňach, univerzitách a výskumných ústavoch, laboratórií úpravy vôd, z laboratórií v priemyselných podnikoch, ako napr. potavinársky priemysel, priemysel chémie, výroby a zpracovania plastov, sklársky a keramický priemysel, dodávame rôzny bežný materiál nutný pre denný chod laboratórií, až po vybavenie nových alebo rekonštruovaných laboratórií na kľúč.

Základom ponuky sú tieto skupiny tovaru:

Pracovné ochranné prostriedky

Pomôcky pre ochranu očí - okuliare, štíty, ďalšie základné ochranné pomôcky ako rukavice, pracovné plášte, nohavice a základný výber z hygienických a kozmetických prostriedkov.

Laboratórne sklo a porcelán

Bežné varné nádoby z borosilikátového skla, ako kadičky, banky, zábrusové diely a aparatúry, odmerné sklo ako valce, odmerné banky, pipety a byrety, fľaše na vzorky, exikátory, misky, žíhacie téglky a pod.

Drobné pomôcky z plastov, gumy a kovu

Nevyhnutné stojany a klemy, kahany, pinzety, skalpely, špachtle, lyžičky, misky z kovu. Nádoby z plastu, ako kadičky, odmerné valce a banky, misky, podložky. Výber hadíc z bežných a špeciálnych materiálov, spojky a rozbočky hadíc. Sortiment fliaš z PE a PP, kanistre. Pomôcky na prečerpávanie kvapalín, na odoberanie vzoriek kvapalín a pevných látok v teréne aj v prevádzkach.

Pomôcky pre filtráciu

Široký sortiment filtračných papierov a membránových filtrov z bežných aj špeciálnych materiálov. Nerezové aj sklenené filtračné zostavy.

Prístroje a pomôcky pre dávkovanie kvapalín

Dávkovače a zásobné fľaše, mikropipety a špičky k nim, digitálne byrety, mikrostriekačky, mechanické a elektrické nástavce pre prácu so sklenenými pipetami.

Prístroje pre ohrev a chladenie

Sušiarne, klimatické komory, inkubátory, sterilizátory, obehové termostaty, vodné kúpele, mraziace boxy, mufľové či trubicové pece.

KAVALIER
SCHOTT glass made of ideas
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Prístoje pre mechanické úpravy vzoriek

Magnetické aj hriadeľové miešadlá, trepačky, dispergátory, mlynčeky, sitá, ultazvukové prístroje, odstredivky. Vývevy membránové aj olejové, zubové aj peristaltické čerpadlá.

Meracie prístroje

Sklenené aj elektronické teplomery, vlhkomery, prístroje pre záznam teploty a vlhkosti, tlakomery, prietokomery. Technické aj analytické elektronické váhy, závažia. Elektrochemické prístroje pre meranie pH, vodivosti, rozpusteného kyslíka, elektródy, kombinované prístroje. Spektrofotometre, prístroje pre vyhodnocovanie farebnosti pevných látok. Refraktometre, polarimetre, viskozimetre. Študentské aj vedecké mikroskopy.

Aparatúry

Kombinované laboratórne prístroje alebo ich zostavy - titrátory, mineralizačné aparatúry. Rotačné odparky. Destilačné prístroje, ionomeničové aparatúry. Elektroforéza a jej príslušenstvo.

Laboratórny nábytok

Bežné aj špeciálne zostavy nábytku s dielmi z ušľachtilých, odolných materiálov. Digestory, bezpečnostné skrine, váhové stoly. Zostavy kancelárskeho nábytku s náväznosťou na design laboratórneho nábytku. Stoličky a kreslá do kancelárií aj do výrobných prevádzok.

Chemikálie

Veľmi široký sortiment bežných a špeciálnych chemikálií v čistote p.a., v malých baleniach. Normanály.

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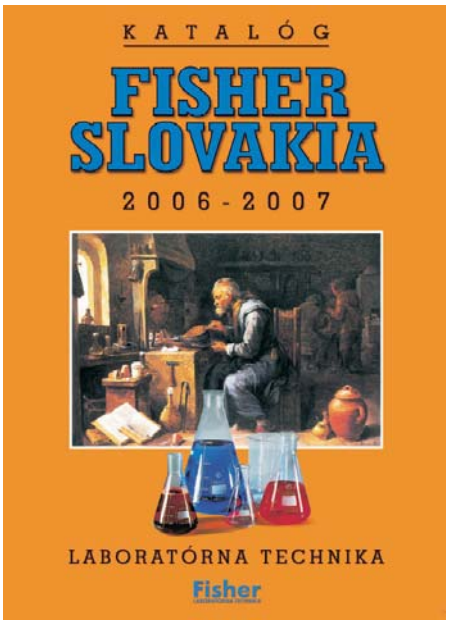


Vyžiadajte si náš aktuálny tlačený alebo elektronický katalóg LABORATÓRNA TECHNIKA 2006-2007

FISHER Slovakia, s.r.o.
Mäsiarska 13
054 01 LEVOČA

Tel.: 053 - 451 1070
Tel.: 053 - 451 0236
Fax: 053 - 469 90 08

VoIP: 069 200 2022
Skype: FisherSlovakia

e-mail: info@fisherww.sk
http://www.fisherww.sk

TRADE

www.ktrade.sk

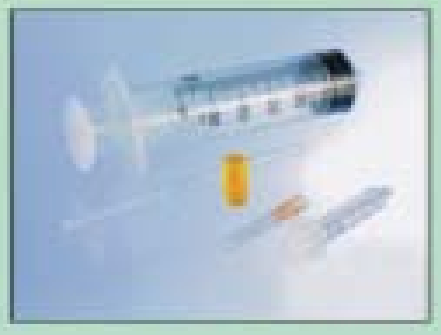
Molekulárna biológia

Bunkové kultúry

PCR diagnostika

Prístroje a zariadenia

Partner pre laboratórium



K-TRADE

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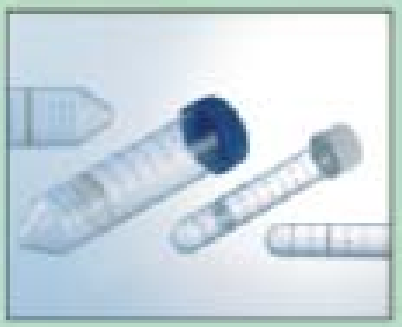
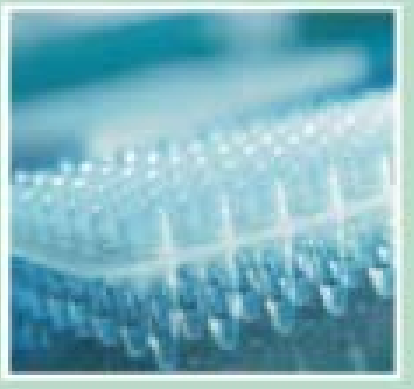


PapilloCheck® HPV-Screening

DNA-Chip for the genotyping of 24 types of genital HPV

1
Sample collection

2
DNA Extraction



3
PCR

4
Hybridisation

5
Washing

6
Scanning

7
Evaluation

Cat.No.
486 060

Platform
HTA™ Slide12

Tests per case:
kit for 60 reactions

distributor: K-TRADE

KRD molecular technologies

cbmb

Inter Active Bio Science

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MOLEKULÁRNÍ BIOLOGIE A BIOCHEMIE

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» přednáškové cykly + kurzy + interaktivní workshopy

nové trendy v:

- › genomice
- › proteomice
- › klonování
- › bioinformatice
- › bioprodukci
- › biodefense

webové prezentace:

www.interactivebioscience.cz

KRD molecular technologies

InterActive BioScience

Podzimní škola 2006

Česká společnost pro biochemii a molekulární biologii
ve spolupráci s firmou KRD s.r.o pořádají podzimní školu
pro středoškolské učitele přírodovědných oborů

Termín konání:	**22.10.-29.10.2006**
Místo konání:	Praha, ÚOCHB AV ČR
Ubytování:	**hotel Krystal,** dvoulůžkové pokoje
Stravování:	plná penze
Laboratorní praktika:	28.10.2006 na VŠCHT

Akce je hrazena grantem **MŠMT ČR** a je akreditována.

teacher.vscht.cz

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přednáškové cykly + kurzy + interaktivní workshopy

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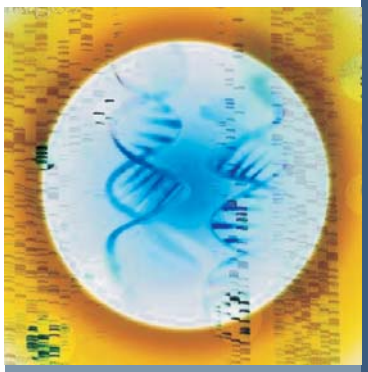
LAMBDA LIFE

SIGMA-ALDRICH Promega MILLIPORE



Lambda Life a.s.

vychutnajte si prácu z produktami
Sigma-Aldrich, Promega, Millipore v oblastiach:

genomiky

- izolácie ultračistej DNA/RNA kolonkovými a magnetickými metódami
- PCR purifikačné kity, kity na izoláciu DNA z agarózy
- restrikčné a modifikačné enzýmy
- kompletné reagencie pre PCR, RT-PCR a Real-Time PCR „Plexor“
- široké spektrum klonovacích vektorov
- systémy pre tvorbu cDNA
- transkripčné a translačné systémy
- kity pre genotypizáciu
- SNP genotypizácia
- komplexné riešenia pre analýzu fyziologického stavu buniek (apoptóza, nekróza, TUNEL)
- systémy pre analýzu aktivity jednotlivých typov kaspáz, kináz, fosfatáz, MDR, MAO, p450, DPP-IV, Calpain a proteolytickej aktivity enzýmov metódou ultrasenzitívnej luminometrie
- kity pre jednoducho modelovateľné experimenty RNA silencingu
- ultračisté chemikálie
- syntéza primerov s dodaním do 7 pracovných dní

proteomiky

- technológie na stanovenie protein-proteín (Pull Down) a proteín/DNA, resp. RNA interakcií
- reagencie na rýchlu a šetrnú izoláciu proteínov
- expresné a fúzne systémy (HIS, FLAG, GST, CAT, GFP, HaloTaq)
- sledovanie expresie, lokalizácie a dynamiky sledovaných proteínov v reálnom čase (HaloTaq)
- syntéza peptidov a tvorba protilátok na požiadanie
- detekcia mutácií v proteínoch (PTT test)
- široká paleta primárnych a sekundárnych protilátok
- reagencie na Western blot, blotovacie membrány
- ultrafiltračné centrifugačné jednotky na zahusťovanie proteínov

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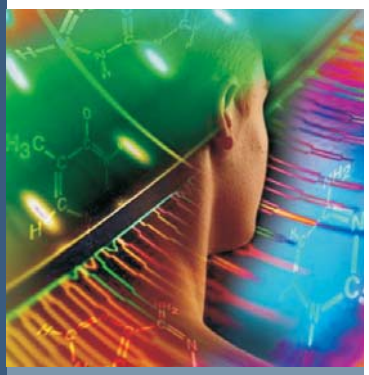

poskytujeme:

technické a aplikačné poradenstvo pre implementáciu nových laboratórnych techník, ako aj odborné semináre a prednášky.
Ich zoznam Vám zašleme na vyžiadanie.

V prípade záujmu organizujeme k jednotlivým témam workshopy a konzultácie na referenčných pracoviskách.

Kompletný filtračný materiál a sortiment prístrojov pre všetky typy laboratórií:
trepačky, vortexy, inkubátory, gél dokumentačné systémy, centrifúgy, elektroforézy, blotovacie systémy, autoklávy, váhy, pipety, termobloky, miešadlá, laminárne a PCR boxy, vodné kúpele, termocykléry, hlbokomraziace boxy, prístroje na prípravu ultračistej vody, ...

Lambda Life a.s.
Bojnická 20, 831 04 Bratislava 3, Slovenská republika
Tel.: 02/44 880 159, 44 880 160, Fax: 02/44 880 165
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Real-time bez limitů

Systémové řešení od Eppendorf

Real-time PCR reagencie

spotřební materiál

informace - kontakty

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My určujeme štandard

Našim zákazníkom dodávame kvalitné a inovatívne produkty materskej firmy s viac než 300 ročnou tradíciou - Merck KGaA Darmstadt, Nemecko.

Súčasťou našej širokej ponuky sú aj produkty a služby z portfólia ďalších renomovaných výrobcov a dodávateľov, ktorých na Slovensku dlhodobo zastupujeme.

Od vstupu na slovenský trh sa spoločnosť Merck spol. s r.o. vo svojom sektore podnikania vypracovala na spoľahlivého partnera a dodávateľa:

- laboratórnych chemikálií a reagencií,
- špeciálnych chemikálií a substancií,
- laboratórnych prístrojov a zariadení,
- priemyselných chemikálií,
- laboratórneho spotrebného materiálu.

Naši oblastní manažéri a produktoví špecialisti spolu s pracovníkmi Zákazníckeho centra Vám radi poskytnú podrobné informácie o produktoch a službách, konzultácie a poradenstvo.

Spoločnosť Merck má komplexné riadenie kvality certifikované podľa požiadaviek normy STN EN ISO 9001:2000.

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Rapid by Nature – Accurate by Design

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CEQ 8000/8800
Jeden gel - stejné kapiláry - jeden software

Genetické analyzátory a DNA sekvenátory umožňují plně automatickou analýzu od denaturace DNA po zpracování dat.

CEQ 8000/8800 využívají:
- 8 univerzálních kapilár
- Lineární polyakrylamid (patent Beckman Coulter) jako univerzální náplň
- Jednotný software na zpracování dat

CEQ 8000/8000 jsou vybaveny **2 diodovými lasery** pracujícími v NIR oblasti. Diodové lasery mají dlouhou životnost, jsou levné a energeticky úsporné. V kombinaci s fluorochromy na bázi Cy získáte při porovnání s argonovými lasery srovnatelnou citlivost za zlomek provozních nákladů.

Tyto vlastnosti umožňují **přepínání mezi aplikacemi bez zásahu operátora, bez přestavování systému.** Můžete kombinovat libovolné techniky fragmentační analýzy (STR, AFLP, LOH, MLPA …) se sekvenováním či SNP analýzou. V roce 2006 rozšíříme menu o analýzu genové exprese.

Systém **CEQ 8800** je určen pro laboratoře s větším počtem vzorků a většími nároky na průchodnost systému. CEQ 8800 má zdvojnásobenou kapacitu zásobníku (2x 96 vzorků) a vestavěnou čtečku čárových kódů. Je také vybaven softwarovým modulem Visualise pro pokročilé zpracování dat.

BECKMAN COULTER.

e-mail pro odborne dotazy BMRIOT@beckman.com a telefon pro odborné dotazy +420 267 008 513

Jak se daří vašim buňkám?

Přesvědčte se na vlastní oči

Vi-Cell™ XR Cell Viability Analyzer

Mějte buňky pod dohledem!

Vi-Cell™ Cell Viability Analyzer Vám umožní monitorovat počet buněk v kultuře, jejich životnost, velikost i distribuci velikosti a tvar buněk.

Vi-Cell™ umožňuje:

- Obrazovou analýzu buněk v rozmezí koncentrací $5x10^4$ - 10^7
- Obrazovou analýzu buněk v rozmezí velikostí 2 - 70 µm
- Pomocí distribuce velikostí rozeznat subpopulace buněk v kultuře
- S využitím trypan blue rozeznat a vyhodnotit podíl živých a mrtvých buněk
- Plně automatickou analýzu trvající 2,5 minuty

Přístroj je vybaven autosamplerem na 12 pozic. Na jednu analýzu potřebujete pouze 0,5 ml vzorku. Pokročilá zobrazovací technologie je vybavena automatickým zaostřováním, CCD kamerou o rozlišení 1394 x 1040 a dodatečným zoomem k rozlišení detailů jednotlivých buněk a jejich shluků. Systém je vybaven uživatelsky příjemným software, který umožňuje kvantitativní zpracování dat, statistické vyhodnocení, 3D grafy či sledování trendů. Data mohou být exportována jako Excelový soubor. Software vyhovuje požadavkům 21 CFR Part 11.

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Špecifikácia zariadenia na Real-Time PCR

Termocyklér - **iCycler**

- termocyklér určený pre najnáročnejších užívateľov
- vyhrievané veko s programovateľným teplotným gradientom 1-25°C
- rýchlosť ohrevu 3,3°C/sek., chladenie 2°C/sek., výnimočná presnosť a stabilita
- integrované monitorovanie teploty
- "dual-block" konfigurácia umožňujúca súbežné spustenie dvoch rôznych protokolov v dvoch nezávislých blokoch
- blok pre 96 skúmaviek 0.2 ml
- blok pre 2x48 skúmaviek 0,2 ml
- blok pre 60 skúmaviek 0.5 ml
- blok pre 384 jamkovú platničku
- ľahko prístupné grafické ovládanie, možnosť uloženia až 255 protokolov
- možnosť priamej úpravy na Real-time PCR cyklér

Real-time PCR cyklér - **iCycler iQ**

- možnosť priamej úpravy z termocykléru iCycler
- možnosť kvantitatívnej i kvalitatívnej PCR analýzy
- "sample multiplexing" - excitácia a emisia v rozmedzí vlnových dĺžok 400-700 nm
- detekcia až 5 fluorescenčných reportérov súbežne
- možnosť použitia rôznych detekčných systémov
- výkonný a ľahko ovládateľný software
- online zobrazovanie dát
- vysoká kapacita - súbežné spracovanie až 96 vzoriek
- vysoká citlivosť - schopnosť detekcie 10-10^{10} kópii sledovaného génu
- "melting curve analysis" - analýza teploty topenia

Firma Bio-Rad ďalej ponúka:

- horizontálne a vertikálne elektroforetické systémy
- imaging systémy
- zariadenie na preparatívnu chromatografiu
- kompletné vybavenie pre proteomiku a genomiku
- spotrebný materiál a reagencie

Mlynské Nivy 54

821 05 Bratislava
Tel.: 02 58265111
Mobil: 0905 531649-51

http//www.bio-rad.com

Dark Reader - Blue light transiluminátor

Clare Chemical Research

Transiluminátor využívající k vizualizaci fluorescenčně barvené DNA, RNA a proteinů viditelné modré světlo. Primárně vyvinutý pro barvičky typu SYBR (SYBR Green, SYBR Gold, GelStar, SYBRO Orange atd.). Vynikající také pro detekci GFP.

Žádné UV záření
Žádné poškození DNA dlouhou expozicí
Vyšší senzitivita než klasický UV transiluminátor
Možnost detekce in vivo

Cena 23 990 Kč + 19% DPH

DR-45 M	14 x 21 cm	**23 990 Kč**
DR-88M	22 x 25 cm	**30 625 Kč**
DR-190M	42 x 28 cm	**48 615 Kč**

UVIprochemi

Uvitec

Cena od 732 000 Kč + 19% DPH

Chemiluminescenční a fluorescenční zobrazovací systém. 12-bitová vysoce senzitivní chlazená CCD kamera, progresivní scan, 1Mpixel rozlišení 1280 (H)x 1024(V). UV transiluminátor a iluminátor s bílým světlem
Epiiluminace bílým světlem
Softwarový balík UVIchemi a UVIband

Cena od 17 300 Kč + 19% DPH

UV transiluminátory

Uvitec

Více než 30 modelů UV transiluminátorů. Různé velikosti od 15x15 až po 20x 40 cm. Vlnové délky 254 nm, 312 nm a 365 nm, také jako duální a nebo s bílým světlem.

Dokumentační systém UVIdoc

Uvitec

Cena od 143 000 Kč + 19% DPH

Jednoduchý dokumentační systém pro univerzální použití. Obrázky ve formátu TIFF nebo JPEG, připojení k síti.

Systém zahrnuje:
- Darkroom a kontrolní jednotku
- UV transiluminátor, monitor
- SONY CCD kameru, objektivy a filtry
- Termotiskárnu Mitsubishi P93
- UVIgeltec software

Uvilink CL 508 - UV crosslinker

Uvitec

Cena 32 800 Kč + 19% DPH

Programovatelný UV crosslinker
Automatický monitoring UV energie
Max. čas UV expozice: 999.9 minut
Vlnové délky: 254 nm, 312 nm a 365 nm
Vější rozměry: 350 x 350 x 300mm
Rozměry vnitřku: 300 x 270 x 140mm
Váha : 10,5 kg

Model	Description	Wavelength (nm)
CL 508S	Crosslinker shortwave	254
CL 508M	Crosslinker midrange	312
CL 508L	Crosslinker longwave	365

Dokumentační systém UVIpro

Uvitec

Vysoce kvalitní dokumentační systém s volitelným příslušenstvím. Jednoduchá obsluha. Přímé připojení k PC.

Systém zahrnuje:
• Darkroom • SONY CCD kameru (až 14-bitů) • Objektivy a filtry • UV transiluminátor • Termotiskárnu Mitsubishi P93 • Analytický software

Cena od 129 000 Kč + 19% DPH

Apollo ATC201 Thermal Cycler

Uvitec

Cena 124 000 Kč + 19% DPH

- Combi blok 96 x 0,2 ml a 48 x 0,5 ml PCR zkumavky.
- Dotykový displej.
- Malé rozměry.
- Přítlačné víko.
- Záruky 2 roky.

Cena od 99 000 Kč + 19% DPH

Dokumentační systém UVIsave

Uvitec

Flexibilní - lze použít s jakýmkoli transiluminátorem. Kompaktní, uživatelsky jednoduchý dokumentační systém.

Systém zahrnuje: Hood + kontrolní jednotku • CCD kameru • Objektivy a filtry • UVIgeltec software

Apollo ATC401 Gradientový cycler

Uvitec

Cena 105 000 Kč + 19% DPH

- Gradientový cycler - teplotní gradient 20°C
- Dotykový barevný displej
- Přítlačné víko
- Malé kompaktní rozměry
- 2 roky záruka

Cena výměnných bloků: 54 000 Kč

ATC401-B1	96 x 0,2 ml gradient block
ATC401-B2	384 well block or flat block (4 slides)
ATC401-B3	48 x 0,5 ml gradient block

UVIchemi

Uvitec

Cena od 689 000 Kč + 19% DPH

Chemiluminescenční zobrazovací systém. 12-bitová vysoce senzitivní chlazená CCD kamera, progresivní scan, 1Mpixel rozlišení 1280 (H)x 1024(V). Maximální velikost vzorku 21x18 cm. Softwarový balík UVIchemi a UVIband.

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- biologicky aktivní a fluorescenčně značené peptidy pro buněčné zobrazení a průtokovou cytometrii
- značení a detekce nukleových kyselin
- imunohistochemie a *in situ* hybridizace
- radioaktivně značené nukleotidy a aminokyseliny (^{3}**H**, ^{14}**C**, ^{32}**P**, ^{33}**P**, ^{35}**S**)
- blotting a autoradiografie
- DNA microarray, DNA chip technologie - paralelní analýza tisíců imobilizovaných genů
- genová diagnostika
- GeneFix - zkumavky pro navázání nukleových kyselin
    - příprava značených ssDNA sond
    - solid-phase sekvenace DNA
    - optimalizace a kvantifikace PCR
    - příprava značených RNA
    - izolace DNA/RNA vázajících proteinů
    - izolace mRNA
    - solid-phase kompletace genů
    - solid-phase transkripce
    - přímá/nepřímá hybridizace
- oligonukleotidy (DNA/RNA)
    - purifikace zdarma : čištění RP-HPLC je zahrnuto v ceně
    - široká nabídka modifikací : u modifikovaných nukleotidů navíc PAGE purifikace zdarma
- široký sortiment radiochemikálií

DÁLE DODÁVÁME: imunodiagnostické soupravy (RIA, FIA, LIA, EIA), luminometry a radiochemikálie pro výzkum.

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MOLEKULÁRNÍ BIOLOGIE
-kity na značení a purifikaci DNA
-detekce DNA
-in Situ hybridizace
-enzymy, membrány, filmy

BUNĚČNÁ BIOLOGIE
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-detekce a odstranění mykoplazmy
-**Cellvition** medium pro zmrazování

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Unikátní kit **Genefishing** k detekci diferenciálně exprim. genů.Systém **Forever DNA ladder** Vás zbaví nutnosti ještě někdy nakupovat DNA hmotnostní markery

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Supercitlivé kity pro značení nukleových kyselin pro použití u DNA arrayí

CYPEX
Lidské CYP koexprimované s lidskou P450 reduktázou v E.coli, protilátky

BioFx
Vysoce citlivé kolorimetrické a chemiluminiscenční substr., substráty pro enzym. detekce na membránách i v roztoku

MOBITEC
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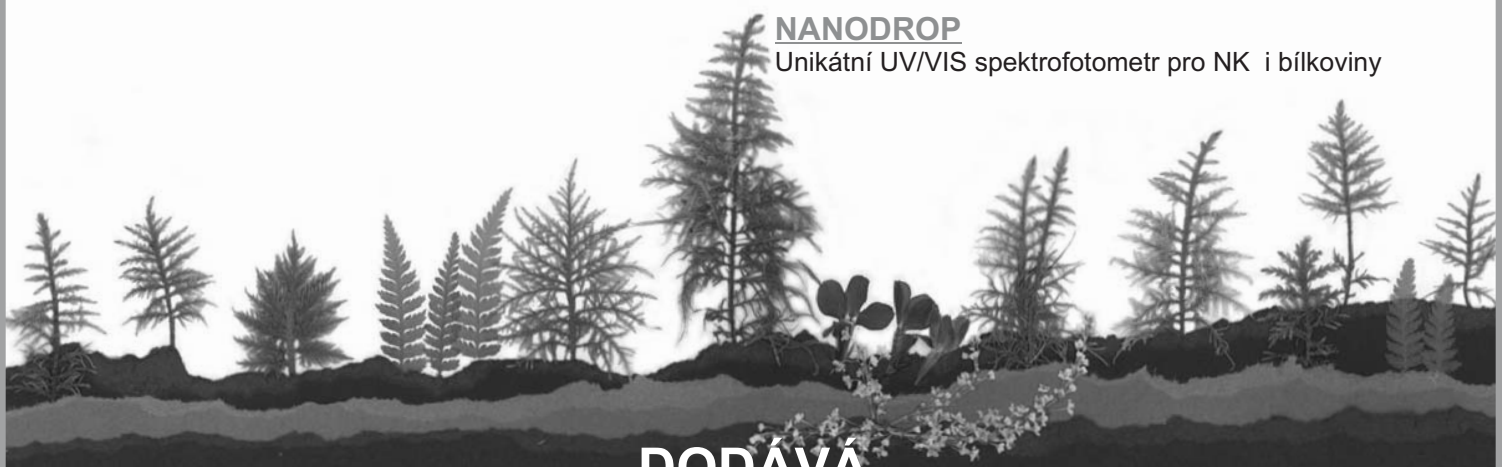

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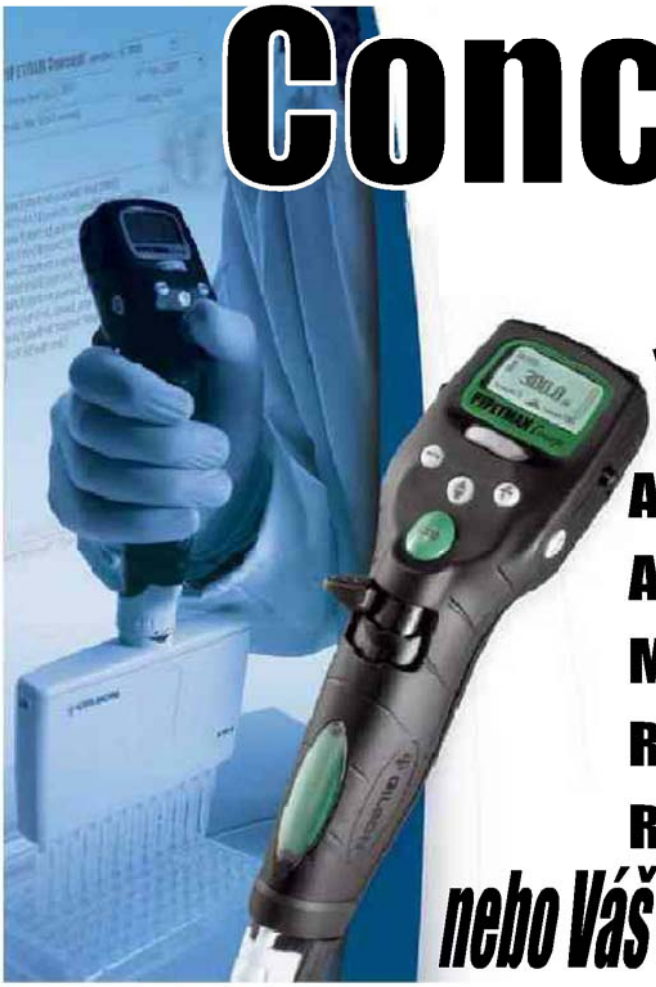

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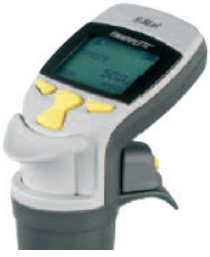

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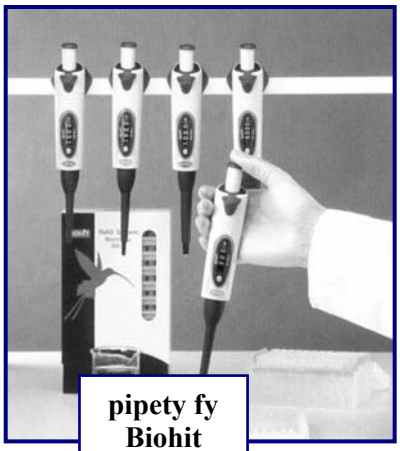
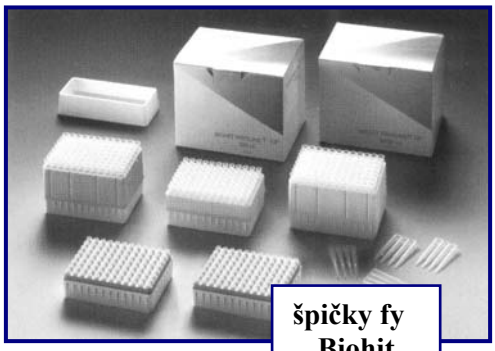
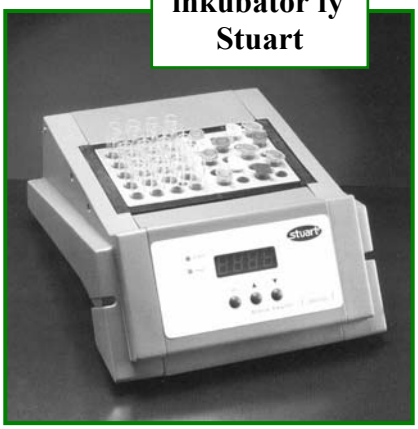


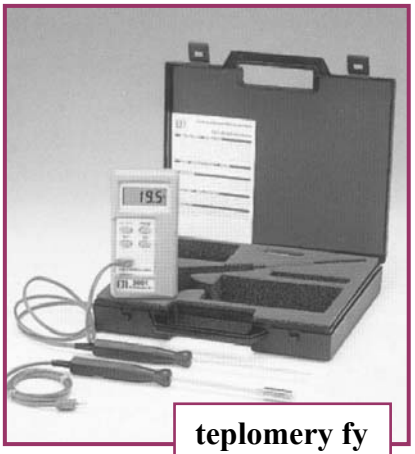
pipety fy Biohit



špičky fy Biohit

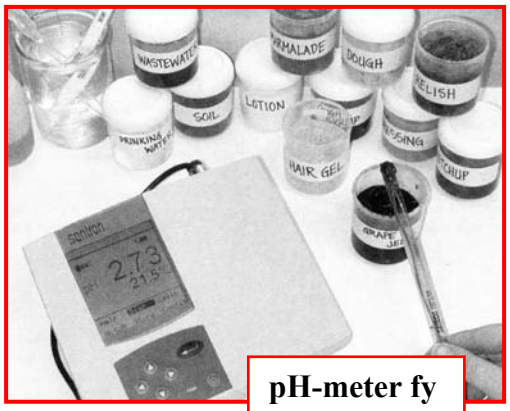
inkubátor fy Stuart





teplomery fy ETI

pH-meter fy Shindengen



pH-meter fy Sentron

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