

**UNIVERZITA KARLOVA V PRAZE,  
LÉKAŘSKÁ FAKULTA V HRADCI KRÁLOVÉ  
A  
FAKULTNÍ NEMOCNICE V HRADCI KRÁLOVÉ**

**VIII. VĚDECKÁ KONFERENCE**

**P R O G R A M**



**28. ledna 2004**

**Velká posluchárna teoretických ústavů Lékařské fakulty UK,  
Šimkova 870, Hradec Králové**

## **T e c h n i c k é   p o k y n y**

***Vědecká konference je zároveň částí oponentního řízení grantů následujících grantových agentur:***

Grantové agentury České republiky

Grantové agentury Univerzity Karlovy v Praze

Interní grantové agentury Ministerstva zdravotnictví ČR

***V programu jsou uvedeny názvy řešených projektů a jména odpovědných řešitelů.***

### ***Ústní sdělení***

1. Doba sdělení 10 minut, diskuse 5 minut.
2. K dispozici je projekce diapositivů 5 x 5 cm, zpětná projekce, video a dataprojekce.
3. Materiály k projekci se předávají nejpozději 15 minut před začátkem sekce v místnosti č. 14 vedle velké posluchárny.

**Středa 28. 1. 2004**

- 8.00 - 8.15            **Z a h á j e n í   k o n f e r e n c e**  
**prof. MUDr. Vladimír Palička, CSc., děkan lékařské fakulty**  
**doc. MUDr. Leoš Heger, CSc., ředitel fakultní nemocnice**
- Sekce I**                    **Předsedající: prof. MUDr. Vladimír Geršl, CSc.**
- 8.15 - 8.30            Studium změn regulačních proteinů myokardu za různých  
patologických stavů  
**doc. MUDr. Michaela Adamcová, Ph.D.**  
GA UK 81/02 (LF)
- 8.30 - 8.45            Význam glutaminu pro BCAA – BCKA cyklus a proteinovou bilanci  
**doc. MUDr. Milan Holeček, DrSc.**  
GA UK 78/03 (LF)
- 8.45 - 9.00            Screening a diagnostika dědičných poruch glykosylace  
**MUDr. Eliška Marklová, CSc.**  
GA UK 85/01 (LF)
- 9.00 – 9.15            Hodnocení změn exprese MRP2 na kanalikulární membráně  
hepatocytů a jejich vlivu na jaterní eliminaci metotrexatu  
**MUDr. Stanislav Mičuda, Ph.D.**  
GA UK 89/02 (LF)
- 9.15 - 9.30            Vnímání rizika a míra zdravotního uvědomění u zaměstnanců různých  
profesí  
**doc. MUDr. Jindra Šmejkalová, CSc.**  
GA UK 90/01 (LF)
- 9.30 - 9.45            Prospektivní sledování změn funkčních parametrů leukocytů periferní  
krve u nemocných s jaterními metastázami karcinomu tlustého střeva  
**MUDr. Pavel Veselý**  
GA UK 75/03 (LF)
- 9.45 - 10.00            Electrophysiological Assessment of Human Cognitive Processes  
**doc. MUDr. Miroslav Kuba, CSc.**  
Společný výzkumný projekt s Fyziologickými laboratořemi Univerzity  
v Oxfordu (contractor - Prof. Colin Blakemore): Grant James S.  
McDonnell Foundation for Cognitive Neurosciences - USA  
99-57EE-GLO.04 (LF)
- 10.00 - 10.30            ***P ř e s t á v k a - o b č e r s t v e n í***

- Sekce II**                      **Předsedající: prof. MUDr. RNDr. Miroslav Červinka, CSc.**
- 10.30 - 10.45                  Vliv acidózy na metabolismus proteinů a aminokyselin  
**doc. MUDr. Milan Holeček, DrSc.**  
GA ČR 305/01/0578 (LF)
- 10.45 - 11.00                  Sociální opora u dětí a dospívajících v psychicky náročných situacích  
**prof. PhDr. Jiří Mareš, CSc.**  
GA ČR 406/01/0659 (LF)
- 11.00 - 11.15                  Stanovení isoprenu ve vydechovaném vzduchu - interpretace vyšetření  
a zavedení do klinického výzkumu poruch metabolismu cholesterolu  
**MUDr. Radomír Hyšpler, Ph.D.**  
GA ČR 203/01/P110 (LF)
- 11.15 - 11.30                  Vliv transparence nitrooční čočky na rozlišovací schopnost oka  
**MUDr. Hana Langrová, Ph.D.**  
GA ČR 309/00/D056 (LF)
- 11.30 - 11.45                  Vliv aminokyselin a hormonů na metabolismus proteinů u  
katabolických stavů  
**doc. MUDr. Milan Holeček, DrSc.**  
IGA MZ ČR NB/6793-3 (LF)
- 11.45 - 12.00                  Imunohistochemická detekce nestinu jakožto diagnostického markeru  
v patomorfologii  
**doc. MUDr. Jaroslav Mokrý, Ph.D.**  
IGA MZ ČR NK/6727-3 (LF)
- 12.00 - 12.55                  *Přestávka - občerstvení*
- Sekce III**                      **Předsedající: doc. RNDr. Jan Krejsek, CSc.**
- 13.00 - 13.15                  Úloha metabolické, endoteliální a trombocytární dysfunkce v procesu  
atherogenesy  
**prof. MUDr. Milan Bláha, CSc.**  
IGA MZ ČR NB/6549-3 (FN)
- 13.15 - 13.30                  Studium aterogenních mechanismů při léčbě LDL-aferézou úloha  
metabolismu mastných kyselin, syntézy cholesterolu a lipoperoxidace  
**doc. MUDr. Vladimír Bláha, CSc.**  
IGA MZ ČR NB/6822-3 (FN)
- 13.30 - 13.45                  Užití nízkomolekulárního heparinu během perkutánní transluminální  
koronární angioplastiky (PTCA)  
**MUDr. Miroslav Brtko**  
IGA MZ ČR NA/6579-3 (FN)

- 13.45 - 14.00 Vliv potažení stentu na výskyt stenóz u nemocných léčených metodou transjugulární intrahepatální portosystémové spojky – TIPS – část II. Prospektivní, randomizovaná, kontrolovaná studie  
**doc. MUDr. Petr Hůlek, CSc.**  
IGA MZ ČR NA/6767-3 (LF)
- 14.00 - 14.15 Kontinuální biochemické monitorování pacientů během kardiochirurgické operace v mimotělním oběhu a v časném pooperačním období užitím intersticiální mikrodialýzy  
**MUDr. Jiří Mand'ák**  
IGA MZ ČR NB/6547-3 (FN)
- 14.15 - 14.30 Změny hemostázy při katetrizační radiofrekvenční ablaci  
**MUDr. Petr Pařízek**  
IGA MZ ČR NA/6603-3 (LF)
- 14.30 - 15.00 *P ř e s t á v k a - občerstvení*
- Sekce IV** **Předsedající: prof. MUDr. Pavel Rozsival, CSc.**
- 15.00 - 15.15 Glutaminem suplementovaná parenterální výživa při transplantaci periferních kmenových buněk u hematologických malignit  
**MUDr. Alice Poznarová**  
IGA MZ ČR NA/7000-2 (FN)
- 15.15 - 15.30 Význam monitorování metabolismu lipidů procesu stárnutí  
**RNDr. Dagmar Solichová**  
IGA MZ ČR NG/6770-3 (FN)
- 15.30 - 15.45 Fotopická elektroretinografie u diabetu  
**prof. MUDr. Jaromír Svěrák, DrSc.**  
IGA MZ ČR NK/6835-3 (FN)
- 15.45 - 16.00 Sledování a porovnání osudu biologických a syntetických materiálů implantovaných do kostních defektů  
**MUDr. Pavel Šponer**  
IGA MZ ČR ND/6853-3 (LF)
- 16.00 - 16.15 Terapeutická ovlivnění kognitivních funkcí u schizofrenie  
**MUDr. Ivan Tůma, CSc.**  
IGA MZ ČR NF/6753-3 (LF)

**U k o n ě n í k o n f e r e n c e**  
**prof. MUDr. Vladimír Palička, CSc., děkan lékařské fakulty**

**NA LF UK A VE FN V HRADCI KRÁLOVÉ SE V ROCE 2003 DÁLE ŘEŠILY  
NÁSLEDUJÍCÍ PROJEKTY**

**(abecedně podle jmen řešitelů)**

*V tomto přehledu nejsou uvedeny všechny smluvně podložené spolupráce na projektech, jejichž odpovědní řešitelé jsou z jiných organizací než z LF UK a FN v Hradci Králové.*

1. Predikace, optimalizace a individualizace výsledku léčebné procedury při LDL-aferéze - multivariantní model v prospektivní studii  
**prof. MUDr. Milan Bláha, CSc.**  
IGA MZ ČR NB/7006-3 (FN)
2. Aterogenní potenciál poruch metabolismu mastných kyselin a cholesterolu v procesu koronární aterogeneze u diabetu mellitu II. typu  
**doc. MUDr. Vladimír Bláha, CSc.**  
IGA MZ ČR NB/6999-3 (FN)
3. Zavedení gelové elektroforézy do praktických cvičení z lékařské biochemie  
**doc. MUDr. Jaroslav Cerman, CSc.**  
FRVŠ 2948/F3/03 (LF)
4. Zkvalitnění výuky ve fantomové laboratoři  
**MUDr. Daniel Černý**  
FRVŠ 2949/F3/03 (LF)
5. Závažná orgánová selhání: experimentální a klinické aspekty, možnosti prevence a terapeutického ovlivnění  
koordinátor: **prof. MUDr. RNDr. Miroslav Červinka, CSc.**  
Výzkumný záměr MSM 111500001 (LF)
6. Proteomové centrum pro studium intracelulárního parazitismu bakterií  
**prof. MUDr. RNDr. Miroslav Červinka, CSc.**  
(nositel: Vojenská lékařská akademie JEP, Hradec Králové,  
odp. řešitel: MUDr. Jiří Stulík, CSc.)  
Výzkumná centra LN00-A033 (LF)
7. Hepatologie - fyziologické, patofyziologické a klinické aspekty  
koordinátor: **doc. MUDr. Zuzana Červinková, CSc.**  
Výzkumný záměr MSM 111500003 (LF)
8. Kardiovaskulární, potenciálně kardioprotektivní a biotransformační účinky nových látek chelatujících železo  
**prof. MUDr. Vladimír Geršl, CSc.**  
GA ČR 305/03/1511 (LF)

9. AV zpracování pitev pro nové kurikulum 1. ročníku stomatologického směru  
**MUDr. Petr Hájek**  
FRVŠ 2950/F3/03 (LF)
10. Změny vybraných metabolických ukazatelů v perioperačním období  
**MUDr. Eduard Havel, Ph.D.**  
IGA MZ ČR NB/7561-3/03 (LF)
11. Morfologické a funkční vyhodnocení radikálních resekcí rekta pro karcinom  
**MUDr. Pavel Hladík, Ph.D.**  
IGA MZ ČR ND/7657-3/03 (FN)
12. Možnosti ovlivnění proteinové bilance u proteokatabolických stavů - vliv inhibitorů proteasomů  
**doc. MUDr. Milan Holeček, DrSc.**  
GA ČR 303/03/1512 (LF)
13. Biochemické markery zánětu ve vydechovaném vzduchu u dětských astmatiků jako nová metoda sledování kontroly astmatu a prostředek pro optimalizaci farmakoterapie  
**Ing. Jaroslav Chládek, Ph.D.**  
IGA MZ ČR NL/7024-3/02 (LF)
14. Expres vazivových bílkovin v jaterních lipocytech potkanů  
**RNDr. Jiří Kanta, CSc.**  
GA ČR 305/03/1513 (LF)
15. Elektrofyzilogické hodnocení věkově závislých změn zrakového vnímání člověka  
**doc. MUDr. Miroslav Kuba, CSc.**  
GA ČR 309/02/1134 (LF)
16. Srovnání účinnosti dvou aplikačních forem alergenové imunoterapie (sublinguální a subkutánní) v léčbě polinózy - v klinických a laboratorních parametrech  
**MUDr. Irena Krčmová, CSc.**  
IGA MZ ČR NI/7470-3/03 (FN)
17. Integrace výuky lékařské mikrobiologie a imunologie  
**MUDr. Jiřina Lesná, PhD.**  
FRVŠ 2952/F3/03 (LF)
18. Patogeneze, diagnostika a terapie nádorových onemocnění  
koordinátor: **prof. MUDr. Jaroslav Malý, CSc.**  
Výzkumný záměr MSM 111500002 (LF)
19. Populační modelování farmakokinetiky a farmakodynamiky v klinické farmakologii  
**prof. MUDr. Jiřina Martínková, CSc.**  
COST OC B15.10 (LF)

20. Metody predikce lékových interakcí na úrovni metabolismu s využitím metod in vitro  
**prof. MUDr. Jiřina Martínková, CSc.**  
COST OC B15.30 (LF)
21. Experimentální model Huntingtonovy chorey: Reakce subependymové vrstvy postranních mozkových komor na neurodegenerativní proces ve striatu a na fetální neurální transplantát  
**doc. MUDr. Yvona Mazurová, CSc.**  
IGA MZ ČR NF/7594-3/03 (LF)
22. Zavedení a využití systémů kultivace lidských hepatocytů pro hodnocení metabolismu léků in vitro.  
**doc. MUDr. Jaroslav Mokrý, Ph.D.**  
COST OC B15.40 (LF)
23. Buněčná terapie: Studium faktorů ovlivňujících míru osídlení tkání příjemce kmenovými buňkami.  
**doc. MUDr. Jaroslav Mokrý, Ph.D.**  
GA ČR 304/03/1515 (LF)
24. Inovace výuky obecné a speciální neurohistologie  
**doc. MUDr. Jaroslav Mokrý, Ph.D.**  
FRVŠ 2953/F3/03 (LF)
25. Zavedení histopatologické diagnostiky nádorů CNS do výuky neurochirurgie  
**prof. MUDr. Stanislav Němeček, DrSc.**  
FRVŠ 2954/F3/03 (LF)
26. Aktivační povrchové leukocytární znaky u novorozenců s rizikem časně infekce  
**doc. MUDr. Eva Pařízková, CSc.**  
IGA MZ ČR NI/7533-3/03 (LF)
27. Sledování kvality péče o nemocné s mimonemocniční oběhovou zástavou ve východočeském regionu  
**doc. MUDr. Miloslav Pleskot, CSc.**  
IGA MZ ČR NA/7254-3 (FN)
28. Využití fluorescenčních metod při výuce buněčné biologie a genetiky  
**PharmDr. Emil Rudolf, Ph.D.**  
FRVŠ 2955/F3/03 (LF)
29. Stanovení hladiny LPA u pacientek s ovariálním karcinomem  
**MUDr. Jana Sedláková**  
IGA MZ ČR NH/7666-3/03 (LF)



30. Magnetická rezonance versus thaliová scintigrafie v detekci viabilního myokardu.  
Prospektivní srovnávací studie.  
**MUDr. Miroslav Solař**  
IGA MZ ČR NA/7248-3 (FN)
31. Inovace kurikula chemie a biochemie pro studující stomatologie  
**doc. MUDr. Alena Stoklasová, CSc.**  
FRVŠ 2956/F3/03 (LF)
32. Vývoj softwarových prostředků pro práci s medicínskými informacemi, pro zpracování biosignálů, pro podporu rozhodování lékaře a pro zvýšení kvality vzdělávání  
**prof. MUDr. Pravoslav Stránský, CSc.**  
Výzkumný záměr MSM 111500004
33. Vliv růstového hormonu a glutaminu na metabolismus proteinů v izolovaném kosterním svalu  
**MUDr. Roman Šafránek – doc. MUDr. Milan Holeček, DrSc.**  
IGA MZ ČR NB/7611-3/03 (LF)
34. Program pro testování znalostí studentů počítačem  
**Ing. Pavel Šiman, CSc.**  
FRVŠ 2960/F3/03 (LF)
35. Sinus lift v dentální implantologii v souvislosti s parametry oseointegrovaných implantátů a s typem augmentačního materiálu  
**doc. MUDr. Antonín Šimůnek, CSc.**  
IGA MZ ČR NK/7711-3/03 (LF)
36. Vztah morfologie plicních žil k fibrilaci síní, se zvláštním zřetelem na izolovaný síňový amyloid  
**prof. MUDr. Ivo Šteiner, CSc.**  
IGA MZ ČR NA/7592-3/03 (LF)
37. Efektivitu biventrikulární stimulace lze hodnotit změnami neinvazivně měřené pulzové amplitudy  
**MUDr. Miloslav Tauchman**  
IGA MZ ČR NA/7261-3 (FN)
38. Návuk rekonstrukce zubní korunky při výuce stomatologické propedeutiky  
**MUDr. Dagmar Vahalová**  
FRVŠ 2959/F3/03 (LF)
39. Metabolismus cholesterolu a jeho změny u nemocných v kritickém stavu  
**prof. MUDr. Zdeněk Zadák, CSc.**  
IGA MZ ČR NB/7589-3/03 (LF)

40. Komplexní využití biomasy Amaranthu  
(nositel projektu: AMR AMARANTH, a.s., Hradec Králové)  
odp. řešitel dílčího úkolu: **prof. MUDr. Zdeněk Zadák, CSc.**  
MPO ČR FD-K2/73 (LF)
41. Vývoj tukové emulze pro parenterální výživu a technické řešení její aplikace  
(nositel projektu: Infusia, a.s., Hořátev, Sadská)  
odp. řešitel dílčího úkolu: **prof. MUDr. Zdeněk Zadák, CSc.**  
MPO ČR FD-K/033/01 (LF)
42. Ultrazvukové zobrazovací metody v praktické výuce lékařské biofyziky  
**Mgr. Jiří Záhora, Ph.D.**  
FRVŠ 2964/H/03 (LF)
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**SOUHRNÝ VÝZKUMNÝCH ÚKOLŮ  
ŘEŠENÝCH NA LF UK A VE FN V HRADCI KRÁLOVÉ  
(ABECEDNĚ)**

**Title of the research project:** Study of changes of cardiac regulatory proteins in different pathological states

**Grant Agency:** Charles University

**Project Number:** 81/2002

**Principal Researcher:** Michaela Adamcová

**Joint Researchers:** Vladimír Geršl, Martin Štěrba, Ivona Klimtová, Tomáš Šimůnek, Michal Šitina, Jiřina Hofmanová, Ludmila Koželuhová

**Starting date:** 1.1.2002

**Duration (years):** 2

**Funds allocated for project - total in Czech crowns:** 650000

#### **Summary of 2003 results**

**Title of the presentation:** Protein profiling following repeated administration of iron chelators  
**Authors:** Michaela Adamcová (1), Vladimír Geršl (2), Martin Štěrba (2), Ivona Klimtová (3), Tomáš Šimůnek (3)

Fac. Med., Charles Univ., Hr. Králové: Dept. of Physiology (1), Dept. of Pharmacology (2),  
Fac. Pharmacy, Charles Univ., Hr. Králové: Dept. of Pharmacology and Toxicology (3)

Iron chelators could be very useful in many pathological states resulting from both the iron-overload and the formation of free radicals. The protein remodeling of myocardium following repeated administration of some newly developed iron chelators (P. Poňka, McGill University, Montreal, Canada) was studied in three groups of Chinchilla male rabbits: 1) Pyridoxal isonicotinoyl hydrazone - PIH (50 mg/kg i.p. dissolved in a 10% Cremophor); 2) Salicylaldehyd Isonicotinoyl Hydrazone - SIH (50 mg/kg i.p. dissolved in a 10% Cremophor); 3) 10% Cremophor solution in water (2 mL/kg i.p.), 4) the control group (saline 1 mL/kg i.v.). Protein fractions were isolated by stepwise extraction from the samples of the left ventricle (Pelouch et al. 1995). After the isolation, SDS-PAGE was carried out; further proteins were immunoblotted onto nitrocellulose membrane and the regulatory proteins (troponin T and tropomyosin) were analysed using JLT-12 and TM 228 MAb (Sigma Chemicals).

Both the non-collagenous or collagenous proteins did not show any change following repeated administration of PIH (134.5 ± 3.8 mg/g; 139.3 ± 7.0 mg/g) or SIH (139.5 ± 4.8 mg/g; 120.8 ± 2.8 mg/g) compared with the control (145.4 ± 8.8 mg/g; 118.4 ± 7.9 mg/g) and Cremophor group (156.3 ± 11.2 mg/g; 124.8 ± 4.7 mg/g). The expression of regulatory proteins troponin T and tropomyosin was not also affected by iron chelators.

We can conclude that this observation confirmed very low cardiotoxicity of SIH and PIH declared in the previous studies which can be valuable from the standpoint of clinical use.

Reference: V. Pelouch et al.: Mol. Cell. Biochemistry 147, 43-49, 1995

Project was supported by the Charles University Grant Agency No. 81/2002.

**Address for correspondence:** Adamcová Michaela, Dept. of Physiology, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 01 Hradec Králové, Czech Republic



**Title of the research project:** The role of metabolic, thrombocytic and endothelial dysfunction in process of atherogenesis

**Grant Agency:** Ministry of Health

**Project Number:** NB/6549-3

**Principal Researcher:** Milan Bláha

**Joint Researchers:** Zdeněk Zadák, Vladimír Bláha, Jaroslav Malý, Melanie Cermanová, Martin Blažek, Radek Hyšpler, Jan Krejsek

**Starting date:** 1.1.2001

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 1483000

**Summary of 2003 results**

**Title of the presentation:** Selectins, monocyte chemotactic peptide and thrombocyte aggregability as markers of atherosclerosis activity

**Authors:** Milan Bláha , Martin Blažek , Vladimír Bláha, Jaroslav Malý, Melanie Cermanová, Jan Krejsek

Faculty Hospital, Charles University, Hradec Králové

**Methods:** We measured the levels of sE-, sP-selectin and MCP-1 in plasma before and after LDL-apheresis. Eighty readings were obtained repeatedly in 6 patients with severe familial hypercholesterolemia (FH) on long-term LDL-apheresis treatment. Our modification of platelet aggregation test was developed and 88 examinations were performed in our long-term patients (with severe familial hyperlipoproteinaemia) both before and after the apheresis. **Results:** Before procedure (mean + SD): P-selectin 204+179 ng/ml, E-selectin 32,1+33,7 ng/ml, MCP-1 323,8+121 pg/l. After the procedure: P-selectin 131,6+134 ng/ml, E-selectin 33,1+51 ng/ml, and MCP-1 200,4+15 pg/l.

**Conclusion:** Levels of P- selectin were increased in the blood of patients with FH in spite of long-term intensive extracorporeal LDL-elimination thus demonstrating the endothelial dysfunction. The levels of P-selectin and MCP-1 decrease after the hypolipidemic procedure significantly and could be used as another marker showing the effectivity of the extracorporeal LDL-cholesterol elimination (immediately post procedure), and, after further verification, may serve as a marker for regulating the therapy intensity. Our modification of platelet aggregation test in patients with severe familial hypercholesterolaemia when LDL-cholesterol level rapidly drops after extracorporeal elimination may be another marker showing immediate success of the procedure.

**Address for correspondence:** Prof.Milan Bláha, M.D., IIInd Clinic of Medicine, Faculty Hospital, Charles University, 50005 Hradec Králové, CZ

**Title of the research project:** Prediction, optimization and individualisation of therapeutic procedure in LDL-apheresis - a multivariate model in a prospective study

**Grant Agency:** Ministry of Health

**Project Number:** NB/7006-3

**Principal Researcher:** Milan Bláha

**Joint Researchers:** Zdeněk Zadák, Vladimír Bláha, Jaroslav Malý, Melanie Cermanová, Vladimír Mašín

**Starting date:** 1.1.2002

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 1482000

**Summary of 2003 results**

**Title of the presentation:** Optimization of LDL-Apheresis - a Multivariate Computerized Model

**Authors:** Milan Bláha(1) , Vladimír Mašín(2), Vladimír Bláha(2), Jaroslav Malý(2), Melanie Cermanová(2)

Fac.Med., Charles University, : Hradec Králové: Faculty Hospital (1), Kat. od Biophysic (2)

**Objectives:** LDL-apheresis is a very effective method in the treatment of resistant hypercholesterolemia when other therapy (dietary, medicamentous) fails. To maximize efficacy of the usage of LDL-adsorbers we aimed to create a multivariate computerized model.

**Methods:** LDL-apheresis was performed (after separating plasma in a continuous-flow blood separator Cobe Spectra, USA) by absorption-desorption automat ADA (Medicap, Germany) controlling the passage of plasma through a pair of columns containing Sepharose 46 carrying an anti-apoprotein B antibody (Lipopak, Pocard). Computerized model was created to control the volume and flow of plasma for optimal adsorption performance.

**Results:** Suitable software has been created, using Microsoft Excel, including HTML version. User loads the data and based on the results of calculation changes optimal plasma flow through separate columns to gain the expected goal. On the contrary to the previously used empiric method a shortening of the procedure has been reached, which has both positive medical and financial consequences.

**Conclusion:** Software for calculation based on the above presumption has been created and validated in the first procedures. It enables to rationalize setting of LDL apheresis, with maximal efficacy of absorption columns. Recently, the software is being tested in a larger number of procedures.

Supported by the grant IGA MH CZ NB/7006-3.

**Address for correspondence:** Prof.Milan Bláha, M.D., IIInd Clinic of Medicine, Faculty Hospital, Charles University, 50005 Hradec Králové, CZ

**Title of the research project:** Study of atherogenic mechanisms during treatment with LDL-apheresis: the role of fatty acid metabolism, cholesterol synthesis and lipoperoxidation

**Grant Agency:** Ministry of Health

**Project Number:** NB/6822-3

**Principal Researcher:** Vladimír Bláha

**Joint Researchers:** MUDr. Pavel Vyroubal, RNDr. Dagmar Solichová, MUDr. Radomír Hyšpler, prof. MUDr. Zdeněk Zadák, CSc., prof. MUDr. Milan Bláha, CSc., RNDr. Petr Žďánský, CSc.

**Starting date:** 1.1.2001

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 2500000

#### **Summary of 2003 results**

**Title of the presentation:** Cholesterol turnover during aggressive hypolipidemic intervention

**Authors:** Vladimír Bláha (1), Pavel Vyroubal (1), Dagmar Solichová (1), Radomír Hyšpler (1), Petr Žďánský (1), Zdeněk Zadák (1), Milan Bláha (2) Faculty Hospital, Charles Univ., Dept. Gerontol. Metabol. Care (1), Dept. Haematology (2)

To determine effect of low density lipoprotein (LDL) reduction on cholesterol biosynthesis and absorption, we studied the changes in cholesterol precursor lathosterol and of markers of its absorption as sitosterol and campesterol in patients with familial hypercholesterolemia undergoing LDL-apheresis. Seven patients had blood taken immediately before and after LDL-apheresis, and afterwards on days 3, 7 and before next LDL-apheresis (day 17,5 + 1,6). Blood samples were analyzed using gas chromatography/mass spectrometry. LDL-apheresis significantly reduced plasma cholesterol (7,8+0,7 mmol/l vs. 2,7+0,2 mmol/l, p=0,025). Plasma lathosterol decreased (10,5+3,5 mmol/l vs. 3,8+1,0 mmol/l, p=0,025) and correlated with the decreased cholesterol (p<0,001), however lathosterol/cholesterol did not change significantly during the study (p=0,778). The sitosterol after LDL-apheresis was significantly decreased (21,0+5,0 mmol/l vs. 11,8+2,7 mmol/l, p<0,001), and reached pre-treatment values before next LDL-apheresis (15,6+2,0 mmol/l, p=0,771). Plasma sitosterol/cholesterol non-significantly increased after LDL-apheresis (3,3+1,0 mmol/l/mmol/l vs. 4,9+1,3 mmol/l/mmol/l, p=0,095), but decreased afterwards (2,2+0,4 mmol/l/mmol/l, p=0,046). Plasma campesterol decreased (25,8+4,2 mmol/l vs. 11,6+1,9 mmol/l, p<0,001), and reached pre-treatment values before next LDL-apheresis (22,3+2,9 mmol/l, p=0,936). Campesterol/cholesterol ratio did not change significantly (p=0,256). The correlation of lathosterol/cholesterol and sitosterol/cholesterol was not significant (p=0,979). We conclude that aggressive lipid lowering procedure with LDL-apheresis has been associated with significant changes in cholesterol homeostasis. Transient increase of intestinal cholesterol absorption preceded its prolonged suppression post LDL-apheresis, and was independent of cholesterol biosynthesis.

**Address for correspondence:** V. Bláha, Department of Gerontology and Metabolic Care, Faculty Hospital, Charles University, Sokolská 581, 50005 Hradec Králové, Czech Republic





**Title of the research project:** Atherogenic role of disorders of fatty acid and cholesterol metabolism in coronary atherogenesis in diabetes mellitus type II

**Grant Agency:** Ministry of Health

**Project Number:** NB/6999-3

**Principal Researcher:** Vladimír Bláha

**Joint Researchers:** MUDr. Dušan Černohorský, RNDr. Dagmar Solichová, RNDr. Petr Žďánský, CSc., MUDr. Pavel Vyroubal, MUDr. Radomír Hyšpler, PhD, prof. MUDr. Zdeněk Zadák, CSc.

**Starting date:** 2002

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 1800000

#### **Summary of 2003 results**

**Title of the presentation:** Biomarkers of risk from stabile coronary atherosclerosis

**Authors:** Vladimír Bláha (1), Dušan Černohorský (1), Dagmar Solichová (1), Radomír Hyšpler (1), Petr Žďánský (1), Pavel Vyroubal (1), Zdeněk Zadák (1), Faculty Hospital, Charles University, Department of Gerontology and Metabolic Care (1)

Patients with stabile angina pectoris were stratified based on the severity of arterial occlusion, measured by angiography to study correlation with the oxidative stress markers. Glycemia, BMI, serum lipoproteins i.e. total cholesterol, very-low-density lipoprotein (VLDL) cholesterol, intermediate-lipoprotein-density (IDL) cholesterol, low-density-lipoprotein (LDL) cholesterol and high-density-lipoprotein (HDL) cholesterol, nor the total triacylglycerols and triacylglycerols in VLDL, IDL, LDL and HDL did not differ significantly between groups 1, 2, 3 or 4 and approached the target values. In our study, serum TBARS did not differ significantly between groups 1, 2, 3 or 4, and did not correlate with the degree of CAD. Thus TBARS was not an exact parameter of lipid peroxidation in stable angina pectoris. These results are in contrast with significantly higher TBARS in the group of patients with unstable angina, who were compared to stable angina or controls. Multiple brief myocardial ischemic episodes are known to be associated with the production of free radicals, and, hence, the consumption of the chain-breaking antioxidants of plasma and elevation of plasma TBARS. There were not any significant differences in serum MUFA and SUFA. PUFAs with a high degree of unsaturation of the n-6 and n-3 series could accelerate cell-mediated LDL peroxidation and thus aggravate the atherosclerotic process. In our study, LDL-PUFAs did not statistically differ significantly between groups 1, 2, 3 or 4. However, there was significant correlation of TBARS with LDL-linoleic acid ( $p=0,01$ ), and HDL-linoleic acid ( $p=0,02$ ). As TBARS are proportional to lipid peroxide-derived malonyldialdehyde and are a measure of lipid peroxidation, the above findings relate to the oxidation hypothesis of coronary atherosclerosis.

**Address for correspondence:** V. Bláha, Department of Gerontology and Metabolic Care, Faculty Hospital, Charles University, Sokolská 581, 50005 Hradec Králové, Czech Republic

**Title of the research project:** The use of low-molecular weight heparin during percutaneous coronary angioplasty (PTCA). Laboratory and clinical evaluation.

**Grant Agency:** IGA, Ministry of Health

**Project Number:** NA 6579-3

**Principal Researcher:** Miroslav Brtko

**Joint Researchers:** Ivo Varvarovsky, Pavel Polansky, Miroslav Pecka, Viera Dytrychova

**Starting date:** 1.1.2001

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 1472000

#### **Summary of 2003 results**

**Title of the presentation:** Unfractionated heparin vs. dalteparin during percutaneous coronary interventions. The final results of a randomized study.

**Authors:** Miroslav Brtko (1), Ivo Varvarovsky (2), Pavel Polansky (1)

Charles University, University Hospital, Dept. of Cardiac Surgery, Hradec Králové (1), Krajska nemocnice Pardubice, Kardio-Troll s.r.o. (2)

Background: Unfractionated heparin (UFH) commonly used during percutaneous coronary interventions (PCI) can activate platelets. The low-molecular weight heparins (LMWH) lack this effect. The aim of this study was to compare the effect of UFH and LMWH – dalteparin – during PCI in patients with stable angina pectoris. Methods: UFH in the dose of 100 IU/kg of body weight or dalteparin in the dose of 80 IU/kg were given intravenously to 340 patients in a randomized study. The clinical course was studied in all patients. The aggregation response of platelets, levels of betathromboglobulin (beta-TG), thromboxan B2 (TxB2), trombin-antithrombin complex (TAT), prothrombin degradation products (F1, F2) and the platelet count before and after PCI were studied in 100 patients. Results: 5 minutes after the administration of UFH the platelet aggregation was higher (compared to dalteparin) after the induction by epinephrine ( $p < 0,01$ ), thrombin and arachidonic acid (both  $p < 0,05$ ). The level of beta-TG decreased 5 minutes after dalteparin bolus from 78,3 to 63,1 ( $p < 0,01$ ), after UFH bolus from 75,9 to 71,2 IU/ml (NS). There was no change in the level of TxB2 in both groups. The level of TAT decreased 5 hours after the administration of dalteparin from 14 to 8,6 mikrog/l ( $p < 0,05$ ), the level of F1,F2 increased after the administration of UFH from 1,1 to 1,6 nmol/l ( $p < 0,05$ ). Platelet count decreased after the PCI in both groups (both  $p < 0,001$ ). Conclusions: The activation of platelets is less pronounced after dalteparin administration. 5 hours after the UFH administration but not after the dalteparin administration the reactivation of coagulation cascade was detectable. Both UFH and dalteparin caused significant decrease of platelet count. The potential advantages of dalteparin have not influenced the clinical course.

**Address for correspondence:** Miroslav Brtko, University Hospital, Dept. of Cardiac Surgery, Sokolska 481, 500 05 Hradec Kralove, Czech Republic, brtkom@seznam.cz

**Title of the research project:**

Introduction of gel electrophoresis into biochemical practical classes

**Grant Agency:** Ministry of Education**Project Number:** 2948/03**Principal Researcher:** Jaroslav Cerman**Joint Researchers:** Alena Stoklasová, Petr Šuba**Starting date:** 1. 1. 2003**Duration (years):** 1**Funds allocated for project - total in Czech crowns:** 139000**Summary of 2003 results****Title of the presentation:** Introduction of gel electrophoresis into biochemical practical classes**Authors:** Jaroslav Cerman, Alena Stoklasová, Petr Šuba

Dept. of Medical Biochemistry, Charles University, Faculty of Medicine in Hradec Králové

The aim of this project was to set up the system for gel electrophoresis (A.L. Instruments, SEBIO) and introduce a set of methods based on a separation of proteins into biochemical practical classes. This innovation was important because of replacement of obsolete and long-lasting paper electrophoresis of serum proteins and thin layer chromatography of lipoproteins. Three methods were modified for reason of practical training: Separation of serum proteins, separation of serum lipoproteins and separation of lactate dehydrogenase isoenzymes. Isoenzyme separation represents a new educational approach that allows better understanding of the importance of both enzymes and isoenzymes in clinical practice. The manuals for students were prepared (in Czech and English versions). These methods are introduced in summer term of the academic year 2003/2004 and in the next academic year 2004/2005.

Ministry of Education supported the project, No. 2948/2003.

**Address for correspondence:** Jaroslav Cerman, Dept. of Medical Biochemistry, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic. E-mail: cerman@lfhk.cuni.cz

**Title of the research project:** Dental phantom laboratory quality improvement

**Grant Agency:** FRVS

**Project Number:** 2949/2003

**Principal Researcher:** MUDr. Daniel Černý

**Joint Researchers:** MUDr. Petr Bednar

**Starting date:** 2003

**Duration (years):** 1

**Funds allocated for project - total in Czech crowns:** 164000

**Summary of 2003 results**

**Title of the presentation:** Dental phantom laboratory quality improvement

**Authors:** MUDr. Daniel Cerny

The project "Dental phantom laboratory quality improvement" is an individual step in sequence of modernization and increase of work effectivity during pragradaul dental education in Hradec Kralove. The sequence of changes and improvements has the goal to make preclinical education as real and similar to clinical practice as possible. Technical development and new assessment protocol should prepare students better for clinical practice and therefore spare time in tight pregradual dental curriculum. All of this is partially caused by the influence of EU commission.

Actual steps realized during this project were:

1. Aquisition of 20 pcs of friction grip handpieces for more precise preparation process.
2. Aquisition of sharpening set
3. Service for 2 handpieces and digital camera.

**Address for correspondence:** daniel.cerny@centrum.cz

**Title of the research project:** Proteome centre for the study of intracellular parasitism

**Grant Agency:** Ministry of Education

**Project Number:** LN00AO3

**Principal Researcher:** Jiří Stulík

**Joint Researchers:** Miroslav Červinka, Jan Krejsek, Emil Rudolf, Hana Andělová, Zdeněk Fiedler, Monika Holická, Jana Havlasová, Martina Loudová, Jakub Novosad

**Starting date:** 1.1.2000

**Duration (years):** 2004

**Funds allocated for project - total in Czech crowns:** 2550000

**Summary of 2003 results**

**Title of the presentation:** Advances in understanding of intracellular parasitism of *Francisella tularensis*

**Authors:** M. Červinka (1), J. Krejsek (2), E. Rudolf (1), H. Andělová (1), Z. Fiedler (1), M. Holická (2), J. Havlasová (2), M. Loudová (2), J. Novosad (2), Fac. Med., Charles Univ., Hr. Králové: Dept. of Medical Biology and Genetics (1), Dept. of Clinical Immunology (2)

The interaction between J774.2 macrophage cell line and *Francisella tularensis* was analyzed. The new approach including MFI calculation and Kolmogorov-Smirnov analysis was used. J774 cells were stimulated by either LPS or interferon gamma in the absence of *F. tularensis*. The rapid increase in the expression of CD54 surface molecule was found. The expression of CD16/CD32 is even higher and is time and dose dependent. The similar changes were found also in the expression of CD86 costimulatory molecule. The increase in the expression of surface molecules is followed by increase in the production of NO. Infection of cell line by *F. tularensis* alone has only limited effect on the expression of examined surface markers. But there is an important influence of previous *F. tularensis* infection on the stimulation of J774 cells by LPS. There is the loss of ability of infected J774 cells to express functionally important molecules. In contrast, the stimulatory effect of interferon gamma was only minimally affected by previous *F. tularensis* infection.

Intracellular trafficking of engulfed *F. tularensis* was analyzed by means of isopycnic centrifugation. Fractions of interest (i.e. those sharing both phagosomal and bacterial markers) were subjected to 2-D electrophoresis for comparative analysis and mass spectrometry to identify proteins expressed during the process of phagocytosis. 13 proteins unique for samples from infected cells were found. 10 out of 13 these proteins were identified using MS technique. All of them were of eukaryotic origin and mostly mitochondrial.

**Conclusion:**

Our data suggest that *F. tularensis* is able to manipulate with innate immunity cell signalling to escape immune response. The contribution of specific cellular immunity mediated by interferon gamma production is very likely crucial to eliminate *F. tularensis* infection.

**Address for correspondence:** M. Červinka, Dept. Med. Biol., Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Rep.



**Title of the research project:** Serious organ failures, experimental and clinical aspects, possibilities for prevention and therapeutic management

**Grant Agency:** Ministry of Education

**Project Number:** MSM111500001

**Principal Researcher:** Miroslav Červinka

**Joint Researchers:** Jaroslav Cerman, Ivo Dřížhal, Vladimír Geršl, Jiří Horáček, Miroslav Kuba, Yvona Mazurová, Naďa Jirásková, Zbyněk Vobořil, Pavel Živný

**Starting date:** 1.1.2000

**Duration (years):** 4

**Funds allocated for project - total in Czech crowns:** 3547000

#### **Summary of 2001 results**

**Title of the presentation:** Serious organ failures - in vitro and in vivo models

**Authors:** M. Červinka (1), J. Cerman (2), I. Dřížhal (3), V. Geršl (4), J. Horáček (5), M. Kuba (6), Y. Mazurová (7), N. Jirásková (8), Z. Kaška (9), P. Živný (10)

Fac. Med., Charles Univ., Hr. Králové, Depts: Med. Biol. (1), Med. Biochem. (2), Stomatol (3), Pharmacol. (4), Microbiol. (5), Path. Physiol. (6), Histology (7), Ophthalmol. (8), Surgery (9), Clin. Biochem. (10)

In this multidisciplinary project the following topics were studied during 2003:

1) The role of apoptosis in pituitary adenomas in the field of conventionally used therapeutic approaches on in vitro and in vivo models (pituitary adenoma cells, leukemic cell line, epithelial cells) using immunocytochemical detection of the specific markers of apoptosis (activated caspases -3,-7, and -9, antigen M30, and PARP) and proliferation index (Ki 67). Experimental study showed pro-apoptotic effect of ionizing radiation and etoposide. 2) The role of intracellular zinc in modulation of death of Hep2 and pituitary cells. 3) The protein remodeling of the left ventricle following administration of daunorubicin and dexrazoxane in rabbits. 4) Verification of a diagnostic value of enlarged set of visual evoked potentials in patients with multiple sclerosis - comparison of sensitivity of evoked potentials and MRI. Motion onset VEPs contribution to brain pathology detection. 5) Infiltrative and invasive growth of foetal neural grafts into the host striatum in an animal model of Huntington's disease. 6) Assessment of the long-term results with the pre-rolled MemoryLens enable us to recommend it for routine use in cataract surgery. 7) Bacterial complications after organ and cell transplantations. 8) Relationships of biochemical markers to surgical trauma, the serum leptin, albumin, haptoglobin, and CRP concentrations demonstrated serious perioperative dynamics, these concentrations are stabilized the 5th postoperative day. 9) Monitoring blood flow and analyte concentrations in an interstitium of different tissues by microdialysis. 10) Evaluation of the efficiency of the initial therapy of inflammatory affections of the periodontium.

Project was supported by the Ministry of Education Research Project, No. MSM 111500001.

**Address for correspondence:** M. Červinka, Dept. Med. Biol., Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Rep.





**Title of the research project:** Hepatology - physiological, pathophysiological and clinical aspects

**Grant Agency:** Ministry of Education

**Project Number:** MSM 11150003

**Principal Researcher:** Zuzana Červinková

**Joint Researchers:** Petr Hůlek, Helena Živná, Jiří Kanta

**Starting date:** 1.1.1999

**Duration (years):** 6

**Funds allocated for project - total in Czech crowns:** 3075000

**Summary of 2003 results**

**Title of the presentation:** Study of acute and chronic liver injury of various etiology in animal experiments and clinical practice

**Authors:** Zuzana Červinková (1), Petr Hůlek (2), Helena Živná (1), Jiří Kanta (3), Halka Lotková (1), Otto Kučera (1)

Fac. Med., Charles Univ., Hr. Králové: Dept. Physiology (1), 1st Dept. Internal Med. (2), Dept. Med. Biochem. (3)

The research project continued according to the plan. Following topics were studied during the last year:

a) Detailed protocol for the evaluation of energy metabolism in permeabilized hepatocytes describing the experimental schemes for oxygraphic evaluation (with the aid of high-resolution oxygraphy) of the individual complexes of the respiratory chain and methods for the assessment of the mitochondrial phosphorylation using the respiratory control index and oligomycin inhibitory effect on ADP-activated respiration has been prepared (Acta Med Suppl., 45 (2): 65-76, 2002).

b) Model of thioacetamide injury in primocultures of rat hepatocytes has been introduced.

c) Using common tests of hepatocyte functions in tissue culture complemented by morphological evaluation and by estimation of mitochondrial function (mitochondrial transmembrane potential measured by accumulation of rhodamine 123) and energy metabolism (high-resolution oxygraphy) the protective effect of S-adenosylmethionine against thioacetamide injury has been documented.

d) Protective effect of lipid emulsions against hepatotoxic action of D-galactosamine in in vivo condition has been documented (Physiol. Res. 52:73-78, 2003).

e) Expression of mRNA for IL-6 has been studied in patients suffering of different liver diseases. These expression has been found in patients with liver fibrosis or cirrhosis but not in patients with liver steatosis.

**Address for correspondence:** Z. Červinková, Department of Physiology, Charles Univ. in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic



*Title of the research project:*

*Grant Agency:*

*Project Number:*

*Principal Researcher:*

*Joint Researchers:*

*Starting date:*

*Duration (years):*

*Funds allocated for project - total in Czech crowns:*

*Summary of 2003 results*

*Title of the presentation:*

*Authors:*

*Address for correspondence:*

*Title of the research project:*

*Grant Agency:*

*Project Number:*

*Principal Researcher:*

*Joint Researchers:*

*Starting date:*

*Duration (years):*

*Funds allocated for project - total in Czech crowns:*

*Summary of 2003 results*

*Title of the presentation:*

*Authors:*

*Address for correspondence:*

**Title of the research project:** VZOR Tissue specific culture models of cell death in vitro  
VZOR

**Grant Agency:** Charles University

**Project Number:** 247/01

**Principal Researcher:** Miroslav Červinka

**Joint Researchers:** Jaroslav Koupil, Zuzana Červinková, Vladimír Geršl, Vladimír Půža, Jiřina Martínková, Jindra Šmejkalová, Zbyněk Vobořil, Vladimír Palička, Zdeněk Nožička

**Starting date:** 1. 1. 2001

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 620000

#### **Summary of 2003 results**

**Title of the presentation:** Cell death induced in vitro by new cardiotoxic agents

**Authors:** Vladimír Geršl (1), Miroslav Červinka (2), Jaroslav Koupil (3), Zuzana Červinková (4), Jiřina Martínková (1), Jindra Šmejkalová (5), Zbyněk Vobořil (6), Vladimír Palička (7)

Fac. Med., Charles Univ., Hr. Králové: Dept. of Pharmacology (1), Dept. of Medical Biology and Genetics (2), Dept. of Dentistry (3), Dept. of Physiology (4), Dept. of Hygiene and Preventive Medicine (5), Dept. of Surgery (6), Dept. of Clin. Biochemistry (7)

Cell death is the most important parameter in toxicological studies. Nevertheless, the mechanism of toxic cell death is not fully understood. It is generally accepted that there are two basic types of cell death: apoptosis and necrosis. Recent data indicate that the situation is more complicated and probably several subtypes of cell death could be distinguished. In our recent study we can ascertain if the type of toxic cell death is tissue specific or not. It is probable that the final stages of cell death are very similar in all cell types. Nevertheless, the dynamics of cell death could be different in different tissue types. Therefore we used time-lapse video-recorder Mitsubishi HS-S5600E to record the dynamics of cell death after the treatment with several model drugs in different specific culture models. Until now we have studied cell death in human epitheloid cell line Hep2, mouse fibroblast line L929, human promyelocytic line HL-60 and human colorectal carcinoma cell line HT 29. Our model drugs include cytostatics (Cisplatin), detergent (TWEEN20), ethanol, hydrogen peroxide, TNF-alpha, and actinomycin D. It seems that xenobiotic-induced cell death vary in great extent in different cell types, and even in one treated cell population we could find cells in various stages of dissimilar types of cell death. This complicates the correlation with published biochemical data.

Literature: J. Palička et al.: J. Clin. Biochem.7, 45-52, 1976

Project was supported by the Charles University Grant Agency, No 247/01

**Address for correspondence:** M. Červinka, Dept. of Medical Biology and Genetics, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic

**Title of the research project:** Cardiovascular, Potentially Cardioprotective and Biotransformational Effects of New Iron Chelating Agents.

**Grant Agency:** Czech Republic

**Project Number:** 305/03/1511

**Principal Researcher:** Vladimír Geršl

**Joint Researchers:** Přemysl Poňka, Eva Kvasničková, Michaela Adamcová, Yvona Mazurová, Jana Kaplanová, Martin Štěřba, Tomáš Šimůnek, Eva Čermáková, Ivona Klimtová

**Starting date:** 1. 2. 2003

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 3445

#### **Summary of 2003 results**

**Title of the presentation:** The effects of new iron chelator (halogenated ortho-derivative of pyridoxal benzoyl hydrazone) on selected parameters of cardiac function in rabbits in vivo.

**Authors:** M. Štěřba (1), J. Kaplanová (2), M. Adamcová (3), Y. Mazurová (4), T. Šimůnek (5), E. Čermáková (6), V. Geršl (1), P. Poňka (7).

Charles Univ.: Fac. Med., Hr. Králové: Dept. of Pharmacol. (1), Dept of Pediatrics (2), Dept. of Physiol. (3), Dept.of Histol. Embryol. (4), Computer Divis. (6); Fac. Pharm., Hr. Králové: Dept.of Pharmacol. (5); McGill Univ., Montreal, Canada: Dept. Physiol. Med. (7).

New halogenated ortho-derivative of pyridoxal benzoyl hydrazone (o-chlorobenzoyl hydrazone; substance o-108 - developed by Ponka et al.) is a lipophilic chelator with high iron-chelating activity. Thus, from the standpoint of its possible indications, the data about its influence on the cardiovascular system in vivo are essential. The effects of repeated administration of three doses of o-108 PBH (25, 50 and 100 mg/kg i.p., once weekly, 10 weeks; partially dissolved in a 10% Cremophor solution) were investigated in three groups of rabbits. The obtained values were compared with data from the control group (saline, 1 ml/kg, i.v.) and the Cremophor group (10% Cremophor solution, 2 ml/kg, i.p.). The effects of chelator on cardiovascular parameters (left ventricular ejection fraction /LV EF/, blood pressure, dP/dtmax., heart rate) were investigated. Mostly no significant and only slight changes were observed following the administration of any dose of the chelator (e.g., LV EF in the 100 mg/kg group was 65.6 % at the beginning and 65.7 % at the end of experiment; in the 50 mg/kg group 65.0 % and 63.8 %, resp.; only mild changes in the blood pressure were present). Moreover, another parameter of cardiac contractility (dP/dtmax., measured at the end of experiment) did not significantly differ between the controls (1245 kPa/s) and the chelator groups (e.g., in the 100 mg/kg group - 1357 kPa/s). Thus, the results can be considered promising from the standpoint of further investigation and possible use of the chelators.

Supported by Grant GA CR No. 305/03/1511.

**Address for correspondence:** V. Geršl, Dept. of Pharmacology, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Rep.





**Title of the research project:** Audio-visual elaboration of dissections for the purposes of new 1st year dental curriculum

**Grant Agency:** Ministry of Education

**Project Number:** 2950/2003

**Principal Researcher:** Petr Hájek

**Joint Researchers:** Olga Procházková, Blanka Pospíšilová

**Starting date:** 1.1.2003

**Duration (years):** 1

**Funds allocated for project - total in Czech crowns:** 169000

**Summary of 2003 results**

**Title of the presentation:** Audio-visual elaboration of dissections for the purposes of new 1st year dental curriculum

**Authors:** Petr Hájek, Olga Procházková, Blanka Pospíšilová

Fac. Med., Charles Univ., Hr. Králové: Dept. of Anatomy

Innovation of dental curriculum brought up the urge to introduce new educational methods and tools into anatomy teaching. Our goal was to create a set of presentations which document dissection of cadaver step by step, covering basic regional, topographic, systematic and functional aspects and so attempt to substitute the unique practical experience as the dissection is.

Anatomical dissection was provided on the regularly donated body. Gradually conducted anatomical dissection of the cadaver and several specimens of the head and neck was taped by digital camcorder Panasonic NV-DS 65. Acquired digital record was processed with the use of up-graded computer equipped with software Pinnacle studio DV V8. Digital recorded material was used for the production of following educational films: Dissection of the head, Dissection of the neck, Dissection of the upper limb, Important topographic regions of the lower limb, Situs viscerum thoracis, Situs viscerum abdominis. Specimens of the head were additionally photographed, described, framed and used as educational tool for students of the 1st and 3rd year of dental curriculum and also for students of the 1st year of general medicine. These documents are also available on departmental www pages ([www.lfhk.cuni.cz/anatomy](http://www.lfhk.cuni.cz/anatomy)) for self study purposes. Camrecorder was also used for filming several unique professional lectures and demonstrations. Specimens and their photo-documentation have been used during this academic year for teaching and learning purposes of 1st and 3rd course of dental curriculum and also 1st year of general medicine curriculum. They serve also for self study purposes of all study specialization groups in regular classes as well as for the self study purposes.

Project was supported by the Ministry of Education Grant Agency, No 2950/2003.

**Address for correspondence:** P. Hájek, Department of Anatomy, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic



**Title of the research project:** The metabolic changes during plan surgery procedure period

**Grant Agency:** Ministry of Health

**Project Number:** NB/7561-3

**Principal Researcher:** Eduard Havel

**Joint Researchers:** Milan Kaška, Martin Motyčka, Radomír Hyšpler, Dagmar Solichová, Zdeněk Zadák

**Starting date:** 2003

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 489000

**Summary of 2003 results**

**Title of the presentation:**

**Authors:**

New informations for racional indication of expensive parenteral nutrition support are needed. Tolerancy of short term perioperative starvation or arteficial nutritional support indication depends on surgery plan procedure and nutritional state of patient. Short term nutritional support before surgery procedure improves result of surgery inspite usual nutritional markers are not improved (body weight, muscle strength, plasmatic protein level).The aim of the study is to describe changes of some metabolic markers (plasmatic level of insuline like growth factor I - IGF-I, insulin, non-cholesterol steroles, cholesterol and its subfractiones, apolipoproteins A-I and B) during different nutritional perioperative strategy: 1. parenteral nutrition for 3 days before, during and after operation, 2. early gastric support after surgery operation, 3. without nutritional support with short several day prolonged period of starvation. The choice of nutritional mode is independent of the study.

The clinical prospective study of monitoring plasmatic metabolic markers changes (IGF-I, cholesterol, apolipoproteins, insulin) during different nutritional perioperative strategies (parenteral, enteral support and starvation) have been started in 2003. The aim of the study is correction of nutritional support decision during surgical procedure based on new arguments and the second aim is to improve knowledge of intestinal metabolic and endocrine anabolic function. There was investigated the first collection of blood samples oriented especially on IGF-I (150 samples). The mesured resultes are know under statistical analysis and first resultes will be publiced during this year.

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**Title of the research project:** Morphological and functional evaluation of radical resections for rectal cancer.

**Grant Agency:** Ministry of Health

**Project Number:** ND/7657-3

**Principal Researcher:** Pavel Hladík

**Joint Researchers:** Zbyněk Vobořil , Jaroslav Vižďa, Iva Pospíšil, Dimitar Hadži Nikolov, Josef Dvořák, Milan Široký, Leoš Ungermann.

Depts. of Teaching Hospital Hradec Králové: Dept. of Surgery, Dept. of Nuclear Medicine, Dept. of Pathology, Dept. of Oncology, II. Internal Clinic.

**Starting date:** 1.1.2003

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 2170000

#### **Summary of 2003 results**

**Title of the presentation:** DETECTION OF SENTINEL NODES DURING THE RECTUM RESECTION DUE TO CARCINOMA; PRELIMINARY RESULTS.

**Authors:** Zbyněk Vobořil , Jaroslav Vižďa, Iva Pospíšil, Dimitar Hadži Nikolov, Josef Dvořák, Milan Široký, Leoš Ungermann., Dušan Šimkovič

#### **Introduction:**

Total mesorectal excision belongs among the radical resection kinds of operations. The exact staging of the disease is the main condition for the successful treatment.

**Aim:** Evaluation of methodology of sentinel node detection performed by using the lymphoscintigraphy and endosonography.

#### **Patients and methods:**

Group of surgically treated patients with histologically proved rectum carcinoma has been evaluated. Before the operation the endosonographic examination was performed. The scintigraphic examination of the pelvis follows the submucosal insertion of colloid 99mTc. During the operation and for examination of operating mount the hand gama probe was used in order to detect radioactivity. The whole mount of mesorectum was examined histologically. That examination is aimed to localizations with higher radioactivity. When the sentinel nodes are detected, the usual H+E staining or immunohistochemical examination by cytokeratin is used.

#### **Results:**

24 patients have been examined. There were scintigraphy results confirmed by histology in 19 cases. In 10 cases the nodes were malignantly infiltrated. Using the ultrasonography the nodes were detected in 8 cases before the operation but without the sentinel node specification.

#### **Conclusions:**

Sensitivity 79% of scintigraphic examination was proved in detection of sentinel node in rectum carcinoma. The endoscopic examination performed before the operation gives information mainly about how much the tumor has infiltrated into the rectum wall.

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E-mail: hladikp@lfhk.cuni.cz



**Title of the research project:** Effect of acidosis on protein and amino acid metabolism

**Grant Agency:** Czech Republic

**Project Number:** 305/01/0578

**Principal Researcher:** Milan Holeček

**Joint Researchers:** Vladimír Teplan, Luděk Šprongl, Roman Šafránek, Jana Kadlčíková

**Starting date:** 1. 1. 2001

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 2061000

**Summary of 2003 results**

**Title of the presentation:** Protein and amino acid metabolism in acidosis - experimental study on rats

**Authors:** Milan Holeček (1), Roman Šafránek (1), Luděk Šprongl (2)

Dept. of Physiology, Fac. Med., Charles Univ, Hr. Králové (1) and Univ. Hosp. Motol, Prague

Acidosis is considered a significant cause of protein wasting in various pathologic situations. As its effect is modulated by other abnormal conditions, it is difficult to separate out the effects solely due to acidosis. We evaluated the direct response to acidosis in vivo, in isolated perfused liver and incubated skeletal muscle in rats.

In the first study, rats were infused for 6 h with HCl resulting in blood pH of 7.3 or saline. The parameters of protein metabolism were evaluated using L-[1-14C]leucine. In the second study, using recirculation and single pass technique with 4,5-[3H]leucine, [1-14C]leucine and [1-14C]ketoisocaproate we compared the response of isolated perfused rat liver to perfusion with solution of pH 7.2 and 7.4. In the third study, soleus and extensor digitorum longus muscles were incubated in medium of pH 7.4 or 7.3. [1-14C]leucine was used to estimate protein synthesis and leucine oxidation. Tyrosine release was used to estimate proteolysis. Negative protein balance and an increase in whole-body proteolysis, protein synthesis, leucine oxidation and amino acid concentration in the blood were observed in rats with acidosis. In the liver perfused with solution of pH 7.2 we observed higher rates of proteolysis, protein synthesis, amino acid utilization, urea production and ketoisocaproate decarboxylation than in controls. Decrease in pH of incubation media had no effect on protein synthesis, proteolysis, leucine oxidation and/or release of amino acids by both types of skeletal muscle.

Supported by a grant of the Grant Agency of the Czech Republic No. 305/01/0578.

**Address for correspondence:** M. Holeček, Dept. of Physiology, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic

**Title of the research project:** The possibilities to affect the negative protein balance in severe illness - the effect of proteasome inhibitors

**Grant Agency:** Czech Republic

**Project Number:** 303/03/1512

**Principal Researcher:** Milan Holeček

**Joint Researchers:** Jana Kadlčíková, Roman Šafránek, Jaroslav Chládek

**Starting date:** 1. 1. 2003

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 1684000

**Summary of 2003 results**

**Title of the presentation:** The protein catabolism in sepsis is influenced by proteasome inhibitors.

**Authors:** Jana Kadlčíková (1), Milan Holeček (2), Roman Šafránek(2), Jaroslav Chládek (3)

Dept. of Pharmacology, Fac. Pharm. (1), Dept. of Physiology, Fac. Med. (2), Dept. of Pharmacology, Fac. Med. (3), Charles Univ, Hr. Králové

Sepsis is very often accompanied with cachexia and muscle wasting that complicate the treatment of sepsis and its recovery. The most probable mechanism of the protein catabolism is the enhanced activity of the ubiquitin – proteasome system. We evaluated the effects of proteasome inhibitors –a peptide aldehyde MG132, and two vinylsulfone inhibitors ZL3VS and AdaAhx3L3VS – on protein metabolism in sepsis.

Sepsis was induced in rats by caecal ligation and puncture. Parameters of protein metabolism were measured in incubated rat skeletal muscles. Total proteolysis was determined according to the rates of tyrosine release into the medium during incubation. The rates of protein synthesis and leucine oxidation were evaluated by incubating muscles in medium containing L-[1-14C]leucine.

Sepsis induced protein catabolism in rats - protein synthesis was decreased and proteolysis increased. In the septic muscles, all three inhibitors tested had no effect on protein synthesis. MG132 decreased proteolysis by more than 50%, AdaAhx3L3VS by 20%, while the effect of ZL3VS was not significant.

We conclude, that MG132 and AdaAhx3L3VS reversed at least partly the protein catabolism in sepsis, as they decreased the proteolysis and did not change the protein synthesis.

Project was supported by the Grant Agency of the Czech Republic No. 303/03/1512

**Address for correspondence:** M. Holeček, Dept. of Physiology, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic

**Title of the research project:** The importance of glutamine in BCAA-BCKA cycle and protein balance

**Grant Agency:** Charles University

**Project Number:** 78/03/C

**Principal Researcher:** Milan Holeček

**Joint Researchers:** Roman Šafránek, Luděk Šprongl, Jana Kadlčíková

**Starting date:** 1. 1. 2003

**Duration (years):** 1

**Funds allocated for project - total in Czech crowns:** 172000

**Summary of 2003 results**

**Title of the presentation:** Effect of glutamine and BCAA on amino acid concentrations in body fluids

**Authors:** Milan Holeček (1), Luděk Šprongl (2)

Dept. of Physiology, Fac. Med., Charles Univ., Hr. Králové (1), Univ. Hosp. Motol, Prague

The aim of the project is to verify the hypothesis, that the favourable effect of glutamine administration on protein balance is related to its influence on metabolism of branched-chain amino acids (BCAA; valine, leucine and isoleucine) (Nutrition 18: 130-133, 2002).

Using male laboratory rats we evaluated the changes in plasma and tissue amino acid concentrations after infusion of glutamine (n=13), BCAA (n=10), glutamine + BCAA (n=7), and saline (n=11). Amino acid concentrations were measured using HPLC. Statistical analysis was performed using ANOVA and Bonferroni test.  $P < 0.05$  was considered significant.

Main results:

1. BCAA infusion - significant increase in glutamine concentration in blood plasma (insignificant changes in skeletal muscle).
2. Glutamine infusion – increase in glutamine and decrease in BCAA concentrations in skeletal muscle.
3. Glutamin+BCAA infusion – significant increase in BCAA concentration in skeletal muscle.

The results confirm the known relationships in metabolism of glutamine and BCAA. We assume, that the decrease in BCAA in skeletal muscle of glutamine infused rats is related to decreased proteolysis and significant increase in BCAA concentrations in skeletal muscle after simultaneous infusion of Gln and BCAA is caused by decreased utilisation of BCAA in pathways of glutamine synthesis.

Supported by a grant 78/03/C of the Charles University

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**Title of the research project:** Effect of amino acids and hormones on protein metabolism in catabolic illness

**Grant Agency:** Ministry of Health

**Project Number:** NB/6793-3/01

**Principal Researcher:** Milan Holeček

**Joint Researchers:** Luděk Šprongl, Ivan Tilšer, Roman Šafránek, Jana Kadlčíková

**Starting date:** 1. 1. 2001

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 1230000

**Summary of 2003 results**

**Title of the presentation:** Protein metabolism in guanethidine treated rats

**Authors:** Milan Holeček (1), Marcela Bielavská (2) and Luděk Šprongl (3)

Dept. of Physiology, Fac. Med. Charles Univ, Hr. Králové (1), Dept. Toxicol., Purkyně Military Medical Academy, Hr. Králové (2), and Univ. Hosp. Motol, Prague (3)

The aim of this study was to evaluate the effect of short-term adrenergic blockade on the rate of whole-body protein turnover and leucine oxidation, and on protein synthesis in tissues. Adrenergic blockade was induced in rats by guanethidine infusion (100 mg/kg b.w.) into the jugular vein. The control group consisted of saline infused animals. Parameters of protein and leucine metabolism were evaluated using a primed constant intravenous infusion of labeled leucine.

Guanethidine treatment caused a decrease in norepinephrine in skeletal muscle. Whole-body leucine oxidation and leucine oxidized fraction were higher in guanethidine treated rats.

There was insignificant effect of guanethidine on whole-body proteolysis, protein synthesis, and leucine clearance. However, protein balance in guanethidine treated animals was more negative because the higher difference between protein synthesis and proteolysis than in controls. In guanethidine treated rats, protein synthesis was higher in gastrocnemius muscle and in the kidneys and lower ones in the liver and spleen. Changes in small intestine and colon were insignificant. A marked decrease in concentration of several amino acids has been observed in the liver, the kidneys and the spleen.

It is concluded that activated proteolysis in muscle and leucine oxidation induced by the adrenergic blockade are associated with significant changes in protein metabolism and amino acid concentrations in several tissues. The most important are increased protein synthesis in skeletal muscle and kidneys, and decreased protein synthesis in the liver and spleen. These changes may be induced also by administration of other sympathetic blocking agents.

Supported by a grant NB/6793-3/01 of the IGA MH of the Czech Republic

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**Title of the research project:**

The Influence of Covering of Stents on the Occurrence of Stenoses in Patients Treated with Transjugular Intrahepatic Portosystemic Shunt - TIPS.  
Part II, Prospective Randomized Control Study

**Grant Agency:** IGA MZ ČR**Project Number:** NA-6767-3**Principal Researcher:**

Petr Hůlek, II.nd Dept. of Internal Medicine, University Hospital, Hradec Kralove

**Joint Researchers:**

Antonín Krajina, Dept. of Rentgenology, University Hospital, Hradec Kralove

Pavel Eliáš, Dept. of Rentgenology

Tomáš Fejfar, II.nd Dept. of Internal Medicine

Miroslav Lojík, Dept. of Rentgenology

Pavel Ryška, Dept. of Rentgenology

**Starting date:** 1. 1. 2000**Duration (years):** 3**Funds allocated for project - total in Czech crowns:** 3589000**Summary of 2003 results**

**Title of the presentation:** The Influence of Covering of Stents on the Occurrence of Stenoses in Patients Treated with Transjugular Intrahepatic Portosystemic Shunt - TIPS  
Part II, Prospective Randomized Control Study

**Authors:** P. Hůlek, A. Krajina, P. Eliáš, T. Fejfar, M. Lojík, P. Ryška

**PURPOSE:** In a prospective randomized control trial to compare occurrence of stenosis in general and inside the stent and in outflow part of the stent particularly while using covered and non covered stents

**METHODS:** Between January 2000 to December 2003 98 patients with symptomatic portal hypertension (bleeding from esophageal varices or refractory ascites) were treated with TIPS using stentgrafts. Control group consisted of another 66 patients of the same age, Child-Pugh classification and etiology of cirrhosis. Wallstent, Spiral Z stent, and Viatorr stentgraft (made from ePTFE) were used.

**RESULTS:** In ePTFE group revision in 8 patients was indicated – in-stent stenosis in 2 cases, outflow stenosis in 5 cases. In control group 30 patients underwent angioplasty for stenosis – in-stent stenosis in 10 cases, outflow stenosis in 20 patients. There were no technical difficulties in placement of stentgrafts.

**CONCLUSION:** The use of ePTFE stentgrafts is technically possible, but more expensive in the beginning of treatment. The use of ePTFE was associated with statistically significant reduction of stenoses. The number of revisions (angioplasty for stenosis) was significantly lower. Finally it means, that use of covered stents is less expensive in long term follow-up in surviving patients.

**Address for correspondence:**

Doc. MUDr. Petr Hůlek, CSc,

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**Title of the research project:** Breath isoprene determination - test interpretation and its application into clinical research of cholesterol metabolism disorders

**Grant Agency:** Czech Republic

**Project Number:** 203/01/P110

**Principal Researcher:** MUDr. Radomír Hyšpler, PhD., Medical Faculty, Šimkova 870, Hradec Králové, 500 02

**Joint Researchers:**

**Starting date:** 1.1.2001

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 630000

**Summary of 2003 results**

**Title of the presentation:** Deuterium incorporation into expired isoprene and its relationship to cholesterol synthesis rate estimation

**Authors:** Hyšpler, R., Tichá, A., Gasparič, J., Zadak, Z.

Isoprene in expired breath originates from inductive part of cholesterol synthesis pathway and has a special properties - quickly reflect the metabolic rate and frequent and non-invasive sampling from expired breath is possible. Deuterium incorporation methodology is based on the rate of incorporation of deuterium-labeled water tracer into de novo synthesized isoprene and subsequently cholesterol. The deuterated water tracer equilibrates in total body water and NADPH, that is a precursor pool from which 3 of the 8 hydrogens in isoprene (and 22 of the 46 hydrogens in cholesterol) derive. Deuterium enrichment of this precursor pool, accessible as plasma water, was analyzed by gas chromatography-mass spectrometry (GC-MS). Deuterium enrichment of isoprene was also analyzed by GC-MS after Solid Phase Micro Extraction of expired breath.

Priming dose of deuterium oxide (3 g per kg of body water) was applied in two equal parts in two hour interval. The detectable enrichment was found after 3 hours and full enrichment was found after 5 hours from the beginning of experiment. The slope of the incorporation curve represent synthetic rate and plateau the deuterium enrichment of NADPH pool multiplied by 3 (maximum number of deuterium atoms incorporated in isoprene molecule).

Selected publications:

Bakalar, B., Hyšpler, R., Páchl, J., Zadak, Z.: Changes in cholesterol and its precursors during the first days after major trauma. *Wien Klin Wochenschr* 115/21-22 (2003):775-779.

Hyšpler, R., Tichá, A., Zadak, Z.: The optimized method for GC-MS analysis of farnesyl groups of proteins. 3 rd Int. Symposium on Separation in BioSciences - SBS 2003, p. 123, Moscow, Russia.

**Address for correspondence:**

MUDr. Radomír Hyšpler, PhD, Klinika gerontologická a metabolická, Fakultní nemocnice, 500 05 Hradec Králové, rhyšpler@lfhk.cuni.cz

**Title of the research project:** Biochemical markers of inflammation in the exhaled breath condensate of children with asthma.

**Grant Agency:** Ministry of Health

**Project Number:** NL/7024-3

**Principal Researcher:** Jaroslav Chládek

**Joint Researchers:** Petr Čáp, František Pehal, Miroslav Průcha, Jiřina Chládková, Jiřina Martínková, Irena Krčmová, Yvona Hanzálková, Lucie Mundlová

**Starting date:** 1.1. 2002

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 3200000

#### **Summary of 2003 results**

**Title of the presentation:** Leukotrienes (LT) and metabolites of nitric oxide (NO) in the exhaled breath condensate of healthy subjects and of patients with seasonal allergic rhinitis.

**Authors:** Jaroslav Chládek (1), Petr Čáp, František Pehal, Miroslav Průcha (2), Jiřina Chládková (3), Irena Krčmová, Yvona Hanzálková (4), Jiřina Martínková (1). Fac. Med., Charles Univ., Hr. Králové: Dept. of Pharmacology (1), Dept. of Pediatrics (3), and Clinical Immunology and Alergology (4), Hospital Na Homolce, Prague (2).

Allergic rhinitis is a serious risk factor for asthma. LT are potent pro-inflammatory mediators in asthma and LT modifiers are currently used in the therapy of asthma. LTs are increased in exhaled breath condensate (EBC) in patients with asthma. The aim of the study was to find out whether the LT levels in EBC are early predictors of the onset of asthma in adult patients with seasonal allergic rhinitis (SAR). Moreover, nitrites (NI) were measured as biomarkers of NO induction. Methods: Twenty-nine nonasthmatic adult patients with SAR underwent measurements of exhaled LTs and NI in EBC during and after the pollen season. Leukotrienes B<sub>4</sub>, C<sub>4</sub>, D<sub>4</sub> and E<sub>4</sub> were analyzed by a specific and sensitive gas chromatography-mass spectrometry (GC/MS) assay and NI were analyzed by HPLC. Values were compared to those from 50 healthy nonsmoking controls. Spirometry, skin prick tests and nonspecific IgE were evaluated. Results: LTB<sub>4</sub>, LTE<sub>4</sub> but not LTD<sub>4</sub> concentrations were significantly increased both in and after the pollen season in EBC of patients compared with controls. In most of the samples, LTC<sub>4</sub> was undetectable. LT concentrations in SAR patients decreased after the pollen season ( $p < 0.05$ ). The highest post-seasonal drops in LT levels were found in those SAR patients with the in-season levels exceeding the upper limits for LT concentrations in healthy nonatopic subjects. Similarly, in-season nitrite levels (mikromol/L) in SAR patients were higher than in controls ( $8.6 \pm 6.7$  vs  $4.8 \pm 2.2$ ,  $p < 0.05$ ) and decreased after the pollen season ( $6.5 \pm 3.0$ ). Conclusion. LT and NI levels in EBC are increased in SAR patients and possibly indicate inflammation in the lower airways.

**Address for correspondence:** Jaroslav Chládek, Dept. of Pharmacology, Charles University, Faculty of Medicine, Šimkova 870, 500 01 Hradec Králové, chladekj@lfhk.cuni.cz

**Title of the research project:** Expression of connective tissue proteins in rat hepatic stellate cells

**Grant Agency:** GAČR

**Project Number:** 305/03/1513

**Principal Researcher:** Jiří Kanta

**Joint Researchers:** Jaroslav Cerman, Irena Hanovcová, Renata Köhlerová

**Starting date:** 1 January 2003

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 1383,000

**Summary of 2003 results**

**Title of the presentation:** Isolation of hepatic stellate cells from normal and cirrhotic rat liver

**Authors:** Jiří Kanta (1), Renata Köhlerová (1), Irena Hanovcová (2), Jaroslav Cerman (1)  
Charles University in Prague, Faculty of Medicine in Hradec Králové, Department of Medical Biochemistry (1) and Faculty Hospital, Department of Clinical Microbiology (2)

Hepatic stellate cells are thought to be responsible for the synthesis of majority of connective tissue proteins in normal and cirrhotic rat liver. They are found in a resting state in normal liver where they are used as a major storage site of retinol esters. In damaged liver they become activated, they migrate to the site of injury, proliferate and begin to express large amounts of fibronectin, various types of collagen and proteoglycans.

Male Wistar rats weighing approximately 300 g were used in our experiments. Liver was perfused with Hanks salt solution to remove blood, hepatocytes were destroyed by perfusion with pronase solution and liver collagen was dissolved by collagenase perfusion. Hepatic stellate cells were isolated from the cell suspension by centrifugation on 19% Optiprep gradient. The cells contained characteristic fat vacuoles and stained with antibodies against alpha-smooth muscle actin. Liver cirrhosis was induced in rats by treating them with carbon tetrachloride twice weekly (1 ml CCl<sub>4</sub> dissolved in olive oil/kg body weight) for 2 months. The cells isolated from cirrhotic livers underwent frequent apoptosis which made their cultivation difficult. To isolate total cellular RNA cells were dissolved in guanidiniocyanate solution and extracted with a mixture of phenol and chloroform. RNA was precipitated from the acidified water phase by isopropylalcohol and subjected to electrophoresis on 1% denaturing agarose gel. RNA stained by ethidium bromide was transferred to Hybond nylon membrane and fixed with UV radiation.

Literature: J.Kanta et al.: Liver 22, 220-227 (2002)

Project was supported by Grant Agency of the Czech Republic, grant No. 305/03/1513

**Address for correspondence:** Jiří Kanta, Department of Medical Chemistry, Charles University in Prague, Faculty of Medicine in Hradec Králové, Czech Republic

**Title of the research project:** Comparison of efficacy of two allergen immunotherapy application forms (sublingual and subcutaneous) in treatment of pollinosis, using clinical and laboratory parameters

**Grant Agency:** Ministry of Health

**Project Number:** NI / 7470-3

**Principal Researcher:** Irena Krčmová; Inst. of Clinical Immunology and Allergy, Faculty Hospital Charles' University Hradec Králové, Czech Republic

**Joint Researchers:** Yvona Hanzálková, Ctirad Andrýs, Doris Vokurková, Petr Souček  
Inst. of Clinical Immunology and Allergy, Faculty Hospital Charles' University Hradec Králové, Czech Republic

**Starting date:** 1.1.2003

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 2278000

**Summary of 2003 results**

**Title of the presentation:** See above - Title of the research project

**Authors:** I.Krčmová, Y.Hanzálková, C.Andrýs, D.Vokurková, P.Souček

Allergen immunotherapy is the only therapeutical method in treatment of allergic diseases which causally intervenes development of specific immunological hypersensitivity. This therapy is based on application of gradually increasing doses of allergen vaccines, reaching a dose which is effective in ameliorating the symptoms. According to present knowledge, helper T-lymphocytes are deemed the target of the modulation intervention of therapy. Therapeutical allergen can be applied in two application forms- sublingual and subcutaneous. The aim of this study is mutual comparison of effectiveness of two AIT forms of application in the treatment of pollinotic patients. Clinical effectiveness (development of skin and nasal reactivity) in context with laboratory changes at immune system level will be evaluated. The group consists of 62 pollen allergics (average age 29 years) who are given therapeutical allergen. Twenty one patients are given depot injection form (Phostal), eighteen patients apply the allergen to themselves sublingually (Staloral). Twenty three patients serve as a control group. Prick tests and nasal challenge tests by the causative allergen were carried out before the start of the study and then will be carried out regularly once in a year. The following laboratory parameters are followed: total IgE, spec. IgE and IgG4, cytokines (IFN gamma, IL-4,5,10,12,13), CAST-ELISA (Cellular Antigen Stimulation Test); intracellular production of cytokines is determined, namely production of IL-4, IFN gamma in cellular sub-population of CD4+ after stimulation by mitogens, and IL-12 in sub-population of CD14 monocytes after stimulation by lipopolysaccharide. From this point of view, the aim of the described study is unique according to the present literary data and its solution has a specific impact in clinical practice.

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**Title of the research project:** Electrophysiological evaluation of age related changes in visual perception of man

**Grant Agency:** Czech Republic

**Project Number:** 309/02/1134

**Principal Researcher:** Miroslav Kuba

**Joint Researchers:** Zuzana Kubová, Jan Kremláček, František Vít, Jana Szanyi, David Gayer, Jana Chlubnová

**Starting date:** 1.1.2002

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 1 038,000.-

**Summary of 2003 results**

**Title of the presentation:** Visual evoked potentials indicate ageing of visual system

**Authors:** Miroslav Kuba, Jana Chlubnová, Jan Kremláček, Zuzana Kubová, František Vít, Shiraz Qamaruddin

Dept. of Pathophysiology, Charles University-Faculty of Medicine in Hradec Králové, CZ

We try to verify a value of non-invasive visual evoked potentials (VEPs) examination for objective evaluation of human visual system ageing. VEP peak latencies and amplitudes were so far evaluated in the group of 43 healthy subjects in the age range of 16 – 80 years.

Two main visual processing subsystems (parvo- and magnocellular) were tested with the use of the following set of visual stimuli (for demonstration see <http://www.lfhk.cuni.cz/elf>):

- A) Standard checkerboard reversal stimulation with frequency of 2 Hz, spatial frequency of 0.75 c/deg and contrast of 96% (activating predominantly the parvocellular system and the striate cortex)
- B) Stimulation with onset of visual motion (activating mainly the magnocellular system with the extrastriate associate visual cortex) in two modifications:
  1. low contrast (C = 10%) isolated checks (40' check size and 0.375 c/deg pattern period) randomly moving in four fundamental directions (motion duration = 200 ms, interstimulus interval = 1 s)
  2. centripetal/centrifugal motion of concentric circles (“implosion/expansion”) with increasing velocity (5-25 deg/s) and decreasing spatial frequency (1 - 0.2 c/deg) toward periphery of the visual field (contrast and timing as in the B1 stimulus)

First results of the continuing study have shown that motion-onset VEPs latency prolongation is the most sensitive criterion that might be used for objective evaluation of visual system ageing. It indicates earlier age-related functional changes in the magnocellular system of the visual pathway that is surprisingly evident already in 50 years old healthy subjects. In contrary, VEP amplitude reduction toward elderly was more evident in the pattern-reversal VEPs, which represents more significant influence of visual acuity changes onto this type of VEPs.

**Address for correspondence:** Charles University-Faculty of Medicine in Hradec Králové, CZ - 500 38, Czech Republic, E - mail: kuba@lfhk.cuni.cz



**Title of the research project:** Electrophysiological assessment of human cognitive processes

**Grant Agency:** J. S. McDonnell Found., USA      **Project Number:** 99-57EE

**Principal Researcher:** Miroslav Kuba

**Joint Researchers:** Jan Kremláček, Zuzana Kubová, Colin Blakemore, Philip Benson

**Starting date:** 1.1.2000

**Duration (years):** 4

**Funds allocated for project – total in Czech crowns:** 1,983,000.-

**Summary of 2003 results**

**Title of the presentation:** Visual cognitive evoked potentials in deaf people

**Authors:** Miroslav Kuba, Jana Chlubnová, Jan Kremláček, Zuzana Kubová, František Vít

Dept. of Pathophysiology, Charles University-Faculty of Medicine in Hradec Králové, CZ

Our pilot study was performed in 6 deaf persons (4 women, 2 men, mean age 17 years) and in 6 normally hearing persons (4 women, 2 men, mean age 17 years). The oddball paradigm was used for obtaining of target/non-target cognitive responses (event related potentials – ERP) to three types of visual stimuli/tasks generated on the 21" PC monitor: A. randomly appearing digits („target“ stimulus with checked counting) changing randomly with the letter X („non-target“ stimulus); B. presentation of fingers (“target” stimulus with checked counting) and a picture of the clenched fist (“non-target”); C. distinction between a meaningless character and standard characters from sign language for the deaf.

Although no significant P300 wave latency and amplitude differences were found between normally hearing and deaf subjects, in some deaf individuals a lower target/non-target P300 amplitude difference was seen. Since this might indicate different level of visual cognitive processes in the deaf subjects, we will expand the group and ERPs will be tested as a tool to prove the ability of the deaf people to cope with the sign language.

Supported by James S. McDonnell Foundation for Cognitive Neurosci., USA,  
Grant No.99-57EE-GLO.04.

**Address for correspondence:** Charles University-Faculty of Medicine in Hradec Králové,  
CZ - 500 38, Czech Republic, E - mail: kuba@lfhk.cuni.cz

**Title of the research project:** Transparence of intraocular lens and its influence on differential ability of the eye

**Grant Agency:** Czech Republic

**Project Number:** 309/00/D056

**Principal Researcher:** Hana Langrová

**Joint Researchers:** Dagmar Hejcmanová

**Starting date:** 1.9.2000

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 707000

**Summary of 2003 results**

**Title of the presentation:** Transparence of intraocular lens and its influence on differential ability of the eye

**Authors:** Hana Langrová, Dagmar Hejcmanová, Lisa Bytton-Diaz, Marcela Hovorková, Alena Feuermannová, Pavel Rozsival

Two groups of patients were examined: Group 1, consisting of 53 patients scheduled for cataract surgery and Group 2 consisting of 20 patients with secondary cataract scheduled for Nd-YAG capsulotomy. All patients with a visual acuity of 20/30 or better on Snellen optotypes at the time of patient selection and no other ocular pathology were evaluated objectively and subjectively for visual function preoperatively and postoperatively. Objective measures were following: best corrected visual acuity (BCVA) was measured on log MAR charts with Landolt rings and CS was tested on: a) CS 8010 system at 6 spatial frequencies between 0.74 and 29.55 c/deg, b) Ginsburg chart at spatial frequencies between 1.15 and 27.25 c/deg. Influence of glare of 342.6 cd/m<sup>2</sup> was evaluated using BAT (Brightness Acuity tester). The subjective report of visual performance assessed by questionnaire in Group 1. Preoperatively, visual BCVA acuity and CS were decreased in both groups of patients. Postoperatively, BCVA and CS in group 1 as well as in group 2 ( after the Nd-YAG capsulotomy) increased significantly, nearly to the values of control group. Glare had only nonsignificant influence. The subjective report of visual performance assessed by questionnaire in Group 1 also correlated with the objective measurements. The significant improvement of both BCVA and CS suggest that cataract surgery improve quality of life in early cataract. In 2003, 2 manuscripts were published and 2 manuscript were submitted for publication.

Supported by Grant of Czech Republic No. 309/00/D056.

**Address for correspondence:** Hana Langrová, Charles University Prague, Facultas Medica Hradec Králové: Dept. Ophthalmology, e-mail: langrovah@lfhk.cuni.cz

**Title of the research project:** Integration of courses of medical microbiology and immunology.

**Grant Agency:** Ministry of Education

**Project Number:** 2952/ F3/03

**Principal Researcher:** Jiřina Lesná

**Joint Researchers:** Jan Krejsek, Irena Hanovcová

**Starting date:** 1.1. 2003

**Duration (years):** 1

**Funds allocated for project - total in Czech crowns:** 150000

**Summary of 2003 results**

**Title of the presentation:** Integration of courses of medical microbiology and immunology.

**Authors:** Lesná Jiřina (1), Hanovcová Irena (1), Krejsek Jan (2),

Fac. Med., Charles Univ., Hr. Králové: Dep. of Clinical Microbiology (1)

Dep. of Clinical Immunology (2)

The object of this project is association of the courses of medical microbiology and immunology on the field of antiinfectious immunity and preparing the lessons in computer form accessible on intranet of the faculty. There were made teaching programmes: Mycobacteria, actinomycetes and related organisms, Enterobacteriaceae, Gramnegative rods (Pseudomonas sp., Haemophilus sp., Vibrio sp., Helicobacter sp., Campylobacter sp.), Nosocomial infections. They correspond to content of practical classes and can be used by students as study material for final exam of microbiology.

The most important topics in clinical immunology were selected as the tasks for this project. These are laboratory diagnosis of multiple myeloma, detection of autoantibodies by immunofluorescence and serology of Lyme's disease. The detection of paraproteins as the markers of multiple myeloma by immunofixation are shown together with the results of immunophenotypisation of multiple myeloma cells by immunofluorescence and flow cytometry. The various staining patterns of antinuclear factors and their relevance in differential diagnosis of connective tissue diseases are presented following ANCA antibodies detection and determination of organ specific autoantibodies.

Literature: M. Votava et al.: Lékařská mikrobiologie speciální

J. Horáček et al.: Základy lékařské mikrobiologie

O. Ryšková et al.: Návody k praktickým cvičením

K. Křepela.: Tuberkulóza dětí a dorostu a její diferenciální diagnostika

Project was supported by the Ministry of Education, No 2952/ F3/03

**Address for correspondence:** J. Lesná, Dept. of Clinical Microbiology, Charles University in Prague, Faculty of Medicine in Hradec Králové, Teaching Hospital, Sokolská, 500 05 Hradec Králové, Czech Republic



**Title of the research project:** Pathogenesis, diagnosis and therapy of malignant diseases.

**Grant Agency:** Ministry of Education

**Project Number:** MSM11150002

**Principal Researcher:** Jaroslav Malý

**Joint Researchers:** Jan Peychl , Emil Rudolf , Miroslav Červinka , Zdeněk Fiedler , Vladimír Geršl(2), Michaela Adamcová , Milan Rešl , Pavel Jandík , Zbyněk Vobořil , Jan Bureš , Kopáčová , Jiří Horáček , Alice Poznarová , Jaroslav Malý , Martin Blažek , Bohuslav Melichar , Pavlína Králíčková , Miroslav Kmoníček , Pavel Žák , Ladislav Jebavý

**Starting date:** 1.1.2000

**Duration (years):** 4

**Funds allocated for project - total in Czech crowns:** 5400000

#### **Summary of 2000 results**

**Title of the presentation:** Pathogenesis, diagnosis and therapy of malignant diseases.

**Authors:** Jan Peychl (1), Emil Rudolf (1), Miroslav Červinka (1), Zdeněk Fiedler (1), Vladimír Geršl(2), Michaela Adamcová (3), Milan Rešl (4), Pavel Jandík (5), Zbyněk Vobořil (5), Jan Bureš (6), Kopáčová (6), Jiří Horáček (6), Alice Poznarová (6), Jaroslav Malý (6), Martin Blažek (6), Bohuslav Melichar (6), Pavlína Králíčková (7), Miroslav Kmoníček (6), Pavel Žák (7), Ladislav Jebavý(7)

We evaluated the first results on the field of the carcinoma cell lines, cardiotoxicity and nephrotoxicity of cytostatics, effect of topoisomerase for diagnostic criteria of atypical carcinoids, hemostatic changes during stem cell transplantation and results of non myeloablative transplantation regimen. In vitro Matrigel invasion assay was used for comparison of invasiveness of five stabilized cell lines. Human colorectal carcinoma cell lines (SW420, SW 480) were selected as the models for future experiments. CTnT is the best predictive marker of cardiofoxicity or cardioprotectivity of new drugs. Fifty-four pulmonary carcinoid tumors of surgically treated patients were diagnosed according to histological criteria . The topoisomerase II-alpha is a marker giving a valuable information for diagnostic approach of pulmonary typical and atypical carcinoids. The clinical course of 105 patients admitted for hematopoietic stem cell transplantation to treat a variety of malignancies was monitored. Hemostatic changes were evaluated. Our data shows that D dimers level was elevated and antithrombine level was lower in patients with serious complications of transplantation. The effectiveness of regional chemotherapy was evaluated in patients with metastatic liver involvement of colorectal cancer and malignant melanoma. The activity of systemic oxaliplatin in combination with 5-fluorouracil and leucovorin was also studied in patients with metastatic colorectal cancer. . Two patients with multiple myeloma, four with non Hodgkin lymphoma, one patient with aplastic anemia and one patient with Grawitz tumour were transplanted with minimal conditioning (Flu/Cy).

Project was supported by the Ministry of Education grant No CEZ -MŠMT 111500002

**Address for correspondence:** J. Malý, Dept. of Medicine, Charles Univ. in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 01 Hradec Králové, Czech Rep.

**Title of the research project:** Biochemical monitoring of metabolic changes and tissue blood flow in skeletal muscle during cardiac operation and postoperative care by interstitial microdialysis

**Grant Agency:** Ministry of Health

**Project Number:** 6547-3

**Principal Researcher:** Jiří Mandáček

**Joint Researchers:** Pavel Živný, Vladimír Lonský, Vladimír Palička, Pavel Kuneš, Jaroslav Kubíček, Magdaléna Holečková, Dagmar Kakrdová, Marie Maršíková

**Starting date:** 1.1.2001

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 1592000

**Summary of 2003 results**

**Title of the presentation:** Biochemical monitoring of metabolic changes and tissue blood flow in skeletal muscle during cardiac operation and postoperative care by interstitial microdialysis

**Authors:** Jiří Mandáček (1), Pavel Živný (2), Vladimír Lonský (1), Vladimír Palička (2), Pavel Kuneš (1), Jaroslav Kubíček (1), Magdaléna Holečková (2), Dagmar Kakrdová (2), Marie Maršíková (1). Charles University Hospital, Hradec Králové, Czech Republic: Dept. Cardiac Surgery (1), Inst. Clinical Biochemistry (2)

Serious complication of cardiac surgery using cardiopulmonary bypass (CPB) could be a hypoperfusion of peripheral tissue and splanchnic organs. The aim of this study was monitoring of metabolism and interstitial blood flow in peripheral tissue (skeletal muscle) during cardiac surgery, CPB and postoperative care by microdialysis.

50 patients operated using CPB, 25 in normothermic (NT) and 25 in hypothermic (HT) CPB, were enrolled to this study. Microdialysis was performed by 2 microdialysis probes inserted to the patient's deltoid muscle. Samples were collected in intervals. Estimations in obtained dialysates were glucose, urea, glycerol and lactate. The tissue blood flow was monitored by dynamic microdialysis with gentamicine as a marker.

There were similar time-depending changes in dialysates of all analytes during operation in both groups (NT vs. HT). Low initial concentrations were followed with successive increase. Transient decrease of concentration of above-mentioned analytes were found after operation, increase later in postoperative time. Changes of concentrations of gentamicine indicated lower perfusion of peripheral tissue during HT CPB. These metabolic changes in skeletal muscle are influenced not only by blood tissue flow and tissue metabolic activity but by dramatic changes in tissue perfusion, interstitial hydrostatic pressure and osmotic changes during CPB.

**Address for correspondence:** Jiri Mandak MD, PhD, Dept. Cardiac Surgery, Charles University Hospital, 50005 Hradec Kralove, Czech Republic

**Title of the research project:** Social Support among Children and Adolescent in Stressful Situation (Psychosocial perspective)

**Grant Agency:** Czech Republic

**Project Number:** 406/01/0659

**Principal Researcher:** Jiří Mareš

**Joint Researchers:** Jaro Křivohlavý, Věra Hubková, Marie Rybářová, Jaroslava Pečenková, Jana Marešová, Jana Ježková, Helena Zdeňková, Alena Vodová, David Komárek, Bohumil koukola, Edita Ondřejová, Miroslav Dostálek, Jaroslava Králová, David Skorunka, Lubomír Hadaš, Tomáš Svatoš, Libor troneček, Romana Ivančáková

**Starting date:** 1.1.2001

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 929000

**Summary of 2003 results**

**Title of the presentation:** Social Support among Children and Adolescents in Stressful Situations (Psychosocial Perspective)

**Authors:** Jiří Mareš, Fac. Med., Charles Univ., Hr. Králové: Dept. Social. Medicine

Within the project, the interdisciplinary team examined both healthy and ill population of children and adolescents in the age of 6-18. In the theoretical part of the study, different concepts of social support were compared. Researchers depicted psychosocial determinants of social support, particularly the factors of personality, family, and peer relationships. They studied social support from different perspectives (provider, recipient etc.) They focused on the viewpoint of a child, of its parents and also of professionals. Detailed studies were carried out in order to examine the methods of social support assessment. Different interventions and strategies appropriate for children and adolescents were overviewed. In the empirical part, the current situation in providing and utilization of social support in three psychologically demanding contexts was surveyed. In school, both teachers and schoolmates were studied. In health care facilities several groups of children were studied regarding the process of providing and accepting the social support (chronic ill patients, patients in ophthalmologic unit, stomatologic patients, haemato-oncological patients). The cases of juvenile individuals who live in threatened families or who join peer groups with risk behavior were also studied. Researchers have developed 4 original methods. They also translated 5 questionnaire methods. The most promising seems to be SSQ6 and CASS Questionnaires. Particular attention was paid to those cases, in which social support provided for children and adolescents fails (lack of interest in support, rejection of support etc.). Publication: two monographies, 3 edited books, 2 handbook, 19 journal articles, 68 articles in reviewed edited books, 9 methods of assessment, 2 CD ROM, 47 lectures in congresses (inclusive 11 lectures in foreign countries) were given.

**Address for correspondence:** J.Mareš, Dept of Social Medicine, Charles University Faculty of Medicine Hradec Králové, Šimkova 870, 500 01 Hradec Králové, Czech Republic

**Title of the research project:**

Congenital disorders of glycosylation (CDG), screening and diagnostics

**Grant Agency:** Charles University**Project Number:** 85/2001**Principal Researcher:** Eliška Marklová**Joint Researchers:**

Jaroslava Vávrová, Ziad Albahri, Václava Menclová, Ondřej Renc, Monika Říhová

**Starting date:** 1. 1. 2001**Duration (years):** 3**Funds allocated for project - total in Czech crowns:** 750 000**Summary of 2003 results****Title of the presentation:** Congenital defects of glycosylation, results of 3-years screening.**Authors:** Eliška Marklová, Ziad Albahri, Jaroslava Vávrová\*; Dept. of Pediatrics and \*Dept. of Clin. Biochem., Charles Univ. in Prague, Fac. Med. and Teaching Hosp. in Hradec Králové

Clinical diagnosis of CDG is complicated by heterogeneous, usually age-related features. Many congenital diseases of unknown etiology might turn out to be CDG, which indicates a need to widen the screening criteria. No single screening test for all 14 CDG subtypes is available. The aim of this study was to determine a screening algorithm and report our results. We have introduced various techniques, namely isoelectric focusing (IEF) of serum transferrin (Tf) with direct immunofixation, and electrophoresis of Tf on agarose with Western-blotting. Effect of Tf pretreatment by affinity chromatography and quantitation of Tf isoforms by HPLC has been studied. Apart from Tf we analyzed also alpha1-antitrypsin. The diagnostic procedure is accomplished by testing enzyme activity in isolated leukocytes. Some practical knowledge and experience gained at the particular steps of investigation are discussed. Besides serum, vacuum-concentrated cerebrospinal fluid, as well as serum/plasma/whole blood-dry spots have been checked out with good results. About 120 healthy individuals of various ages and over 800 children/adults with signs of a congenital metabolic defect have been examined. Beside these we screened out several groups of patients with single symptoms or clear diagnoses. CDG-positive samples obtained from other laboratories, as well as serum of newborns, alcohol abusers, and patients with liver diseases serve as positive reference samples. Pathological findings in our set account for about 7 % of the patients examined.

The most prominent abnormality found in a 4-y old child corresponds to CDG type II; the examination is pending. Another serum sample showing a CDG-suspicious IEF pattern appeared to be a rare protein variant, as proved by neuraminidase treatment and testing the parents of the affected adolescent boy.

Supported by the Charles Univ GA, grant No 85/2001/C/LFHK

**Address for correspondence:**

E. Marklová, Dept. of Pediatrics, Charles University in Prague, Faculty of Medicine and Teaching Hospital in Hradec Králové, 500 05 Hradec Králové, Czech Republic.



**Title of the research project:** Population modelling of wanted and unwanted clinical outcome and surrogate parameters

**Grant Agency:** Ministry of Education

**Project Number:** OC B15.10

**Principal Researcher:** Jiřina Martínková

**Joint Researchers:** Jaroslav Chládek, Jiří Grim, Marie Šimková, Jaroslava Vaněčková, Vlasta Koudelková, Marie Nožičková

**Starting date:** 1.1. 2000

**Duration (years):** 5

**Funds allocated for project - total in Czech crowns:** 2400000

#### **Summary of 2003 results**

**Title of the presentation:** Modelling of low-dose oral methotrexate (MTX) pharmacokinetics (PK) and pharmacodynamics (PD) in the therapy of severe psoriasis (PS).

**Authors:** Jaroslav Chládek (1), Jiří Grim (2), Jiřina Martínková (1), Marie Šimková (3), Jaroslava Vaněčková (3), Vlasta Koudelková (3), Marie Nožičková (3). Fac. Med., Charles Univ., Hradec Kralove: Dept. of Pharmacology (1), Internal Medicine (2) and Dermatology (3)

Low-dose oral methotrexate (LDMTX) is an effective therapy of generalized and palmoplantar psoriasis resistant to tar, retinoids, psoralens and UVA-light. The interindividual variability in PK of LDMTX leads to a different exposure to the drug and variable therapeutic/toxic effects in patients given similar doses of MTX. The aims of the study were, first, to analyze PK and PD of LDMTX in patients with PS, and, second, to formulate the principles of PK/PD-guided dose individualization. Methods: The PK and PD were evaluated over an initial 13-week period. The patients were treated with either 7.5 mg or 15 mg MTX a week administered as a bolus or in 12-h intervals 3 times a week. Results: Twenty of 41 patients responded by a higher than 50% drop from the initial PASI-score (The Psoriasis Area and Severity Index). The higher was the area under the plasma concentrations-time curve of MTX (AUC) at week 1, the less was the PASI score at week 13 ( $r_s = -0.65$ ,  $p < 0.01$ ). The steady-state concentrations of MTX-polyglutamates in erythrocytes at week 9 ( $C_{ss-ery}$ ) were linearly correlated with the AUCs at week 1 ( $r_s = 0.87$ ;  $p < 0.05$ ). The  $C_{ss-ery}$  values were also good (but late) predictors of the final PASI ( $r_s = -0.84$ ;  $p < 0.01$ ). The therapeutic range for the AUC of MTX at the start of therapy is 3000 to 3900 nmol.h/L. Values below this range are associated with insufficient response (7% vs 93% of patients,  $p < 0.01$ ). Values above this range are associated with early mild hepatotoxicity of MTX (a higher than 100% increase in ALT activities from the baseline occurred in 50% of patients compared with only 4% of patients with the  $AUC < 3900$  nmol.h/L). The divided-dose schedule for LDMTX is preferable since it leads to a lower frequency (4% vs. 47%) of acute adverse effects (nausea, headache). Published in Br J Clin Pharmacol 2002; 54:147-156 and Clin Pharmacokinet 2003;42:139-51.

**Address for correspondence:** Jaroslav Chládek, Dept. of Pharmacology, Charles University, Faculty of Medicine, Šimkova 870, 500 01 Hradec Králové, chladekj@lfhk.cuni.cz



**Title of the research project:** Prediction of metabolic drug-drug interactions based on in vitro experiments.

**Grant Agency:** COST

**Project Number:** B15.30

**Principal Researcher:** Jiřina Martínková

**Joint Researchers:** Stanislav Mičuda, Jolana Cermanová, Jaroslav Chládek, Lucie Mundlová

**Starting date:** 1.1.2000

**Duration (years):** 4

**Funds allocated for project - total in Czech crowns:** 900000

**Summary of 2003 results**

**Title of the presentation:** Memantine: new selective in vitro inhibitor of CYP2B6

**Authors:** Mičuda S, Mundlová L, Anzenbacher P, Chládek J, Cermanová J, Martínková J.

The in vitro inhibitory effects of memantine on cytochrome P450 (CYP) 1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 activities were examined using pooled human liver microsomes. The in vivo drug interactions of memantine were predicted in vitro using the  $[I]/([I] + K_i)$  values. Memantine inhibited CYP2B6 activity, with a  $K_i$  (IC<sub>50</sub>) value of 101.55 (279.7) microM. Memantine exhibited smaller inhibitory effects on CYP2D6 activity, with a  $K_i$  (IC<sub>50</sub>) value of 250.0 (491.9) microM. Both inhibitions were mixed-competitive. In addition, cDNA-expressed CYP2B6 and CYP2D6 were used to confirm these results. Indeed, memantine strongly inhibited recombinant CYP2B6 activity with IC<sub>50</sub> value of 1.12 microM and activity of recombinant CYP2D6 with IC<sub>50</sub> value of 242.4 microM. With concentrations up to 500 microM, memantine showed no appreciable effect on CYP1A2, CYP2A6, CYP2C9, CYP2D6, CYP2E1, and CYP3A4 activities. Based on  $[I]/([I] + K_i)$  values calculated using peak total plasma (tissue) concentration of memantine, 1% (16.4%), and 0.4% (1.2%) inhibition of the clearance of CYP2B6, and CYP2D6 substrates could be expected, respectively. In conclusion, memantine exerts weak selective inhibition of CYP2B6 activity at clinically relevant concentrations, nevertheless extent of inhibition exclude possibility of significant drug-drug interaction. Inhibition of other CYP isoforms during memantine therapy is also unlikely. In contrast, memantine represent new, potent selective inhibitor of recombinant CYP2B6 and may prove useful for screening purposes during early phases in vitro drug metabolism studies with new chemical entities.

This study was performed within the framework of the COST B15.30 project.

**Address for correspondence:** Stanislav Mičuda, MD. PhD., Department of Pharmacology, Faculty of Medicine in Hradec Králové, Šimkova 870, PO Box 38, 500 38 Hradec Králové, Czech Republic

**Title of the research project:** The experimental model of Huntington's disease: The reaction of the subependymal layer of lateral ventricles to neurodegenerative process within the striatum, and to the fetal neural graft.

**Grant Agency:** Ministry of Health

**Project Number:** NF/7594 - 3

**Principal Researcher:** Yvona Mazurová

**Joint Researchers:** Jan Österreicher, Ivan Látr, Jaroslav Cerman

**Starting date:** 1.1.2003

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 2210000

**Summary of 2003 results**

**Title of the presentation:** An animal model of Huntington's disease: A phenotypization of cells in the subependymal layer of lbrain ventricles in response to the ibotenic acid-lesioned striatum using double-staining methods.

**Authors:** Mazurová Yvona (1), Österreicher Jan (2), Látr Ivan (3), Cerman Jaroslav (4)  
Fac. Med., Charles Univ., Hr. Králové: Dept. of Histol. and Embryol. (1), Neurosurg. Clin. (3),  
Dept. of Med. Biochem. (4); Dept. of Radiobiol., Purkyně Military Acad., Hr. Králové (2)

Intrastratial injection of the ibotenic acid (IA) induces the neurodegenerative process in a rat brain similar to that of Huntington's diseased (HD) patients. The subependymal layer (SEL) in the lateral brain ventricles is known as a source of neural stem and precursor cells not only during the developmental period but also in adulthood (1). The level of proliferative activity in the SEL, the phenotype of newly generated cells, and their possible capability to migrate into the denervated striatum was studied using different immunohistochemical, preferably double-staining methods. With respect to long-term development of HD, four groups of male rats (Long-Evans strain, n = 22) surviving 1, 3, 6 and 9 months after lesion were observed. The neurodegenerative process in the striatum invoked increased proliferation/division of the stem cells within the SEL and their differentiation to the precursor cells – i.e. mild increase in number of NESTIN-, NCAM- and BrdU-positive cells. The persistence of BrdU labelling showed that stem and precursor cells continued in their differentiation only very slowly. Most of those cells differentiated to the GFAP-positive reactive astrocytes (BrdU-GFAP+) inside the SEL and some of them can be found to migrate for a short distance to the striatum. Interesting findings were obtained using the combined GFAP+NCAM detection. Small number of such double-marked cells with morphological characteristics of precursor cells, and less, but already with the characteristics of typical reactive astrocytes was present in SEL.

Literature: (1) Alvarez-Buylla A et al.: Prog Brain Res 2000;127: 1-11

Supported by the Ministry of Health of CR (NF 7594).

**Address for correspondence:** Y. Mazurová, Dept. Histol. Embryol., Charles Univ. in Prague, Fac. Med. in Hradec Králové, Šimkova 870, P.O.Box 38, 500 38 Hradec Králové, Czech Republic

**Title of the research project:** Evaluation of changes in expression of MRP2 on canalicular membranes of hepatocytes and their influence on hepatic elimination of methotrexate.

**Grant Agency:** GAUK

**Project Number:** 89/2002/C

**Principal Researcher:** Stanislav Mičuda

**Joint Researchers:** Jolana Cermanová, Lucie Mundlová, Jiřina Martínková

**Starting date:** 1.1.2002

**Duration (years):** 2

**Funds allocated for project - total in Czech crowns:** 475000

**Summary of 2003 results**

**Title of the presentation:** Hepatic expression of multidrug resistance transporters Mdr1 and Mrp2: Influence of Dexamethasone.

**Authors:** Mičuda S, Cermanová J, Mokry J, Čížková D, Mundlová L, Osterreicher J, Rudolf E, Martínková J

The aim of presented study was to evaluate effect of dexamethasone (DEX) on hepatic morphology and expression of multidrug resistance proteins Mdr1 and Mrp2 in rat liver using methods of immunohistochemistry and Western blot. In addition, light microscopy of oil-red-stained sections was performed to assess fat accumulation in rat liver. Oral administration of DEX (1 mg/kg/day) over 4 days resulted in 22-fold increase in fat deposition together with 4.2- and 1.9-fold increase in expression of Mdr1 and Mrp2, respectively. Moreover, intraperitoneal administration of different DEX doses (1, 10, 25, and 40 mg/kg/day over 4 days) produced dose-dependent increase in expression of Mdr1 and Mrp2, respectively. These results indicate that dexamethasone is a potent inducer of Mdr1 and Mrp2, and produce significant hepatic steatosis.

This study is supported by the grant from the Grant Agency of the Charles University in Prague (GAUK No. 89/2002/C/LFHK).

**Address for correspondence:** Stanislav Mičuda, MD. PhD., Department of Pharmacology, Faculty of Medicine in Hradec Králové, Šimkova 870, PO Box 38, 500 38 Hradec Králové, Czech Republic

**Title of the research project:** Establishment and exploitation of human hepatocyte culture systems for the in vitro evaluation of drug metabolism

**Grant Agency:** COST

**Project Number:** OC B15.40

**Principal Researcher:** Jaroslav Mokrý

**Joint Researchers:** Dana Čížková, Jana Karbanová, Stanislav Mičuda, Jiřina Martínková, Jaroslav Chládek, Luice Mundlová, Jolana Cermanová

**Starting date:** 1.1.2002

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 1971000

**Summary of 2003 results**

**Title of the presentation:** Characterisation of human foetal hepatocytes of the WRL 68 cell line

**Authors:** Dana Čížková (1), Jaroslav Mokrý (1), Stanislav Mičuda (2), Jiřina Martínková (2), Jana Karbanová (1)

Charles Univ. Prague, Fac. Med. Hradec Králové: Dept. of Histology and Embryology (1), Dept. of Pharmacology (2)

We describe our first findings with cultivation of human foetal hepatocytes of the WRL 68 cell line. We characterized the phenotype of these elements and compared morphological properties of hepatocytes in vitro and in situ.

We chose markers suitable for immunophenotypisation of hepatocytes: hepatocytic marker OCH1E5, pancytokeratin, cytokeratin 18 and alpha-fetoprotein, which are expressed in hepatic cell line WRL 68 as well as in rat and human foetal or adult liver. We also focused on the expression of ABC transporters MDR1 (P-glycoprotein) and MRP2 (cMOAT). Their immunopositivity was detected in the liver in later stages of rat and human intrauterine development and in adult liver tissues. In WRL 68 cell line, after 3 days in vitro (DIV) there was no immunoreactivity for these proteins, at 6 DIV a weak signal appeared, while at 9 DIV all cells expressed these transporters. Our data indicate that MDR1 and MRP2 start to be expressed in the course of hepatocyte differentiation; the signal is first scattered throughout the cytoplasm and then it is gradually concentrated in the area of the canalicular membrane. We proved the inductive effect of dexametason on the expression of above mentioned ABC transporters in situ as well as in vitro: in rat liver tissue and in human foetal hepatocytes of the WRL 68 cell line. We can conclude that morphological properties and antigenic profile of hepatocytes in situ and in vitro are closely similar and hepatic cell line WRL 68 represents a useful in vitro model for the study of hepatocyte functions.

Project was supported by the COST grant No. OC B15.40.

**Address for correspondence:** Doc. MUDr. Jaroslav Mokrý, Ph.D., Dept. of Histology and Embryology, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové



**Title of the research project:** Innovation of topics "General and Special Neurohistology"

**Grant Agency:** Ministry of Education

**Project Number:** 2953/F3a

**Principal Researcher:** Jaroslav Mokrý

**Joint Researchers:**

**Starting date:** 1.1.2003

**Duration (years):** 1

**Funds allocated for project - total in Czech crowns:** 117000

**Summary of 2003 results**

**Title of the presentation:** General and special neurohistology: Innovation of lectures and laboratory classes from histology

**Authors:** Jaroslav Mokrý

Department of Histology and Embryology, Charles University in Prague, Medical Faculty in Hradec Králové

Lectures and programmes for laboratory classes covering the themes of "General Histology of the Nervous Tissue" and "Special Histology of CNS Organs" were supplemented with new pieces of knowledge, coloured versions of 3-D reconstructions, digitalized images of neural cells and organs of the central nervous system and prepared as Power Point presentations. New histological sections of human or mouse neocortex, cerebellum, spinal cord, trigeminal ganglion, subependymal zone, hippocampus and olfactory bulb were cut and processed for routine staining and peroxidase immunohistochemistry. The latter detections were focused on cell specific markers (NeuN, Pan-NF, MAP-2, beta-III tubulin, NCAM, GFAP, O4, nestin), proliferation markers (PCNA, Ki-67) or markers specific for neural structures like peripheral nerves (S100, pan-NF), myelin (MBP), synapses (synaptophysin) etc. Immunoreactive structures were photographed and used to prepare the database of digitalized images as well as lectures and laboratory classes. Immunostained sections were inserted in sets (boxes) of slides used by teachers and students. Supported by the grant from Ministry of Education No. 2953/F3a/2003.

**Address for correspondence:** Doc. MUDr. Jaroslav Mokrý, Ph.D., Department of Histology and Embryology, Charles University in Prague, Medical faculty in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic



**Title of the research project:** Factors influencing the extent of colonization of recipient's tissues by stem cells.

**Grant Agency:** Czech Republic

**Project Number:** 304/03/1515

**Principal Researcher:** Jaroslav Mokřý

**Joint Researchers:** Stanislav Filip, Jana Karbanová, Dana Čížková

**Starting date:** 1.1.2003

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 2321000

**Summary of 2003 results**

**Title of the presentation:** Clonal analysis of neural stem cells in vitro and in vivo

**Authors:** Jana Karbanová (1), Jaroslav Mokřý (1), Dana Čížková (1), Stanislav Filip (1, 2)

Fac. Med., Charles Univ., Hr. Králové: Dept. of Histology and Embryology (1), Dept. of Oncology and Radiotherapy (2)

The aim of this study was to evaluate the self-renewal and differentiation potential of separate neural stem cells (NSCs) in in vitro culture and in vivo following transplantation into the mouse brains.

Neural stem cells were isolated from the forebrains of the E20 mouse fetuses and cultured as neurospheres in serum-free medium supplemented with basic fibroblast growth factor and epidermal growth factor. Neurospheres were dissociated and individual NSCs were plated separately into each well of the 96-well plate. Cells were allowed to proliferate and following ten days individual neurospheres were dissociated and cultured in separate Petri dishes. Cells from individual clones were then transferred into the medium containing 1 - 10 % serum and allowed to differentiate on fibronectin- or laminin-coated coverslips.

Immunohistochemical detections proved that NSCs derived from individual stem cells differentiated into beta-III-tubulin+ neurons, GFAP+ astrocytes and O4+ oligodendrocytes. The composition of medium had appreciable effect on ratio of differentiating individual cell types: in medium with 10 % serum astrocytes prevailed, medium with 1% FCS induced prevalence of neurons and astrocytes and in neurobasal medium supplemented with 1µM retinoic acid neurons and oligodendrocytes prevailed. Following neural grafting, NSCs gave rise to neurons and glia.

Our results demonstrate that only a small proportion (approx. 5%) of tested cells (present in neurosphere culture) is able to self-renew (and therefore represent the true stem cells) and each single NSC has capacity to differentiate at least into two cell types.

Project was supported by the Grant Agency of the Czech Republic, No. 304/03/1515.

**Address for correspondence:** Doc. MUDr. Jaroslav Mokřý, Ph.D., Department of Histology and Embryology, Charles University in Prague, Medical Faculty in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic

**Title of the research project:** Immunohistochemical detection of nestin as a diagnostic marker in pathomorphology

**Grant Agency:** Ministry of Health

**Project Number:** 6727-3/2001

**Principal Researcher:** Jaroslav Mokřý

**Joint Researchers:** Dana Čížková, Jiří Ehrmann, Jana Karbanová, Stanislav Němeček, Danuše Šubrtová, Zdeněk Kolář, Miloslav Uher

**Starting date:** 1.1.2001

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 1953000

#### **Summary of 2002 results**

**Title of the presentation:** Nestin: a marker of undifferentiated cells in muscle tissue, blood vessels and tissues of neuroectodermal origin

**Authors:** Jaroslav Mokřý (1), Jiří Ehrmann (2), Dana Duspivová (1), Jana Karbanová (1), Zdeněk Kolář (2)

Fac. Med., Charles Univ., Hr. Králové: Dept. of Histology and Embryology (1), Fac. Med., Purkinje Univ., Olomouc: Dept. of Pathology (2)

In the course of organogenesis, protein nestin is temporarily expressed in muscle tissue, endothelial cells and cells derived from the neuroectoderm. After terminal differentiation, nestin levels are downregulated being replaced with another IF. In adult tissues, processes of regeneration or neoplastic transformation are accompanied by appearance of undifferentiated cells. We tested our hypothesis that these processes are associated with nestin re-expression. To identify immunoreactive cells we screened lots of biological samples taken from embryos, intact adult tissues, injured tissues or tumours with immunohistochemistry using anti-nestin antibodies that recognize nestin epitopes in rat, mouse and human tissues. Our findings confirmed that in the muscle tissue, nestin is expressed during development and in adult tissues it re-appears in newly formed elements, e.g. smooth muscle cells in the wall of newly formed blood vessels, myotubes of transplanted skeletal muscle and newly formed cardiomyocytes in the infarcted hearts. Nestin-positive cardiomyocytes were observed in the myocardium of all patients suffering from the ischemic disease including elderly patients. Nestin positive endothelium was observed in human embryonic tissues as well as adult tissues like regenerating myocardium or corpus luteum. In tissues of neuroectodermal origin, nestin was expressed in human gliomas, medullomas, melanomas, Merkel cell carcinoma etc. Our findings indicate that nestin is a unique marker that can reveal the presence of undifferentiated cells in neoplastic and regenerating tissues.

Project was supported by the grant No. 6727-3/01 from IGA MZ.

**Address for correspondence:** Doc. MUDr. Jaroslav Mokřý, Ph.D., Department of Histology and Embryology, Charles University Medical Faculty, Šimkova 870, 500 38 Hradec Králové

**Title of the research project:**

Introducing histopathology of the CNS tumours into the curriculum of neurosurgery

**Grant Agency:** Ministry of Education

**Project Number:** 2954/03

**Principal Researcher:** Stanislav Němeček

**Joint Researchers:** Jiří Náhlovský, Jaroslav Cerman

**Starting date:** 1. 1. 2003

**Duration (years):** 1

**Funds allocated for project - total in Czech crowns:** 133000

**Summary of 2003 results****Title of the presentation:**

Introducing histopathology of the CNS tumours into the curriculum of neurosurgery

**Authors:** Stanislav Němeček (1,2), Jiří Náhlovský (1), Jaroslav Cerman (3)

Dept. of Neurosurgery (1), Dept. of Histology and Embryology (2), Dept. of Medical Biochemistry (3), Charles University, Faculty of Medicine in Hradec Králové

A major achievement of the WHO project has been to propose a standardised, widely accepted classification with the uniform terminology comprehensive for every participating medical discipline and to establish a grading system of brain tumours, indicative of the degree of malignancy, based on histological grounds. New clinical-pathological entities appear; some terms gain a new conception. Clinical practice, however, is somehow retarded in this respect. The aim of the present project has been to bring this new information to the undergraduate (lectures, seminars), and postgraduate neurosurgical education. Anyway, the neurosurgeon will not teach histopathology, but will concentrate on biological and prognostic aspects.

Results: 1) Paraffin blocks of about 7000 tumours of nervous system, collected in the Laboratory of Neuropathology, were used to find typical cases and to examine them with a battery of classical and immunohistochemical techniques.

2) The main result of the project is represented by a database of digital pictures of neuroepithelial tumours accompanied with brief microscopic characteristics, and with basic biologic and prognostic aspects. This will become a part of the surgeon's lectures.

3) A detailed description of clinical neuropathology of all presently defined neuroepithelial tumours has been elaborated in order to bridge the gap between neurosurgery and microscopy. This remains to be an auxiliary text destined mainly to the teacher.

Literature: P. Kleihues, W.K. Cavenee: Pathology and Genetics. Tumours of the Nervous System. IARC Press, Lyon 2000, 314 pp.

Ministry of Education supported this project, No. 2954/2003.

**Address for correspondence:** Stanislav Němeček, Dept. of Neurosurgery, Charles University in Prague, Faculty of Medicine in Hradec Králové, University Hospital, 500 05 Hradec Králové, Czech Republic.

**Title of the research project:** Hemostatic changes during radiofrequency catheter ablation

**Grant Agency:** Ministry of Health

**Project Number:** NA 6603-3

**Principal Researcher:** Petr Pařízek

**Joint Researchers:** Jaroslav Malý, Miloslav Pleskot, Martin Hodač, Pravoslav Stránský, Miroslav Pecka, Luděk Haman

**Starting date:** 17.7.2001

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 1710000

#### **Summary of 2003 results**

**Title of the presentation:** Hemostatic changes during electrophysiologic study and radiofrequency catheter ablation

**Authors:** Petr Pařízek (1), Luděk Haman (1, 4), Jaroslav Malý (2), Miroslav Pecka (2), Martin Hodač (2), Miloslav Pleskot (1), Josef Bukač (3), Pravoslav Stránský (3)

Fac.Med., Charles Univ., Hradec Králové: 1st (1) and 2nd (2) Dept. of Internal Medicine, Dept. of Medical Biophysics (3), Purkyne Military Medical Academy Hradec Králové (4)

The aim was the study of chosen hemostasis activation markers during electrophysiologic study (EPS) with consequent radiofrequency catheter ablation (RFA).

63 patients were studied prospectively during routine EPS with RFA for supraventricular tachycardias. Blood samples were drawn before the insertion of venous sheaths (T0), at the end of EPS (T1), and 30 minutes after completion of RFA (T2). To study coagulation, fibrinolytic, and platelets activation we measured concentrations of thrombin-antithrombin III (TAT), D-dimers (DD), plasminogen inhibitor activator (PAI-1), plasminogen tissue activator (tPA), and circulating platelet aggregates (CPAi).

Levels of DD increased from  $0.30 \pm 0.20$  (mean $\pm$ SD) mg/l at T0 to  $0.44 \pm 0.25$  mg/l at T1 ( $p < 0.001$ ) and to  $0.87 \pm 0.74$  mg/l at T2 ( $p < 0.001$ ). TAT levels increased from  $12.90 \pm 12.83$  mcg/l at T0 to  $36.07 \pm 15.59$  mcg/l at T1 ( $p < 0.001$ ) and decreased to  $28.85 \pm 13.14$  mcg/l at T2 ( $p < 0.001$ ). PAI-1 concentration decreased from  $37.49 \pm 23.20$  mcg/l at T0 to  $29.74 \pm 14.00$  mcg/l at T1 ( $p < 0.001$ ) and to  $28.13 \pm 12.86$  mcg/l at T2 ( $p = 0.07$ ). There was no significant difference between the concentrations of tPA at T1 vs. T0. Dependence of concentration of DD at T2 vs. T1 on the number of RF energy applications ( $p < 0.001$ ,  $R^2 = 0.462$ ) was found. Marked platelet activation (CPAi  $0.59 \pm 0.29$ ) was observed from the start of the procedure without changes during the procedure.

Our results confirmed activation of hemostasis during EPS and RFA. Dependence of concentration of DD on the number of RF energy applications was found. Platelets activation was observed even before the start of the procedure.

Project is supported by the IGA MH CR, No. NA 6603-3/2001.

**Address for correspondence:** P. Pařízek, 1st Dept. of Internal Medicine, Charles University, Faculty Hospital, Hradec Králové, 500 05, Czech Republic, parizek@fnhk.cz



**Title of the research project:** Surface leukocyte markers in newborns with risk for early sepsis

**Grant Agency:** IGA MZ ČR

**Project Number:** 7533-3

**Principal Researcher:** Doc. MUDr. Eva Pařízková, CSc.

**Joint Researchers:** MUDr. Božena Buriánková, MUDr. Pavel Rozsival, MUDr. Eva Šimáková, PharmDr. Doris Vokurková, RNDr. Ctirad Andryš

**Starting date:** 1/2003

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 850000

**Summary of 2003 results**

**Title of the presentation:** Surface leukocyte markers in newborns with risk for early sepsis

**Authors:** E. Pařízková, P. Rozsival

The study „Surface leukocyte markers in newborns with risk for early sepsis“ is targeted at early diagnosis possibilities in newborns, utilizing modern immunology methods, assessing expression of surface markers and production of interleukins. Early infection or sepsis affects 1-10% of newborns and is the leading cause of newborn deaths.

Laboratory markers (increased erythrocyte sedimentation rate, CRP, differential count changes), X-ray and clinical signs (fever, circulation disorders, dyspnea ...) used in older patients are unreliable and late in newborns.

Utilization of surface markers – in our study HLA-DR, CD45RA/RO, CD23, CD69 and CD64 that are expressed on leukocyte surface and interleukins (namely IL-6, IL-8, IL-10) excreted during inflammatory reaction on encounter with microbial antigens, we try to find early and precise markers of ongoing infection in newborns. Surface markers are assessed with flow cytometry, interleukin levels with ELISA.

Results will be correlated with histological finding in placentae and routine clinical and laboratory follow-up. A group of specialist pediatricians, immunologists and pathologist cooperates on the grant.

Due to late funding assignment by IGA initial tests were carried out in 2003, method calibration and conservation agent was evaluated.

**Address for correspondence:** pavel.rozsival@seznam.cz

**Title of the research project:** Monitoring quality of care in patients with out-of-hospital circulatory arrest in the east Bohemian region

**Grant Agency:** Ministry of Health

**Project Number:** NO/7254-3

**Principal Researcher:** Miloslav Pleskot

**Joint Researchers:** Jaroslav Kajzr, Zdeněk Tušl, Petr Pařízek, Miroslav Měšťan, Miloslav Tauchman, Jakub Střítecký, Jiří Kvasnička, Vladimír Černý

**Starting date:** 1.1.2002

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 765000

#### **Summary of 2003 results**

**Title of the presentation:** Out-of-hospital cardiac arrests in the East Bohemian region

**Authors:** Miloslav Pleskot (1), Jaroslav Kajzr (1), Zdeněk Tušl (1), Petr Pařízek (1), Miroslav Měšťan (1), Miloslav Tauchman (1), Jakub Střítecký (1), Jiří Kvasnička (1), Anush Babu (1), Vladimír Černý (2), Eva Čermáková (3)

Charles Univ. Prague, Fac. Med. Hr. Králové: Ist Dep. Med. (1), A/E Clinic (2), Dept. Medical Biophysics (3)

**Introduction:** Long-term results in patients with cardiac arrest (CA) depend on the quality and organisation of mainly out-of-hospital care.

**Aim:** Analysis of clinical data and diagnostic and therapeutic approaches in individual stages of care in patients with out-of-hospital CA in the East Bohemian region. Authors mainly surveyed the number of cases that are hospitalised and the number that are discharged.

**Method:** From 1.1.2002 to 31.12.2003 authors collected data, with the aid of questionnaires, from 25 rescue centres in the East Bohemian region in a group of 523 patients (375 men, 148 women) with an age range of 16 to 97 years (67+/-13 years) with out-of-hospital CA. CA was defined as sudden unexpented non-traumatic loss of consciousness as a consequence of circulatory arrest.

**Results:** From the total 523 patients, 363 (69,4%) died "on-the-spot" and 47 (9%) during transportation to hospital. Layman resuscitation was provided in 162 (31%). ECG findings of ventricular fibrillation was seen in 174 cases (33,3%), polymorphic ventricular tachycardia in 10 (1,9%), monomorphic ventricular tachycardia in 8 (1,5%) and asystole in 326 (62,3%).

113 patients (21,6%) were admitted to the intensive care unit. Acute myocardial infarction was diagnosed in 44 cases (38,9% of the total hospitalised patients). 31 patients (5,9%) were discharged home.

**Conclusions:** Results of this project show a high mortality in patients with out-of-hospital CA. The patients who survived, had in mediate layman cardiopulmonary resuscitation, short arrival times of ambulance and prompt invasive procedures for acute myocardial infarction. Supported by Grant IGA MZČR NO/7254-3.

**Address for correspondence:** M. Pleskot, Ist Dep. of Internal Medicine, Faculty Hospital, Sokolská 581, 500 05, Hradec Králové, Czech Republic





**Title of the research project:** A randomized, double blind comparative study of parenteral nutritional support with or without glutamine in autologous stem cell transplantation for hematologic malignancies

**Grant Agency:** Ministry of Health

**Project Number:** NA/7000-2

**Principal Researcher:** Alice Poznarova

**Joint Researchers:** Jiri Horacek, Pavel Zak, Miloslav Kmonicek, Jaroslav Maly

**Starting date:** 1.1.2002

**Duration (years):** 2

**Funds allocated for project - total in Czech crowns:** 360000

**Summary of 2003 results**

**Title of the presentation:** A randomized, double - blind comparative study of parenteral nutritional support with or without glutamine in autologous stem cells transplantation for hematologic malignancies

**Authors:** Alice Poznarova, Jiri Horacek, Pavel Zak, Miloslav Kmonicek, Jaroslav Maly  
Faculty Hospital, Department of Hematology, Hradec Kralove

Standards of nutritional support in bone marrow transplantation have recently been questioned vdue to advent of newer techniques of supportive care that shorten the critical post-transplant period. Our aim was to evaluate the possible significance of prophylactic parenteral nutrition (PN) in stem cells transplantation and the suggested advantage of glutamine (Gln) supplementation. Since 8/2000 43 adult patients (13 with multiple myeloma, 15 with non-Hodgkin´s lymphomas, 8 with Hodgkin´s disease and 7 with leukemias) were randomized to receive either prophylactic PN starting with the cytoreductive regimen (P group, n=20), or PN given if oral intake became inadequate (control C group, n=23). In each group they were further randomized to receive standard PN or isocaloric, isonitrogenous PN with L-Ala-L-Gln (0,5 g Gln/kg). The groups were compared in the hospitalization period and during follow-up. The results are expressed as medians, significance was tested using Mann-Whitney and Wilcoxon tests, as appropriate. For survival analysis, Kaplan-Maier method was used. The higher cost in the P group was not compensated for by better outcome. There was no significant difference in the length of hospital stay, time to engraftment, G-CSF consumption, or in the nutritional markers. On the contrary, in the P group there were significantly ( $p<0,05$ ) more days with fever  $>38^{\circ}\text{C}$ , higher antibiotics (atb) consumption and more blood transfusion unitsvwere needed. Gln-supplemented patients (in both C and P groups) did not do better in any of the parameters tested. There was no overall difference in survival or relaps rate between C and P groups. Surprisingly, more relapses were found in Gln - supplemented patients and these also had worse overall survival. Difference was not found in more homogenous group too - in patients with multiple myeloma and non-Hodgkin s lymphoma.

**Address for correspondence:** A.Poznarova, dept. of Hematology, Faculty Hospital, Hradec Kralove, Sokolska 581, 500 00 Hradec kralove, Czech Republic



**Title of the research project:** The use of fluorescent methods in cell biology and genetics classes

**Grant Agency:** Ministry of Education

**Project Number:** 2955/F3

**Principal Researcher:** Emil Rudolf

**Joint Researchers:** Jana Kolářová, Miroslav Červinka

**Starting date:** 1.1. 2003

**Duration (years):** 1

**Funds allocated for project - total in Czech crowns:** 183000

**Summary of 2003 results**

**Title of the presentation:** The use of fluorescent methods in cell biology and genetics classes

**Authors:** Emil Rudolf, Jana Kolářová, Miroslav Červinka

Fac. Med., Charles Univ., Hr. Králové: Department of Medical biology and Genetics

Many biological and medical disciplines take advantage of fluorescence and its applications. Due to the fact that nowadays fluorescent techniques undergo dynamic development, every medical student should know their principle along with possible applications. During practical classes in cell biology and genetics our students use light microscopy in different applications which have been made possible due to support of the Grant agency of Ministry of Education over past years. In order to introduce the concept of fluorescence to our classes we have selected and prepared six simple fluorescent tasks which will help illustrate basic biological phenomena while demonstrating to our students the importance of fluorescence in medicine. The tasks include staining of various organelles such as actin or nucleus, distinguishing between living and dead cells as well as visualization of cell death-specific markers. Some of the tasks are scheduled for direct use by students, others will be demonstrated. Also, students will have a chance to participate on documentation of the results using digital fluorescence cameras or camcorders. Furthermore, we prepared the manual on fluorescence and its applications in microscopy which will be placed onto the departmental homepage: [www.biologie-lfhk.cz/](http://www.biologie-lfhk.cz/). There it will serve as an education as well as reference material for our students and academic workers of different related medical disciplines. We believe that thus innovated practical classes will broaden working knowledge of medical students and put emphasis on current developments in medical and biological sciences. This project was supported by Ministry of Education Grant No. 2955.

**Address for correspondence:** E. Rudolf, Dept. of Medical Biology and Genetics, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové

**Title of the research project:** Effect of growth hormone and glutamine on protein metabolism in isolated skeletal muscle.

**Grant Agency:** Ministry of Health

**Project Number:** NB/7611-3/03

**Principal Researcher:** Roman Šafránek

**Joint Researchers:** Milan Holeček, Jaroslav Chládek, Jana Kadlčíková

**Starting date:** 1.1.2003

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 309000

**Summary of 2003 results**

**Title of the presentation:** Effect of glutamine on protein synthesis, proteolysis and leucine oxidation by isolated skeletal muscle of rat.

**Authors:** Roman Šafránek (1), Milan Holeček (1), Jana Kadlčíková (2)

Dept. of Physiology, Fac. Med. (1), Dept. of Pharmacology, Fac. Pharm. (2), Charles Univ, Hr. Králové

The essential donor of nitrogen for glutamine synthesis in skeletal muscle are branched-chain amino acids (i.e. valine, leucine, isoleucine). It may be suggested that exogenous glutamine could affect positively protein and amino acid metabolism in skeletal muscle.

The aim of the present study was to evaluate the direct effect of the lack and supplementation of glutamine on protein synthesis, leucine oxidation and protein breakdown in skeletal muscle.

Isolated soleus and extensor digitorum longus muscles from normal Wistar rats were incubated in medium containing 0.5, 2 mmol/l or no glutamine. L-[1-14C]leucine was used to evaluate protein synthesis and oxidation of leucine. Tyrosine release was used to estimate protein breakdown. Statistical analysis was performed using ANOVA and Bonferroni test. Statistical significance was accepted at  $P < 0.05$ .

Supplementation of incubation media with glutamine to concentration 2 mmol/l decreased significantly oxidation of leucine in both muscles and did not change protein synthesis and protein breakdown. The lack of glutamine had no effect on measured parameters. There was no difference between response of soleus and extensor digitorum longus muscle. We conclude that acute changes of glutamine concentration in vitro do not have the regulatory role in protein synthesis and protein breakdown. However, glutamine supplementation decreases leucine oxidation. We suppose that this is due to decrease of glutamine synthesis.

Supported by a grant NB/76111-3/03 of the IGA MH of the Czech Republic

**Address for correspondence:** M. Holeček, Dept. of Physiology, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic



**Title of the research project:** Determination of LPA level in ovarian cancer patients

**Grant Agency:** Ministry of Health

**Project Number:** 7666-3

**Principal Researcher:** Iva Sedláková

**Joint Researchers:** Jindřich Tošner, Jaroslava Vávrová

**Starting date:** 1.1.2003

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 535000

**Summary of 2003 results**

**Title of the presentation:** Determination of LPA level in ovarian cancer patients

**Authors:** Iva Sedláková (1), Jindřich Tošner (1), Jaroslava Vávrová (2)

Fac.Med., Charles Univ., Hr. Králové: Dept. of Gynecology and Obstetrics (1), Dept. of Clin. Biochemistry (2)

Lysophosphatidic acid (LPA, 1-acyl-2-lyso-sn-glycero-3-phosphate) has been shown to stimulate proliferation of ovarian cancer cells. LPA has multiple effects on ovarian cancer cells including increased cell proliferation, increased cell survival by decreasing apoptosis, decreased sensitivity to cisplatin, increased invasiveness and increased production of LPA itself. A higher plasma level of LPA at patients with ovarian cancer than in healthy women was reported. LPA may represent a more sensitive marker for ovarian cancer, particularly for the stage I of this disease. The methodics for the determination of LPA level with its specification by capillary electrophoresis using indirect ultraviolet detection has been implemented and a cut-off value for LPA plasma level will be determined in the sample of ovarian cancer patients in this project. Since beginning of this project venous blood samples from 66 patients (16 patients with ovarian cancer, 50 patients without ovarian cancer) have been obtained. An advantage of the determination of LPA by capillary electrophoresis using the ultraviolet detection is a possibility to determinate single types of lysophosphatidic acid in biological material. There are LPA (M) 1-myristoyl-2-hydroxy-sn-glycerophosphatidic acid, LPA (P) 1-palmitoyl-2-hydroxy-sn-glycerophosphatidic acid, LPA (S) 1-stearoyl-2-hydroxy-sn-glycerophosphatidic acid and LPA (O) 1-oleoyl-2-hydroxy-sn-glycerophosphatidic acid and LPA (D) 1-decanoyl-2-hydroxy-sn-glycerophosphatidic acid which will be used as an internal standard. The detection limits for various molecular species of LPA are: LPA (O) 2,8  $\mu\text{mol/l}$ , LPA (P) 2,9  $\mu\text{mol/l}$ , LPA (M) 1,4  $\mu\text{mol/l}$ , LPA (S) 2,1  $\mu\text{mol/l}$ .

Project is supported by the Ministry of Health Grant Agency, No 7666-3

**Address for correspondence:** Iva Sedláková, Dept. of Gynecology and Obstetrics, Faculty Hospital Hradec Králové, 500 05 Hradec Králové, Czech Republic



**Title of the research project:** Program for knowledge testing of students by computer

**Grant Agency:** Ministry of Education

**Project Number:** 2960/F3

**Principal Researcher:** Pavel Šiman

**Joint Researchers:**

**Starting date:** 2003

**Duration (years):** 1

**Funds allocated for project - total in Czech crowns:** 87000

**Summary of 2003 results**

**Title of the presentation:** Program TEST

**Authors:** Pavel Šiman

The computer program TEST is designed for knowledge testing of the chosen subject (e.g. chemistry, biology, physics ...). For each of them the program manages the database of questions, answers and explanation of the problem in question. The subjects can be divided into (sub)branches up to three levels (e.g. CHEMISTRY: inorganic chemistry – s,p-elements – 1. subclass). Testing questions can be chosen according to these (sub)branches. Multiple-choice questioner is used.

During testing, the program offers questions of chosen (sub)branch (or chosen by other way – e.g. manually) in random sequence. Answers for each question are also „mixed“. Hence, there is nearly impossible to cheat („crib“) even if each student has the same pool of questions. The testing is terminated by exhausting of relevant questions or can be earlier terminated manually. The program then will evaluate the answers of each student. If the student is registered, his/her result is saved into the database for future use. It is important that during self-testing (self-learning) students can find the explanation of problem in question.

Due to cooperation with MS-Word, it is possible to use also structural formulas, pictures, maps etc. The program also permits to use examples for testing. The author of such question determines equation for calculation and limits and precision of all parameters. The values of parameters are randomly chosen (within limits) by computer.

The program permits comfortable adding of new subjects, (sub)branches, questions, answers and explanations and their future editing. The program can be, therefore, widely used for testing of a variety of subjects in schools of any degree or in self-learning in study rooms or at home. The program is primary designed for use in computer net.

The program will be continually improved.

**Address for correspondence:** LF UK, Dept. Biochemistry, Šimkova 870, 500 01 Hradec Králové, e-mail: siman@lfhk.cuni.cz



**Title of the research project:** Sinus lift in dental implantology referring to osseointegrated implant parameters and type of augmentation materials

**Grant Agency:** Ministry of Health

**Project Number:** 7711-3

**Principal Researcher:** Antonín Šimůnek

**Joint Researchers:** Dana Kopecká, Aleš Kohout

**Starting date:** 1.1.2003

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 1593000

**Summary of 2003 results**

**Title of the presentation:** Sinus lift in dental implantology

**Authors:** Antonín Šimůnek (1), Dana Kopecká (1), Aleš Kohout (2)

Faculty of Medicine, Charles University, Hradec Králové, Department of Stomatology (1), Faculty Hospital, Hradec Králové, Department of Pathology (2)

One of the most common problems in dental implantology is insufficient alveolar bone in the posterior region of the upper jaw due to maxillary sinus pneumatization. In these cases the sinus lift procedure is used to achieve the necessary bone dimensions. An autogenous bone is the most common augmentation material used for this procedure. However this is the reason for relatively large invasiveness of this operation.

The aim of our project is to reduce the invasiveness of the sinus lift procedure by using only a non-autogenous material possibly mixed with a little amount of bone harvested from the same surgical field. This modified method will be performed in local anesthesia and without hospitalization. It is less uncomfortable and more acceptable for the patients. The authors want to judge the most adequate type of implants for use in the augmented bone (with a sand-blasted surface, a hydroxyapatite coating or with a new developed bioactive titanium surface "Bio").

In the first year of the research work the papers "A six-year study of hydroxyapatite-coated root-form dental implants", "Pětileté sledování dentálních implantátů Impladent", and "The use of oxidized regenerated cellulose (Surgicel®) in closing Schneiderian membrane tears during the sinus lift procedure" were written. 18 two-staged sinus lift operations with the bone substitutes Cerasorb ( $\beta$ -tricalciumphosphate), Cerasorb mixed with autogenous bone, Bio Oss (deproteinized bovine bone) a Bio Oss mixed with autogenous bone were performed.

In year 2004 the authors will continue with sinus lift operations, they will harvest the samples of augmented bone and they will perform a histomorphometric examination of these samples.

Project was supported by the IGA MZ ČR, No 7711-3.

**Address for correspondence:** A. Šimůnek, Faculty Hospital, Department of Stomatology, 500 05 Hradec Králové, Czech Republic



**Title of the research project:** Risk perception and level of health self-assessment of employees of different professions

**Grant Agency:** Charles University

**Project Number:** 90/2001

**Principal Researcher:** Jindra Šmejkalová

**Joint Researchers:** Zdeněk Fiala, Lenka Hodačová, Lenka Borská, Dana Fialová

**Starting date:** 1.1.2001

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 359000

**Summary of 2003 results**

**Title of the presentation:** Socioeconomic inequalities in attitudes to own health

**Authors:** Jindra Šmejkalová (1), Hana Skalská (2), Marcela Bendová (3), Lenka Hodačová (4), Lenka Borská (5), Jaroslav Tejral (1), Dana Fialová (6)

Fac. Med., Charles Univ., Hr. Králové: Dept. of Hygiene and Prev. Medicine (1), Dept. of Social Medicine (4), Dept. of Pathological Physiology (5); University Hr. Králové: Dept. of Informatics and Quantitative Methods (2), Dept. of Physical Education and Sports; (6) ERGO Hr. Králové (3)

It has been known that health state, life style, health attitudes and behaviour are strongly affected by the type of profession. We tried to describe the associations between socio-economic status and prevalence of health risk factors and their perception in the Czech Republic. Data were collected using self administered anonymous questionnaire. We analysed 825 questionnaires from 242 teachers, 172 factory workers of mostly risk professions, 267 health workers and 144 clerks. Results obtained showed that our respondents were exposed to number of risk factors resulting from their professions, but above all from their way of living. The highest risk accumulation, though being largely underestimated, was found in the group of factory workers. Workers are not aware of these risks (47%), and if they are, they do not want to solve the situation (29%). The same goes for appreciating the health prevention - 37% of workers do not participate in preventive check-ups by general practitioners and 32% in stomatological prevention. 72% of workers do not know their blood pressure level and 94% of them the level of cholesterol. Nevertheless, we confirmed significant risk underestimation of other occupational groups as well - 51% of medical workers and 40% of clerks do not participate in general prevention. The worst situation was among medical doctors where the ignorance reached 70%! Our findings indicate that educational prevention should target the whole population regardless of the nature of their occupation.

Project was supported by the Charles University Grant Agency, No. 90/01.

**Address for correspondence:** Jindra Šmejkalová, Dept. of Hygiene and Preventive Medicine, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 50038 Hradec Králové, Czech Republic.

**Title of the research project:** Contrast-enhanced Magnetic Resonance Imaging versus Thallium Scintigraphy in the Detection of Myocardial Viability prospective comparative study

**Grant Agency:** Ministry of Health

**Project Number:** NA/7248-3

**Principal Researcher:** Miroslav Solař

**Joint Researchers:** Jan Zizka<sup>2</sup>, Jirí Dolezal<sup>3</sup>, Ludovit Klzo<sup>2</sup>, Jaroslav Vizda<sup>3</sup>, Jaroslav Ťintěra<sup>4</sup>, Jiri Ceral<sup>1</sup>, Jiří Kvasnička<sup>1</sup>, Vladimír Lonský<sup>5</sup>, Pavel Žáček<sup>5</sup>  
1Ist. Department of Internal Medicine, 2Department of Radiology, 3Department of Nuclear medicine and department of 5Cardiac surgery University Hospital Hradec Kralove, 4Institute of Clinical and experimental Medicine Czech Republic

**Starting date:** 1/2003

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 1 500 000

#### **Summary of 2003 results**

**Title of the presentation:** Contrast-enhanced Magnetic Resonance Imaging versus Thallium Scintigraphy in the Detection of Myocardial Viability

**Authors:** Miroslav Solař, Jan Zizka, Jirí Dolezal, Ludovit Klzo, Jaroslav Vizda, Jaroslav Ťintěra, Jiri Ceral, Jiří Kvasnička, Vladimír Lonský, Pavel Žáček

Contrast-enhanced magnetic resonance imaging (MRI) is a new method in the assessment of myocardial viability. The aim of this study was to compare it to SPECT-Thallium scintigraphy.

The study enrolled the patients with documented coronary artery disease and impaired left ventricular systolic function defined by ejection fraction less than 45%. Myocardial viability study was performed both by SPECT using 201Thallium and contrast-enhanced magnetic resonance imaging. Short axis views of the myocardium were divided into segments. In each segment myocardial viability was scored semiquantitatively according to the 201Thallium activity (SPECT) and the relative amount of contrast enhanced tissue (MR). The results of viability assessment were compared in corresponding segments.

25 patients (22 men, 3 women) were included. Myocardial viability assessed by SPECT was normal in 52.9%, impaired in 13.9% and absent in 26.8% of segments evaluated. On MR viability study there were 59.7% of segments with no contrast enhancement showing no irreversible injury, 37.2% of segments contained both contrast enhanced and viable tissue and in 3.2% there was a predominance of contrast-enhanced irreversibly changed tissue.

Comparing the two methods the results of viability assessment corresponded in 51.3% of segments. 42.7% showing no irreversible injury, 5.3% displaying impaired viability and 3.2% with prevailing irreversible injury. In 23.7% of segments that were assessed as non-viable by Thallium scintigraphy there were signs of viability using contrast-enhanced MR. According the results of our study it seems possible that in comparison to Thallium scintigraphy the contrast-enhanced MR imaging can more accurately diagnose irreversible myocardial injury and better detect viable myocardium. The latter finding may be important in selecting the eligible candidates for myocardial revascularisation.

**Address for correspondence:** Miroslav Solař, Ist. Department of Internal Medicine University Hospital Hradec Kralove, Czech Republic



**Title of the research project:** The importance of lipid metabolism monitoring in aging

**Grant Agency:** Ministry of Health

**Project Number:** NG/6770-3

**Principal Researcher:** Dagmar Solichová

**Joint Researchers:** Vladimír Bláha, Miloš Klejna, Božena Jurašková, Radomír Hyšpler, Petr Žďánský, Zdeněk Zadák

**Starting date:** 1.1.2001

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 1200000

**Summary of 2003 results**

**Title of the presentation:** Development of new HPLC method for the determination of alpha tocopherol in human erythrocytes for clinical applications

**Authors:** Dagmar Solichová (1), Lucie Korecká (2), Iveta Svobodová (1), František Musil (1), Vladimír Bláha (1), Petr Žďánský (1), Zdeněk Zadák (1)

Department of Metabolic Care and Gerontology, Teaching Hospital, Hradec Králové (1), Faculty of Chemical Technology, University of Pardubice (2)

In this work, a simple isocratic reversed phase HPLC method for the determination of alpha tocopherol in human erythrocytes has been developed and validated. After separation of plasma the erythrocytes were washed three times with 0,9 % sodium chloride containing 0,01% butylated hydroxytoluene (BHT) as antioxidant and then were diluted with the same solution 1:1(v/v). In the LLE procedure, 2 500 mL of n-hexane was added to 500 mL of erythrocytes. After 2 minutes this mixture was deproteinized by cool methanol (500 mL, 5 min) containing alpha tocopherol acetate (20 mmol/L) as internal standard and then extracted 5 min by Vortex. After centrifugation (10 min, 1 600 x g) the aliquot (2 000 mL) of clean extract was separated and evaporated under nitrogen. The residue was dissolved in 400 mL methanol and analysed by reversed-phase HPLC technique, the mobile phase 100% methanol, flow rate 1,2 ml/min, column Pecosphere C18 4,6 x 150 mm, 5mm, injected volume 100 mL, and detected by diode-array detector at wavelength 295 nm.

The extraction recovery of alpha tocopherol from human erythrocytes was 100 ( $\pm 2$ ) %.

Detection limit was 0,1 mmol/l and linear calibration curve was obtained in the concentration range 0,5-20 mmol/l. Within determination precision was 5,2 % RSD (n = 10), between determination precision was 6,1 % RSD (n = 10). The method was applied successfully in the clinical study for the patients with acute pancreatitis, for elderly patients and for determination of the reference values in healthy Czech population.

Project was supported by Ministry of Health Czech Republic, No. NG/6770-3.

**Address for correspondence:** Dagmar Solichová, Dept. of Metabolic Care and Gerontology, Teaching Hospital, 500 05 Hradec Králové, Czech Republic

**Title of the research project:** Observation and comparison of biologic and synthetic materials implanted into bone defects.

**Grant Agency:** Ministry of Health

**Project Number:** ND6853-3

**Principal Researcher:** Pavel Šponer

**Joint Researchers:** Karel Urban, Karel Karpaš, Elen Urbanová

**Starting date:** 1.1.2001

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 913000

**Summary of 2003 results**

**Title of the presentation:** CANCELLOUS BONE ALOGRAFTS IN THE MANAGEMENT OF THE BONE DEFECTS.

**Authors:** Pavel Šponer (1), Karel Urban (1), Elen Urbanová (2), Karel Karpaš (1)  
Fac.Med., Charles Univ., Hr. Králové: Dept. of Orthop.Surgery (1), Dept. of Nuclear Med. (2)

**Introduction:** The purpose of this study is to assess the long-term results of the cancellous bone allografts used in the treatment of the bone defects.

**Materials and methods:** Only patients with involvement of the long bones (the femur, tibia and humerus) treated by curettage of the benign lesion and full filling of the bone defect with cancellous allografts without recurrence were included in our study. In 2003 19 patients (10 male and 9 female) showed up for the follow-up. Their average age was 10 years (range 5 to 15 years). The primary diagnoses were unicameral bone cyst in 10, nonossifying fibroma in 5, aneurysmal bone cyst in 3 patients and enchondroma in 1 patient. Clinical examination, plain X-rays and three-phase bone scintigraphy with diphosphonates labelled by <sup>99m</sup>Tc were performed at the follow-up with range 2 to 14 years after surgery and average 5 years.

**Results:** Seventeen patients had no pain, slight pain was reported by two patients. No inflammatory changes of the soft tissues with normal carrying capacity of the treated extremity and full range of movements were found in all cases. No signs of bone grafts loosening with no structural changes were observed on plain X-rays in 8 cases, irregular bone structure without bone graft loosening signs was observed in 11 cases. Bone scintigraphy finding was normal in 15 patients and minimally increased uptake of the radionuclide was found in 4 patients.

**Conclusion:** We observed normal or minimally increased uptake of the radionuclide in the bone graft transplanted area. There was no scintigraphy finding difference in the metaphyseal region and the shaft of long bones. These results are compared to group of patients after implantation of BAS-0 bioactive glass-ceramics and group of patients after transplantation of bone autografts.

Project was supported by the Ministry of Health Grant Agency, No 6853-3/2001.

**Address for correspondence:** Pavel Šponer, M.D.,  
Smetanova 719, 551 01 Jaroměř.

**Title of the research project:** The relation of morphology of pulmonary veins to atrial fibrillation, with particular attention to isolated atrial amyloid

**Grant Agency:** Ministry of Health

**Project Number:** NA/7592-3

**Principal Researcher:** Ivo Šteiner (1)

**Joint Researchers:** Iva Kholová (1), Jiří Kvasnička (2)

Department of Pathology (1) and 1st. Department of Internal Medicine (2),  
Faculty Hospital, Hradec Králové

**Starting date:** 1 January 2003

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 1934000

**Summary of 2003 results**

**Title of the presentation:**

**Authors:**

Atrial fibrillation is the most common supraventricular arrhythmia, moreover with an increasing prevalence. Attacks of the arrhythmia are triggered by ectopic activity originating mostly from the pulmonary veins. Left atrial myocardium frequently extends onto pulmonary veins (so called myocardial sleeves). The myocardial "sleeves" have been histologically examined for the presence, quantity, and character of isolated atrial amyloid - one of the "senile" amyloids.

A hypothesis is put forward that amyloidosis of pulmonary veins may play a role in pathogenesis of atrial fibrillation.

During the first year of the project 40 necropsy specimens of heart + pulmonary veins from patients both with atrial fibrillation and negative controls were examined for the presence and character of amyloid by means of histochemical (Saturn red) and imunohistochemical (atrial natriuretic peptide; transthyretin) staining methods.

The low number of hearts examined so far does not allow us to make conclusions. The clinico-pathological correlation will be made after examining a total of 100 hearts.

Literature: Kholova, I., Kautzner, J.: Anatomic characteristics of extensions of atrial myocardium into the pulmonary veins in subjects with and without atrial fibrillation. Pacing Clin Electrophysiol. 26, 2003:1348-1355.

**Address for correspondence:** Prof. I. Steiner, M.D., Dept. of Pathology, Faculty Hospital, 500 05 Hradec Králové, Czech Republic, steiner@lfhk.cuni.cz



**Title of the research project:** Innovation of dental curriculum - chemistry and biochemistry

**Grant Agency:** Ministry of Education

**Project Number:** 2956/03

**Principal Researcher:** Alena Stoklasová

**Joint Researchers:** Věra Hubková

**Starting date:** 1.1. 2003

**Duration (years):** 1

**Funds allocated for project - total in Czech crowns:** 52000

**Summary of 2003 results**

**Title of the presentation:** Innovation of dental curriculum - chemistry and biochemistry

**Authors:** Alena Stoklasová (1), Věra Hubková (2)

Fac. Med., Charles Univ., Hr. Králové: Dept. of Medical Biochemistry (1), Dept.  
of Dentistry (2)

The aim of this project was to introduce the new curriculum of chemistry and biochemistry that was tailored to the need of dental study programme. A set of digitalized X-ray photographs, slides and schemes was also prepared. First of all, the proportions between lectures, seminars and practical classes were established. This resulted in preparation of detailed syllabi of lectures, seminars and practical classes and modification of examination questions. The lectures and seminars covering chemistry of dental materials and biochemistry of connective tissue, teeth, mouth and saliva were prepared including digitalized slides and X-ray photographs.

This curriculum has been introduced in the academic year 2003/2004.

This project was supported by Ministry of Education - No 2956/2003.

**Address for correspondence:** A. Stoklasová, Dept. of Med. Biochemistry, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové

**Title of the research project:** The development of software for processing of medical information and biosignal supporting improvement of and for quality of education

**Grant Agency:** Ministry of Education

**Project Number:** 111500004

**Principal Researcher:** Pravoslav Stránský

**Joint Researchers:** Miroslav Červinka, Josef Hanuš, Nad'a Jirásková, Vladimír Mašín, Emil Rudolf, Zdeněk Fiala

**Starting date:** 1.1.1999

**Duration (years):** 4

**Funds allocated for project - total in Czech crowns:** 2080000

#### **Summary of 2003 results**

**Title of the presentation:** Infrastructure for information technologies and software for biomaterial analysis and image-processing.

**Authors:** Pravoslav Stránský, Vladimír Mašín, Josef Hanuš, Fac. Med., Charles Univ., Hr. Králové: Dept. of Medical Biophysics

In 2003 we supported two main research projects, based on computer-assisted 3D navigation within human body, both using a newly purchased magnetic tracker Ascension miniBIRD 800.

One project is called „Mini navigation“, basically it is a simplified form of neuronavigation, based on CT data and data from the magnetic tracker. It computes a 3D reconstruction showing both anatomical structures (from the CT data) and position and direction of an instrument.

The second project, called “3D reconstruction of peripheral nerves”, combines data from the magnetic tracker with ultrasound images to create 3D reconstructions of peripheral nerves. This is intended mostly for examination of carpal tunnel syndrome patients.

Rest of IT spending was used for IT infrastructure improvements:

- 1) A new publication system for web publishing was build, which should help a lot in our effort to ease organizing of scientific meetings at our faculty.
- 2) Upgrades of data storage facilities (up to 1,5 TB capacity) created enough headroom for our most demanding projects, from 3D navigation to electrophysiological research.
- 3) New PCs equipped with statistical and image-processing software should help in the analysis of research data from all supported projects.

LabView software and peristaltic pump acquired from the project means in the past years were used for measurement and comparison of mechanical properties of Nitinol stents.

**Address for correspondence:** Pravoslav Stránský, Dept. of Medical Biophysics, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 01, Hradec Králové 1, Czech Republic

**Title of the research project:** Photopic electroretinography in diabetics

**Grant Agency:** Ministry of Health

**Project Number:** NK/6835

**Principal Researcher:** Jaromír Svěrák

**Joint Researchers:** Jaroslav Peregrin, Eva Rencová, Hana Langrová, Josef Kvasnička, Hana Dvořáková

**Starting date:** 01.01.2001

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 106000

**Summary of 2003 results**

**Title of the presentation:** Photopic electroretinography in diabetics

**Authors:** Jaromír Svěrák, Eva Rencová, Hana Langrová, Josef Kvasnička, Hana Dvořáková

Charles University in Prague, Faculty of Medicine in Hradec Králové, Dept. of Ophthalmology

The importance of the scotopic electroretinography (ERG) for diagnosis and prognosis of the diabetic retinopathy is doubtless. The aim of this study is to detect possible abnormalities of the photopic ERG in diabetics. The ERG examinations of three groups of diabetics were continued: 1. diabetics without diabetic retinopathy (n=38), 2. diabetics with simple diabetic retinopathy (n=24), 3. diabetics with preproliferative and proliferative diabetic retinopathy before and after panretinal photocoagulation (n=20). Besides the rod and the maximal ERG responses, the photopic ERG responses were evaluated. The abnormalities of the photopic responses were confirmed in all three group, so that the photopic ERG is further important tool in prevention and management of the ocular involvement in diabetes mellitus. The manuscript was published in Czech and Slovak Ophthalmology. The spectrum of examinational methods was enlarged about the scotopic 15 Hz flicker. Also with this new method the abnormalities were found.

The subnormal oscillatory potentials were found in another group of patients with the optic nerve pits complicated by the maculopathy. It was the first description of such finding in the literature. Further the ERG potentials were examined in 40 patients with stenosis of the carotid artery. The stenosis of the carotid artery caused the significant reduction of the ERG rod responses and delayed latencies of the scotopic bright flash a-waves, of the oscillatory potentials and photopic flicker responses on the ipsilateral side.

We will continue in examination and evaluation of scotopic 15 HZ flicker and photopic 30 Hz in groups of patients mentioned.

**Address for correspondence:** Prof. MUDr. Jaromír Svěrák, DrSc., Charles University, Dept. Ophthalmology, Sokolská 158, 500 05 Hradec Králové, e-mail: sverak@fnhk.cz.

**Title of the research project:** Effectivity of the biventricular stimulation assessed by changes induced pulse amplitude

**Grant Agency:** Ministry of Health

**Project Number:** NA 7261-3

**Principal Researcher:** Miloslav Tauchman

**Joint Researchers:** Jiří Kvasnička, Anush Babu, Miloslav Měšťan, Zdeněk Tušl, Miloslav Pleskot, Petr Pařízek, Pavel Rejchrt, Martin Sivák

**Starting date:** 1.1.2002

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 405000

**Summary of 2002 results**

**Title of the presentation:** Effects of different stimulation sites on hemodynamic performance estimated by changes in pulse amplitude

**Authors:** Tauchman M, Měšťan M, Tušl Z, Kvasnička J, Pleskot M, Pařízek P, Babu A, Rejchrt P, Sivák M

In 2003 there were 10 patients included in the study. The patients were implanted with pacemakers with biventricular modality of pacing because of treatment of heart failure. Five of them suffered from malignant ventricular tachyarrhythmias and heart failure were implanted with implantable cardioverter-defibrillators with modality of biventricular pacing. Index of pacing site contribution to pulse pressure is estimated on beat-to-beat basis. Our data showed an essential importance of absolutely constant rate of atrial and ventricular stimulation. During manipulations with the AV delay, the index of site contribution to pulse pressure changed significantly. However this changes were in a large extent influenced by the force-frequency phenomena. Parameters received from the blood pressure (registered by Finometer device) are much more stable in comparison with pulse oximetry. It seems that atrial contribution to pulse pressure plays greater role in patients with severe heart failure than in healthy population.

**Address for correspondence:** Dr.Miloslav Tauchman, 1st Department of Internal Medicine, Faculty Hospital, Hradec Králové, Czech Republic

**Title of the research project:** Therapeutic impact on the cognitive functions in schizophrenia

**Grant Agency:** Ministry of Health

**Project Number:** NF67533/2001

**Principal Researcher:** Ivan Tůma

**Joint Researchers:** Marek Pérez, Zuzana Lenderová

**Starting date:** 17.7.2001

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 1951000

**Summary of 2003 results**

**Title of the presentation:** Donepezil in the treatment of cognitive dysfunction in schizophrenia

**Authors:** Ivan Tuma (1), Zuzana Lenderová (1), Marek Pérez (2)

Charles University in Prague, School of Medicine and Teaching Hospital in Hradec Králové, Dept of Psychiatry (1)

Psychiatric Hospital Opava (2)

Cognitive deficit is common and related to functional outcome in patients with schizophrenia. The possible role of adjunctive pharmacology for cognitive deficit is receiving consideration. Considerable evidence exists suggesting an important role for cholinergic system in schizophrenia. The aim of this project was to evaluate the effectiveness of augmentation of risperidone by inhibitor of acetylcholinesterase donepezil in the treatment of cognitive impairment in schizophrenia. Thirty patients with schizophrenia on individual stable dose of risperidone were randomly assigned to double blind treatment with donepezil or placebo. They were administered the neuropsychological battery at baseline and again following 12 weeks and 16 weeks of experimental treatment. Donepezil did not produce significant improvements in any cognitive parameter compared with placebo. Our results are consistent with the other placebo controlled studies of Friedman et al. 2002 and Tugul and Yazici 2003.

Project was supported by Grant Agency of Ministry of Health, No NF67533/2001

**Address for correspondence:** Ivan Tuma Charles University in Prague, School of Medicine and Teaching Hospital in Hradec Králové, Dept of Psychiatry, 500 05, Czech republic

**Title of the research project:** Tooth crown reconstruction during preclinical dental instruction

**Grant Agency:** Ministry of Education

**Project Number:** 2959/2003

**Principal Researcher:** Dagmar Vahalová

**Joint Researchers:** Jiří Bittner, Lenka Vavříčková

**Starting date:** 1.1.2003

**Duration (years):** 1

**Funds allocated for project - total in Czech crowns:** 207000

**Summary of 2003 results**

**Title of the presentation:** Tooth crown reconstruction during preclinical dental instruction

**Authors:** Dagmar Vahalová (1), Jiří Bittner (1), Lenka Vavříčková(1)

Fac. Med., Charles Univ., Hr. Králové: Dept. of Dentistry (1)

The authors of this project have introduced several techniques of destroyed tooth crown reconstruction in preclinical dental instruction. The procedures of tooth root preparation to achieve any restoration must be trained very carefully as early as possible because the preparation inside the root canal doesn't allow any visible access into the post hole being prepared and the operator is guided during her/his preparation only by her/his own previous experience with this procedure, that they have gained during first two years of preclinical dental instruction. We have bought specialized plastic teaching teeth with destroyed clinical crowns and endo-treated root canals, that may be properly prepared with appropriate instruments to achieve one of many kinds of root inlays restoring together with all-veneer crowns completely the whole tooth crown.

From funds allocated for our project we have bought all necessary instruments, materials, specialized plastic teeth and teaching models and have enriched by that our dental preclinical instruction during the 2<sup>nd</sup> schoolyear of our dental curriculum.

Project was supported by the Ministry of Education Grant Agency, No 2959/2003

**Address for correspondence:** D. Vahalová, Dept. of Dentistry, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic

**Title of the research project:** Prospective evaluation of the functional parameters of peripheral blood leukocytes in patients with colorectal cancer liver metastases

**Grant Agency:** Charles University

**Project Number:** 75/03/C

**Principal Researcher:** MUDr. Pavel Veselý

**Joint Researchers:** doc. MUDr. Bohuslav Melichar, Ph.D., MUDr. Josef Dvořák

**Starting date:** 4/2003

**Duration (years):** 1

**Funds allocated for project - total in Czech crowns:** 61000

**Summary of 2003 results**

**Title of the presentation:** A colorimetric lymphocyte proliferation assay in the assessment of peripheral blood mononuclear cell (PBMC) function in cancer patients

**Authors:** Pavel Veselý, Bohuslav Melichar, Department of Oncology & Radiotherapy, Department of Medicine

Advanced cancer is accompanied by immune dysfunction. The aim of present study was to investigate the immune status of patients with colorectal cancer liver metastases using a nonradioactive lymphoproliferative assay. The MTT test was utilized based on the method originally described by Mosmann. PBMC were separated by density centrifugation, resuspended in Dulbecco's Minimal Essential Medium (DMEM) and washed thrice. The cells were then incubated in DMEM supplemented with 10% fetal calf serum with or without mitogens in a 96-well plate with PBMC numbers ranging from 0.5 to 2 x 10<sup>5</sup> cells/well. Increasing concentrations of phytohemagglutinin, concanavalin A and interleukin-2 were used for mitogenic stimulation. After 72 - 96 h MTT was added. Incubation for 96 h appears to be optimal. After additional 3 h the untransformed MTT was removed and blue formazan crystals were dissolved in dimethylsulfoxide (DMSO). Optical density was measured on spectrophotometer at 570 nm (reference wavelength 450 nm). We observed significant stimulation of PBMC proliferation (100-250% over baseline) for wide range of concentrations of mitogens, but the results varied significantly from patient to patient. On the other hand, the measurements in the same individual were reproducible. Studies are under way using this method in a larger patient population.

**Address for correspondence:** MUDr. Pavel Veselý, Department of Oncology & Radiotherapy, Charles University in Prague, Medical School & Teaching Hospital in Hradec Králové, Sokolská, Hradec Králové 50005

**Title of the research project:** The changes in the metabolism of cholesterol and its precursors in the patients in critical care

**Grant Agency:** Ministry of Health

**Project Number:** NB/7589-3

**Principal Researcher:** Zdeněk Zadák

**Joint Researchers:** Radomír Hyšpler, Eduard Havel, Vladimír Černý, Tomáš Dědek, Vladimír Palička, Jan Krejsek, Martina Loudová, Luboš Sobotka

**Starting date:** 1.1.2003

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 2150000

#### **Summary of 2003 results**

**Title of the presentation:** The changes in the metabolism of cholesterol and its precursors in the patients in critical care

**Authors:** Zadák Z. (1), Hyšpler R. (1), Havel E. (2), Černý V. (3), Dědek T. (2), Palička V. (4), Krejsek J. (5), Loudová M. (5), Sobotka L. (1)

(1) Dept. of Metabolic Care and Geront., (2) Dept. of Surgery, (3) Dept. of Anesth. and Int. Care., (4) Inst. for Clinical Biochem. and Diagn., (5) Dept. of Clin. Immun. and Allergy, Charles Univers. in Prague, Faculty of Medicine, Hradec Králové

During high stress situations, patients dependent on cholesterol supply from the diet ("absorbers") will develop a larger decrease of the plasma concentration of LDL lipoproteins comparing to "synthesizers". The absorption - dependent patients have a lack of enzymes in cholesterol synthetic pathway and there are only cholesterol-free formulas for enteral and parenteral nutrition available on the market. Consequently, an insufficiency of adrenals and granulocytes develops as a result of a low LDL cholesterol level.

Cholesterol is probably a conditionally essential nutrient and the possibility of its deficiency cannot be overlooked during high requirement periods. Pilot studies were already performed in our department and a much lower rate of cholesterol and lathosterol synthesis was found in intensive-care patients.

Serum concentrations of squalene, lanosterol, and lathosterol were measured on admission, and then at 24 and 48 hours after injury using gas chromatography coupled with mass spectrometry. Serum concentrations of total low-density and high-density lipoprotein cholesterol were measured on admission and every day in the first week after injury.

Results: 83 consecutive patients with multiple trauma were examined. Significant drops in concentrations of lanosterol and lathosterol were found in the patients in comparison with the control group. The most profound drop was in lathosterol.

Conclusion: Decreased synthesis of cholesterol precursors is the major cause of hypocholesterolemia in patients with multiple trauma. Lathosterol concentration is proposed as a marker of cholesterol synthesis.

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**Title of the research project:** Complex utilization of Amaranth biomass

**Grant Agency:** Ministry of Industry and Trade      **Project Number:** FD - K2/73

**Principal Researcher:** Zdeněk Zadák

**Joint Researchers:** Petr Žďánský, Dagmar Solichová, Radomír Hyšpler, Alena Tichá, Jana Tilšarová, Jana Krejcarová

**Starting date:** 1.1.2002

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 800000

**Summary of 2003 results**

**Title of the presentation:** Complex utilization of Amaranth biomass

**Authors:** Zdeněk Zadák, Petr Žďánský, Dagmar Solichová, Radomír Hyšpler, Alena Tichá

The monitoring of nutritive effectivity of Amaranth plant in the selected growth phases was performed and possibilities for its utilization as a foodstuff (incl. its fortification) were evaluated.

The aim of the project is a development and realization of the technology for fractionation of amaranths biomass, nutritionally important components, i.e. flavonoids, fibre, protein, natural vitamins. Next research step is intensive utilization of amaranth biomass as a substrate for fermentation to biogas and monitoring of fermentative procedure for energy technology. Fibre fermentation in human digestive tract was simulated and versatile methods for its evaluation were developed.

The project is focused on some basic aspects:

1. Dietetic products -fibre extracts ant supplements for food fortification.

Amaranth biomass are rich in fibre and calcium. Fresh leaves of amaranth contain in average 1,3% of fibre, dried leaves from 7 to 14,3%. For comparison, raw spinach contains 0.6% of fibre, dried spinach 6%.

2. Amaranth biomass is rich in protein (dry mass contents about 20%) of high quality and rich in lysine.

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**Title of the research project:** Development of fat emulsion for parenteral nutrition and technical realization of its application

**Grant Agency:** Ministry of Industry and Trade      **Project Number:** FD-K/033

**Principal Researcher:** Jan Mikeska

**Joint Researchers:** Ladislav Červinka, Tadeáš Graichman, Alois Kozubík, Jiřina Hofmanová, Zdeněk Zadák, Radomír Hyšpler, Luboš Sobotka, Iva Macková, Irena Francová, Kateřina Stankeová, Zdeněk Koblíček, Marie Mňuková

**Starting date:** 19.7.2001

**Duration (years):** 3

**Funds allocated for project - total in Czech crowns:** 3000000

**Summary of 2003 results**

**Title of the presentation:** Report of solution project in programme KONSORCIA for Ministry of Industry and Trade of the Czech Republic (2003)

**Authors:** Jan Mikeska (1), Tadeáš Graichman (1), Alois Kozubík (2), Jiřina Hofmanová (2), Zdeněk Zadák (3), Luboš Sobotka (3), Radomír Hyšpler (3), Kateřina Stankeová (1) Infusia a.s. Hořátek (1), Institute of Biophysics, Academy of Sciences of the Czech Republic (2), Fac. of Medicine, Charles University, Hradec Králové (3)

In the Report are described all steps and practical results of team work in development of new fat emulsion for parenteral nutrition. The project is based on a wide co-operation of laboratory of cytokinetics, clinical centre and manufacturer. Thus, basic research, development and application is connected.

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**Title of the research project:** Ultrasound imaging methods in the practical teaching of medical biophysics

**Grant Agency:** Ministry of Education

**Project Number:** 2964/H/2003

**Principal Researcher:**

**Joint Researchers:** Jiří Záhora

**Starting date:** 1.1.2003

**Duration (years):** 1

**Funds allocated for project - total in Czech crowns:** 860000

**Summary of 2003 results**

**Title of the presentation:** Ultrasound imaging methods in the practical teaching of medical biophysics

**Authors:** Jiří Záhora

Charles University in Prague, Faculty of Medicine in Hradec Králové, Dept. of Medical Biophysics

The aim of this project was to modernize the laboratory for practical teaching of medical biophysics at medical faculty. The new practical assignment was developed: "Blood flow rate measuring by means of ultrasound". The laboratory was equipped with ultrasound imaging unit and phantom for measuring. The imaging unit is able to work in B-mode, in Color Flow and Power Doppler mode. It contains also hard disk and software for storing and managing patient data. Our own dynamical phantom was developed. The phantom is simulating blood vessel system, blood is simulated by means of water with small air bubbles, the flow rate is approximately 5 L/min. The students must localize the vessels, measure their diameters, confirm the equation of continuity, create 3D reconstruction of the vessel system and complete a final report. The teaching program was developed too. This program is able to simulate propagation of ultrasound in various biological tissues, its absorption and reflection on interfaces between tissues with different acoustical impedance. A set of instruction images was prepared too.

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