II. VĚDECKÁ KONFERENCE

LÉKAŘSKÉ FAKULTY UNIVERZITY KARLOVY
A
FAKULTNÍ NEMOCNICE V HRADCI KRÁLOVÉ

P R O G R A M

16. - 17. prosince 1997

Velká posluchárna teoretických ústavů LF UK,
Šimkova 870, Hradec Králové

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

GA ČR	Grantová agentura České republiky
GA UK	Grantová agentura Univerzity Karlovy
NPZ	Projekt podpory zdraví Ministerstva zdravotnictví ČR
IGA MZ	Interní grantová agentura Ministerstva zdravotnictví ČR
FRVŠ	Fond rozvoje vysokých škol

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

Postery budou vyvěšeny po celou dobu konání konference. Panelová diskuse k plakátovým sdělením je na programu **po oba dny od 15.00 do 15.30 hodin.**
Po tuto dobu bude u panelu přítomen řešitel nebo spoluřešitel projektu.

T e c h n i c k é p o k y n y

1. Doba sdělení 10 minut, diskuse 5 minut.
2. K dispozici je projekce diapozitivů 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.

Úterý 16. 12. 1997

13.00 - 13.15	**Z a h á j e n í** **prof. MUDr. Ivo Šteiner, CSc., děkan fakulty**
Sekce I	**Předsedající: prof. MUDr. Jiří Kvasnička, CSc.**
13.15 - 13.30	Vývoj antioxidační rovnováhy a kvalitativní změny sérových lipoproteinových frakcí během hypolipidemické terapie **MUDr. Eduard Havel** GA UK 153/95 **(LF)**
13.30 - 13.45	Metabolismus aminokyselin u odlišných forem jaterního poškození **doc. MUDr. Milan Holeček, CSc.** GA UK 152/95 **(LF)**
13.45 - 14.00	Troponin T v neonatologii **doc. MUDr. Zdeněk Kokštein, CSc.** GA UK 158/95 **(LF)**
14.00 - 14.15	Rozšíření praktické výuky molekulární biologie **doc. MUDr. RNDr. Miroslav Červinka, CSc.** FRVŠ 1260/97 **(LF)**
14.15 - 14.30	Vývoj programů pro výuku lékařské chemie a biochemie **Ing. Pavel Šiman, CSc.** FRVŠ 1420/97 **(LF)**
14.30 - 15.00	***P ř e s t á v k a - občerstvení, prohlídka plakátových sdělení***
15.00 - 15.30	**Panelová diskuse k plakátovým sdělením**
Sekce II	**Předsedající: prof. MUDr. Stanislav Němeček, DrSc.**
15.30 - 15.45	Vývoj a validizace nových metod pro zjišťování nežádoucích účinků stomatologických materiálů **doc. MUDr. RNDr. Miroslav Červinka, CSc.** IGA MZ 3263-3/95 **(LF)**
15.45 - 16.00	Lokalizační diagnostika inzulárních nádorů pankreatu **doc. MUDr. Pavel Eliáš, CSc.** IGA MZ 2949-3
16.00 - 16.15	Stanovení algoritmu pro komplexní diagnostiku Lymeské boreliózy a faktorů humorální a buněčné imunity mající vliv na persistenci borelií v organismu **MUDr. Karel Honegr** IGA MZ 2962-3

16.15 - 16.30	Význam hladin sérového interleukinu 1beta u vlasatobuněčné leukémie, korelace s nádorovou masou a hladinami s IL-2R **prof. MUDr. Ladislav Chrobák, CSc.** IGA MZ 3690-2
16.30 - 16.45	Endoskopická adenotomie **MUDr. Viktor Chrobok** IGA MZ 2946-3
16.45 - 17.00	Studium střevní propustnosti v závislosti na poškození tenkého střeva a možnosti léčebného ovlivnění **MUDr. Pavel Kohout** IGA MZ 2951-3
17.00 - 17.15	Využití dopplerovské ultrasonografie pro screening renovaskulárního typu hypertenzní nemoci **doc. MUDr. Pavel Eliáš, CSc.** IGA MZ 2965-3
17.15 - 18.00	**Prohlídka plakátových sdělení**

Středa 17. 12. 1997

Sekce III	**Předsedající: prof. MUDr. Zbyněk Hrnčíř, DrSc.**
13.00 - 13.15	Klinické využití cytotoxických buněk aktivovaných lymfokiny - adoptivní imunoterapie maligního melanomu a karcinomu ledvin **MUDr. Otakar Kopecký, CSc.** IGA MZ 2945-3
13.15 - 13.30	Algoritmus vyšetřování potravinových alergií, zavedení dvojitě slepého expozičního testu kontrolovaného placebem **MUDr. Pavel Kohout** IGA MZ 2952-3
13.30 - 13.45	Využití průtokové cytometrie a dvojího značení buněk pro imunofenotypizaci leukémií **MUDr. Otakar Kopecký, CSc.** IGA MZ 2944-3
13.45 - 14.00	Nová elektrofyziologická vyšetření pro neurooftalmologickou diagnostiku **doc. MUDr. Miroslav Kuba, CSc.** IGA MZ 3230-3/95 **(LF)**

14.00 - 14. 15	Imunocytochemie adenohypofysy a hypofysárních tumorů. Korelace mezi imunocytochemickými a klinickými nálezy. **MUDr. Radovan Lomský** IGA MZ 2979-3
14.15 - 14,30	Kultivace nervových kmenových buněk a jejich transplantace do mozku příjemce **MUDr. Jaroslav Mokrý** IGA MZ 3233-3/95 **(LF)**
14.30 - 15.00	***P ř e s t á v k a - občerstvení, prohlídka plakátových sdělení***
15.00 - 15.30	**Panelová diskuse k plakátovým sdělením**
Sekce IV	**Předsedající: prof. MUDr. Pavel Rozsíval, CSc.**
15.30 - 15.45	Změny energetického metabolismu a tělesných kompartmentů během absolutního hladovění **doc. MUDr. Luboš Sobotka, CSc.** IGA MZ 2963-2
15.45 - 16.00	Multimodální evokované potenciály ve včasné diagnostice roztroušené sklerózy mozkomíšní **doc. MUDr. Gerhard Waberžinek, CSc.** IGA MZ 2980-3
16.00 - 16.15	DAR - Děti a rodiče **doc. MUDr. Eva Pařízková, CSc.** NPZ 28/97 **(LF)**
16.15 - 16.30	Ke zdravotnímu riziku azbestu a jiných vláknitých materiálů v pracovním prostředí **MUDr. Jaroslav Tejral, CSc.** NPZ 51/122/96 **(LF)**
16.30 - 16.45	Motion related visual evoked potentials (VEPs) and their diagnostic application in neuro-ophthalmology **doc. MUDr. Miroslav Kuba, CSc.** Projekt v rámci programu COST **(LF)**
16.45 - 17.00	**Ukončení konference**

PŘEHLED PLAKÁTOVÝCH SDĚLENÍ

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1. Dětské postupy při zvládání zátěžových situací
   doc. PhDr. Jiří Mareš, CSc.
   GA ČR 406/97/0158 **(LF)**
2. Kultivace neurálních prekurzorových buněk a modifikace jejich dalšího vývoje in vitro
   MUDr. Jaroslav Mokrý
   GA ČR 304/97/1117 **(LF)**
3. Sledování vybraných diferenciačních markerů u lidských maligních gliomů kultivovaných in vitro
   MUDr. Viktor Bartanusz, CSc.
   GA UK 54/97 **(LF)**
4. Nová cytostatika a imunosupresiva
   doc. MUDr. RNDr. Milan Mělka
   GA UK 69/96 **(LF)**
5. Kvalitativní změny sérových lipoproteinových frakcí a vývoj antioxidační rovnováhy během hypolipidemické terapie
   MUDr. Vladimír Bláha, CSc.
   IGA MZ 2967-3
6. Lymeská borrelióza: konstrukce rekombinantní DNA jako vnitřní pozitivní kontroly polymerázové řetězové reakce (PCR) při molekulární detekci borrelií
   MUDr. Zdeněk Fiedler
   IGA MZ 3321-3
7. Nová cytostatika - problematika jejich kardiotoxicity a potenciální kardioprotektivní účinnosti
   doc. MUDr. Vladimír Geršl, CSc.
   IGA MZ 4212-3/97 **(LF)**
8. Familiární hypertrofická kardiomyopatie: detekce mutací na exonu 13 v genu pro těžký řetězec beta myosinu
   PharmDr. Radovan Haluza
   IGA MZ 2971-3
9. Vývoj počítačem řízené multitermočlánkové soupravy pro účely měření teploty v hypertermii
   Ing. Josef Hanuš, CSc.
   IGA MZ 3771-3/96 **(LF)**
10. Vztah hormonální léčby žen k průtoku krve žilním systémem dolních končetin a extrakraniálním úsekem mozkových tepen s využitím duplexní sonografie
    MUDr. Ivo Kalousek
    IGA MZ 2975-3

11. Kardiovaskulární systém u fyziologických a patologických novorozenců
    doc. MUDr. Zdeněk Kokštein, CSc.
    IGA MZ 2938-3
12. Predikce dávkovacího schématu vankomycinu a aminoglykosidů u nedonošených novorozenců v prvním týdnu postnatálního života
    PharmDr. Jana Kopecká
    IGA MZ 2950-3/95
13. Screening poruch metabolismu tryptofanu
    MUDr. Eliška Marklová, CSc.
    IGA MZ 4097-3/97
14. Kompozitní dermoepidermální štěp pro léčbu popálených
    MUDr. Pavel Měřička
    IGA MZ 3696-3
15. Protilátky a cytokiny v séru a v buněčných kulturách u dětí s primárními imunodeficity
    doc. MUDr. Eva Pařízková, CSc.
    IGA MZ 4098-3/97
16. Diagnostika proarytmického účinku antiarytmik
    prof. MUDr. Vladimír Pidrman, DrSc.
    IGA MZ 2948-3
17. Vliv antioxidační rovnováhy na klinický stav a biochemické parametry dlouhověkých pacientů - otevřená prospektivní klinická studie
    RNDr. Dagmar Solichová
    IGA MZ 2953-3
18. Recividující mykotické infekce ženského genitálu. Optimalizace diagnostiky, terapie a profilaxe.
    MUDr. Jiří Špaček
    IGA MZ 3694-3
19. Vysokodávkovaná chemoterapie s podpůrnou léčbou plné krve bohaté na kmenové buňky
    MUDr. Jaroslav Vaňásek, CSc.
    IGA MZ 3679-3
20. Ovlivnění kvality štěpu stupněm rozvoje postagresivních mediátorových sítí u dárce ledvin
    MUDr. Helena Živná, CSc.
    IGA MZ 3689-3/96
21. Trojrozměrná organizace synapsí a endoplazmatického retikula dendritických trnů
    prof. MUDr. Josef Špaček, DrSc.
    NIH BETHESDA TW 00178

PŘEHLED OSTATNÍCH PŘEDANÝCH SOUHRNŮ

1. Nové možnosti tympanometrické diagnostiky
   MUDr. Petr Čelakovský
   IGA MZ ČR 4 100-3/97
2. Studium poškození a reparace hepatocytů po působení hepatotoxickými látkami in vivo a in vitro
   doc. MUDr. Zuzana Červinková, CSc.
   GA UK 67/96
3. Stanovení racionálních diagnostických a léčebných postupů u purulentních meningitid se současným zánětlivým postižením ucha, nosu nebo vedlejších nosních dutin v rámci interdisciplinární péče
   doc. MUDr. Václav Dostál
   IGA MZ 3687-3
4. Environmental Exposure of Children to PAH in Czech Republic and Canada
   Ing. A. Vyskočil, Université de Montréal, Canada - odpovědný řešitel
   Ing. Zdeněk Fiala, CSc. - spoluřešitel
   NATO ENVIR.CRG 970418
5. Citlivost na kontrast a rozlišovací schopnost oka při laserové fotorefraktivní keratektomii
   doc. MUDr. Dagmar Hejcmanová, CSc.
   GA UK 59/97
6. Regulace syntézy a rozpadu proteinů
   doc. MUDr. Milan Holeček, CSc.
   IGA MZ 3772-3
7. Kvantifikace HBV DNA a určení sérotypového HCV - rozšíření kritérií výběru nemocných VHB a VHC pro léčbu interferonem
   doc. MUDr. Jiří Horáček, CSc.
   IGA MZ ČR 3692-3
8. Histopatologie sluchového orgánu a spánkové kosti u fetu a novorozence
   MUDr. Viktor Chrobok
   IGA MZ 3682-3
9. Matematické modelování zrakového vnímání pohybu
   Ing. Jan Kremláček
   GA UK 56/97
10. Počítačové zpracování elektrické aktivity mozku pro hodnocení funkcí centrálního nervového systému
    doc. MUDr. Miroslav Kuba, CSc.
    GA ČR 309/96/0959

11. Stanovení polymorfismu cytochromu P450 3A za pomoci modelového substrátu - endogenního kortizolu
    MUDr. Stanislav Mičuda
    GA UK 57/97
12. Interleukin IL-6 v oční komorové vodě
    MUDr. Jan Novák, CSc.
    IGA MZ 4096-2/97
13. Dekomprese obalů zrakového nervu
    prof. MUDr. Pavel Rozsíval, CSc.
    GA ČR 308/96/0756
14. Ke stavu životního prostředí a zdraví člověka
    prof. RNDr. Vladimír Srb, DrSc.
    GA UK 70/96
15. Účinek a farmakokinetika methotrexátu v léčbě psoriázy
    MUDr. Marie Šimková
    IGA MZ 2960-3

SOUHRNY VÝZKUMNÝCH ÚKOLŮ

ŘEŠENÝCH NA LF UK A FN V HRADCI KRÁLOVÉ

(ABECEDNĚ)

Name of the research project:

Differentiation markers in human malignant glioma explant cultures

Grant Agency: GA of the Charles University ***Project number:*** 54/97

Researcher: Viktor BARTANUSZ

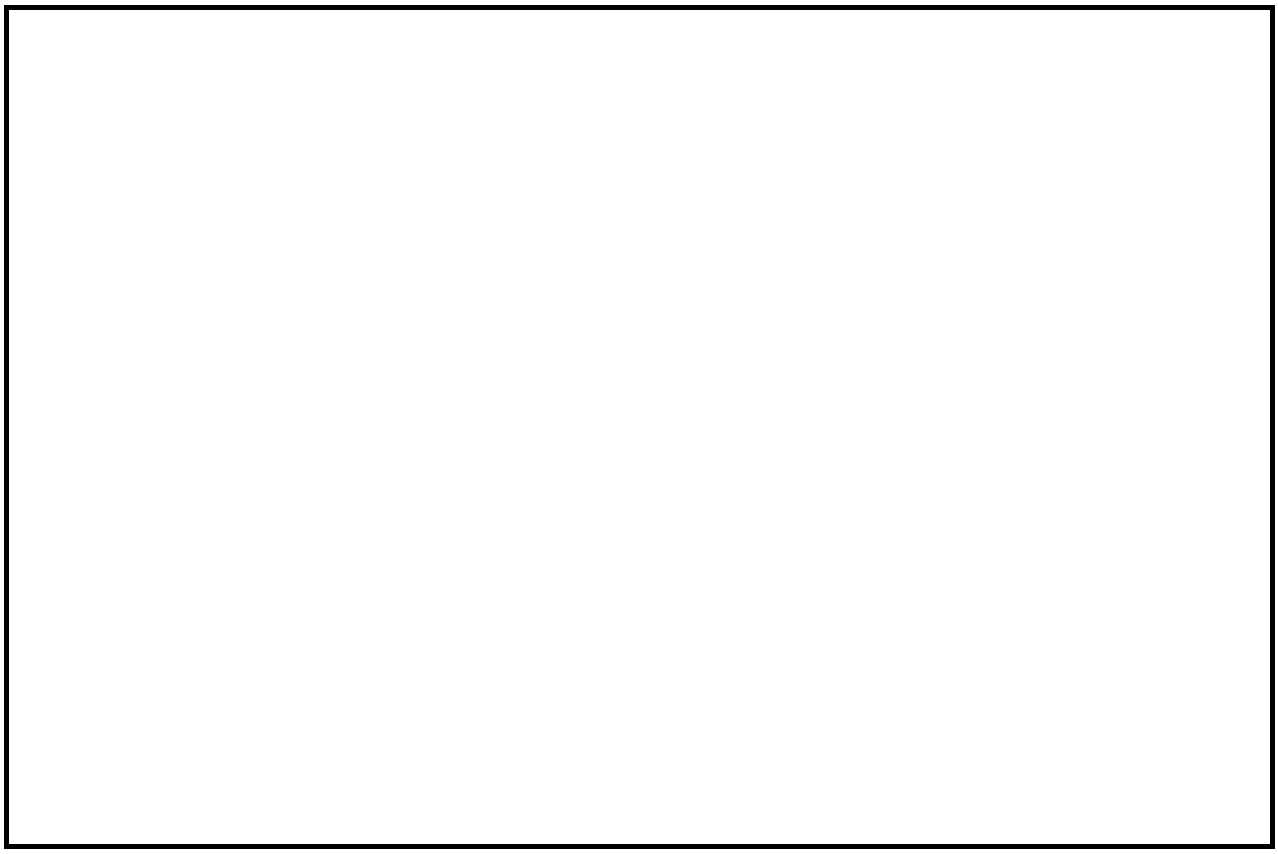
Joint Researchers: Jaroslav MOKRÝ, Stanislav NĚMEČEK, Jiří NÁHLOVSKÝ,

Starting date: 1997 ***Duration:*** 3 yrs.

Funds allocated for project - total: 100.000,- CZK

S u m m a r y o f r e s u l t s :

Differentiation inducing agents, such as hexamethylene bisacetamide, sodium butyrate, retinoic acid, phenylacetate or lovastatin are agents in experimental use that are capable of inducing morphological and biochemical differentiation in malignant tumor cell lines in culture and are believed to be a promise for the future treatment of glial tumors. Intermediate filament proteins, like GFAP, vimentin and nestin are being used as tumor markers to evaluate the degree of differentiation - malignancy of glial tumors; GFAP being positive mainly in low-grade astrocytomas and nestin in anaplastic astrocytoma and glioblastoma multiforme. In this respect, detection of differentiation antigens in testing the therapeutic effectiveness of differentiation inducing drugs is a methodological prerequisite. In our study, the immunoreactivity for GFAP, vimentin and nestin was detected in human glioma explants grown in vitro. A specimen of tumor obtained at the operation theater was dissected in ice cold glucose-saline medium and put in culture as an explant of 2-3 mm in diameter on poly-L-lysine coated slides. The other part of the tumor was processed for neuropathological diagnosis. Tumor explants were cultured in DMEM supplemented with 10% FCS, L-glutamine (2 mM), and gentamicine (10 mg/ml) at 37 °C in an incubator with humidified atmosphere with 5% CO_2. Immunoreactivity for the above mentioned differentiation markers of fixed monolayers of tumor cells was compared with histological sections of the same resected specimen.



Name of the research project:

Consequences of reduced plasma cholesterol fractions during hypolipidemic therapy, lipoperoxidation activity and the distribution of antioxidant vitamin E in lipoprotein fractions.

Grant Agency: IGA MZ CR ***Project number:*** 2967-3

Researcher: **MUDr. Vladimír Bláha, CSc.**

Joint Researchers:

MUDr.Eduard Havel, MUDr.Pavel Kohout, RNDr.Miluše Brátová, RNDr.Dagmar Solichová,.

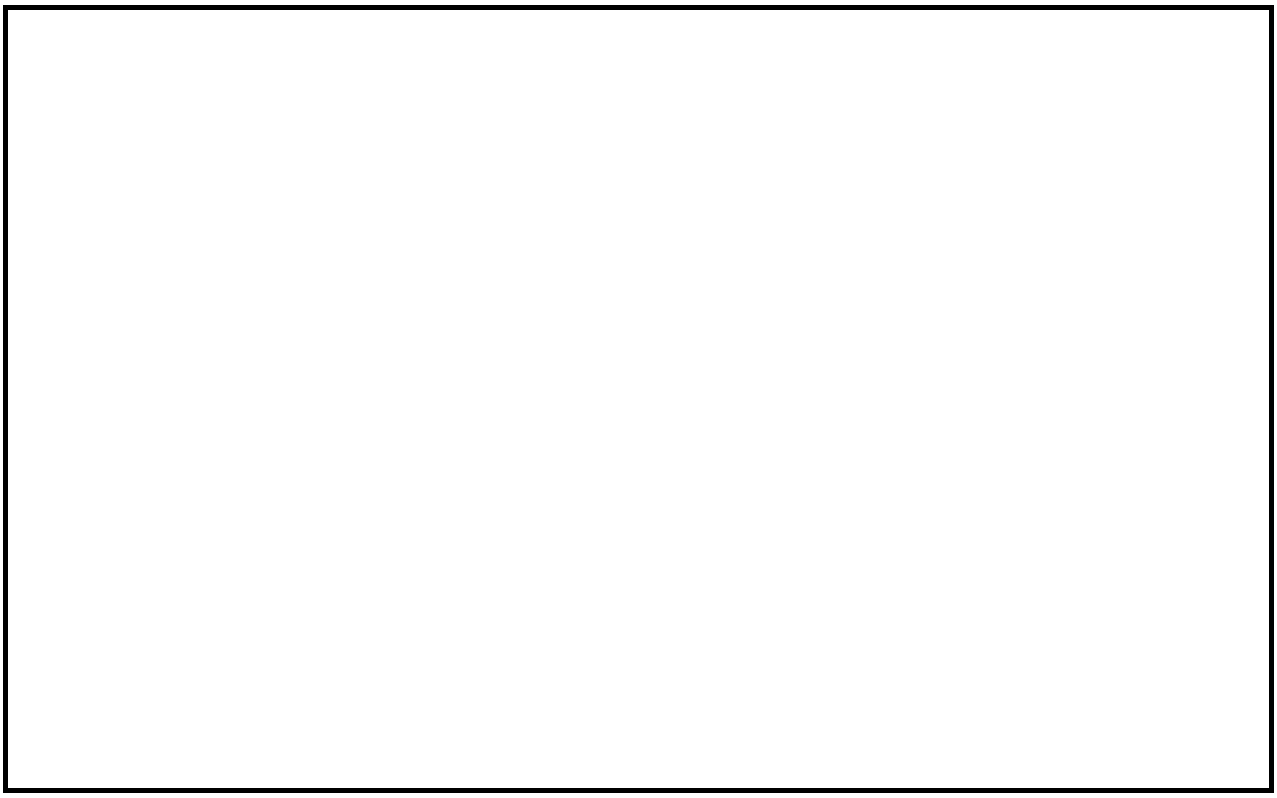
Prof. MUDr Zdeněk Zadák,CSc.

Starting date: 1995 ***Duration:*** 3 years

Funds allocated for project - total: 1.487.000 CZK

S u m m a r y o f r e s u l t s :

Inroduction: There is evidence that statins may have other anti-atherogenic effects besides their lipid-lowering activity, including effects on oxidability of lipoproteins. Thus the aim of present study was to examine consequences of reduced plasma cholesterol during hypolipidemic therapy, lipoperoxidation activity and the distribution of antioxidant vitamin E in lipoprotein fractions.
Material and Methods: A group of 54 patients with hypercholesterolaemia was treated using simvastatin (14 patients, 20mg daily), pravastatin (15 patients, 40mg daily) or fluvastatin (10 patients, 40mg daily). To investigate antioxidant effects of fibrates, another group of 15 patients was treated with fenofibrate (200mg daily). Blood samples were examined before treatment, after 4 and 8 weeks of therapy. After ultracentrifugation, samples were analyzed for vitamin E content in lipoprotein fractions. Antioxidant status was examined using serum thiobarbituric acid reacting substance (TBARS) activity. Results: Both simvastatin and pravastatin were effective hypolipidemic agents I. With simvastatin, total serum vitamin E was reduced during hypolipidemic therapy (44.54±3.62 vs. 36.85±1.72 μmol/l; p=0.06). However, the ratio of serum vitamin E/total serum cholesterol (4,86±0.31 vs. 5.63±0.28 μmol/mmol; p=0.09) and ratio of LDL-C vitamin E/LDL-C (3.57±0.31 vs. 3.67±0.31 μmol/mmol; n.s.) did not change, and the ratio of IDL-C vitamin E/IDL-C (4.44±0.32 vs. 5.40±0.61 μmol/mmol; p<0.01) and HDL-C vitamin E/HDL-C (3.78±0.41 vs. 5.83±0.49 μmol/mmol; p=0.01) significantly increased. Serum TBARS significantly decreased (6.97±0.69 vs. 4.72±0.48 μmol/l; p<0.001). II. With pravastatin, serum vitaminu E was decreased in the fractions of total cholesterol, LDL1, LDL2 a VLDL-cholesterol. However, the ration vitamin E/total cholesterol (4.57±0.32 vs. 5.12±0.37 mmol/l/mmol/l; p<0.05) a ratio LDL2-C vitamin E/LDL2-C (3.92±0.07 vs. 4.64±0.37 mmol/l/mmol/l; p=0.08) increased vs. baseline. III., IV. The results obtained with fluvastatin and fenofibrate will be available within one month.
Conclusion: We conclude, that effective hypolipidemic treatment with statins is associated with improved antioxidant status and proportional increase in the serum content of vitamin E in HDL and IDL cholesterol fraction, and that the content of vitamin E in total and LDL-cholesterol did not change despite the decreased concentration of its lipid carrier. With regard to functions of vitamin E, this may be an additional anti-atherogenic effect of such therapy.



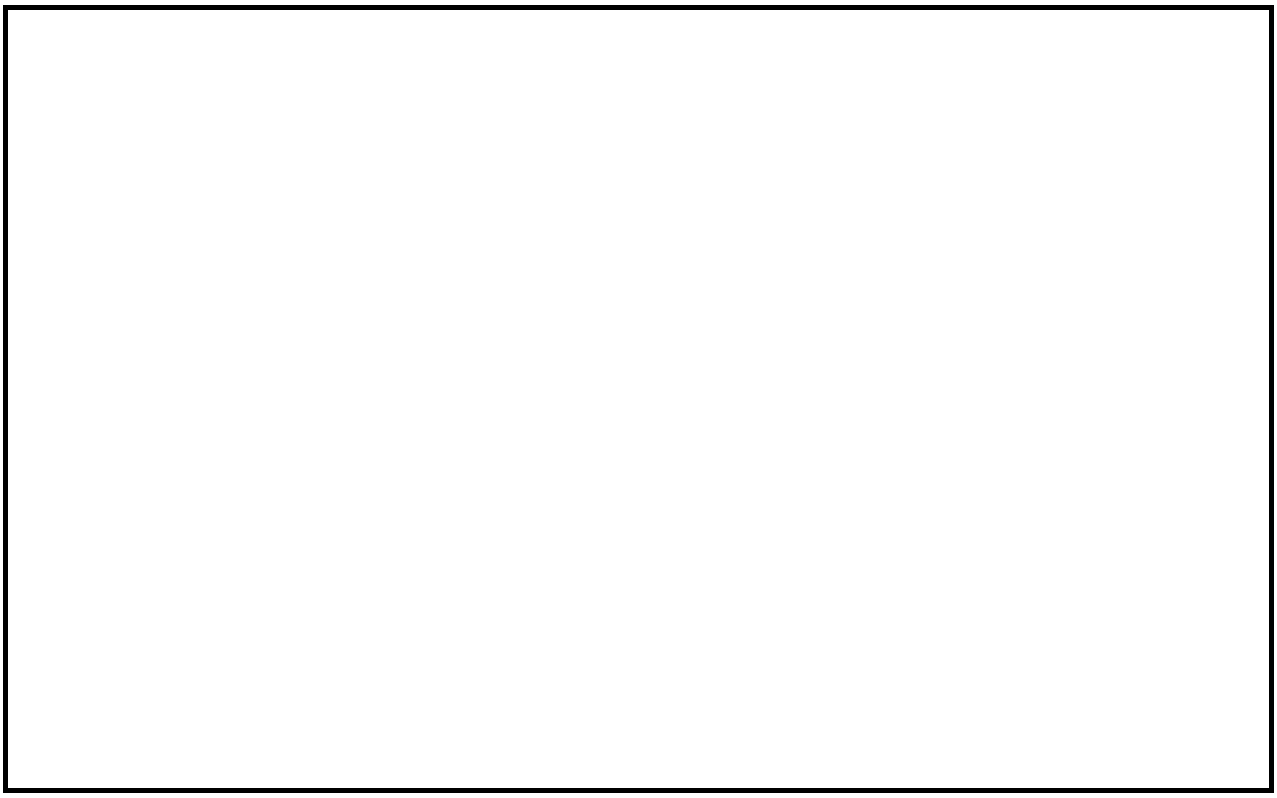
Name of the research project:
The new possibilities of tympanometric investigation

Grant Agency: *IGA Ministry of Health*	***Project number:*** *IGA 4 100-3 93-82*
Researcher: *Petr Čelakovský*	
Joint Researchers: *: Viktor Chrobok Ivan Hybášek*	
Starting date: *1.1.1997*	***Duration:*** *3 years*
Funds allocated for project - total: *326 OOO for the first year*	

Summary of r esults:

Results, published in 1997:
P. Čelakovský, V. Chrobok: Causes of a Positive Pressure in the Middle Ear Cavity in Tympanometric Examination

The authors evaluate a group of 62 patients, where they diagnosed in 1993 - 1995 66times a positive pressure in the middle ear cavity during tympanometric examination. Based on their own experiance and data in the literature they found that a positive pressure in the middle ear may be caused by an air current during politzerization or catheterization of the auditory tube / a similar effect can be produced also by sneezing , blowing of the nose, crying /, acute tubotympanic catarrh, acute otitis media in the initial or regressing stage, or as a result of pressure changes in the middle ear during general inhalation anesthesia using nitrous oxide. Based on statistical processing of the results by the non-paired t-test the authors reach the conclusion, that in the majority of patients the positive pressure in the middle ear is not associated with conduction deafnes and that there is no relationship between the pressure and the hearing loss. There is however a relationship between the elasticity of the conduction system and the presence of conduction disorder. The stapedial reflex can be evoked in cca half of ears with positive pressure in the middle ear. However, there is no relationship between the pressure and evoking of the stapedial reflex. The stapedial reflex is however more readily evoked on ears with a greater elasticity of the conduction system



Name of the research project:

Improvement of practical training in molecular biology at Charles University Faculty of Medicine in Hradec Králové

Grant Agency: **FRVŠ**	***Project number:*** **1260**
Researcher: Doc. MUDr. RNDr. Miroslav Èervinka, CSc.	
Joint Researchers: PharmDr. Radovan Haluza Doc. MUDr. Jiøí Horáèek, CSc.	
Starting date: 1. 1. 1997	***Duration:*** 1 year
Funds allocated for project - total: 355000,-Kè	

S u m m a r y o f r e s u l t s :

Molecular biology is one of the most rapidly growing biomedical disciplines with immediate impact on clinical practice, mainly in the field of diagnosis. Therefore it is essential to accommodate curricula at medical faculties. This is true not only for graduate training but for postgraduate training as well. The curricula will be insufficient without practical courses in important basic techniques of molecular biology.

The main problem connected with the introduction of molecular biology is a high demand for financial resources. Therefore teachers from several departments at our faculty (biology, biochemistry, microbiology, immunology, genetic) joined and prepared a co-ordinated scheme for teaching practicals focused on molecular biology.

The main goal of this project is further improvement of practical classes from molecular biology. In our original proposal we would like to introduce the following new techniques: isolation of genomic DNA from human cells, agarose gel electrophoresis of DNA, PCR, blotting and hybridisation of nucleic acid. All these methods belong to basic standard in modern molecular laboratory. Due to the fact that the proposed budget was substantially lowered (to the 35 % of proposed value), we prepared a new reduced version which will still allow introduction at least some essential techniques. During this year we prepare protocols for new practical classes. This laboratory classes are now obligatory for students of biology. Newly built laboratory facilities are utilised also by several postgraduate students.

Name of the research project:

Development and validation of new methods for the assessment of toxic effects of stomatological materials

Grant Agency:	**Ministry of Health**	***Project number:***	**3263-3**
Researcher:	Doc. MUDr. RNDr. Miroslav Červinka, CSc.		
Joint Researchers:	Doc. RNDr. Miroslav Hroch, CSc. MUDr. Jana Kolářová, CSc. MUDr. Jaroslav Koupil Prof. MUDr. Lubor Novák, DrSc. MUDr. Jan Peychl Prof. MUDr. Vladimír Půža, DrSc. Mgr. Emil Rudolf		
Starting date:	1. 1. 1995	***Duration:***	3 years
Funds allocated for project - total:	1 181 000 Kč		

S u m m a r y o f r e s u l t s :

The goal of this project is to compare tests recommended for toxicity assessment of stomatological materials by the international standard ISO 10933 (Millipore filtration test, Agar diffusion test) with tests recommended by the Czech Committee for New Dental Materials (Cell proliferation assay, Direct contact assay) with newly developed photometric tests based on cultivation of cells in microtitration plates (96 well plates). During the last three years we have tested the toxicity of metal dental alloys (TI 45), root filling materials (AH26, Dexamethasone), and this year we have focused our attention on composite restorative materials (Evicrol Solar LC) and dental amalgams (ANA 2000).

Our results demonstrate a basic correlation between the different cytotoxicity tests. Nevertheless, in several cases we have found surprising differences. Therefore we recommend using a battery of the following basic tests as a standard: Agar diffusion test, Cell proliferation assay and Mitochondrial activity assessment by WST-1 assay. Based on our results we will prepare a proposal for the Committee for New dental materials. Optimised standard operating protocols for each tested protocol are available.

Results of this project were presented at 15 scientific meetings, six of them abroad, and 25 scientific papers related to the project were published.

Name of the research project:

Study of hepatocyte injury and repair induced by hepatotoxic agents *in vivo* and *in vitro*

Grant Agency: **GAUK** ***Project number:*** **67/1996**

Researcher: Doc. MUDr. Zuzana Červinková, CSc.

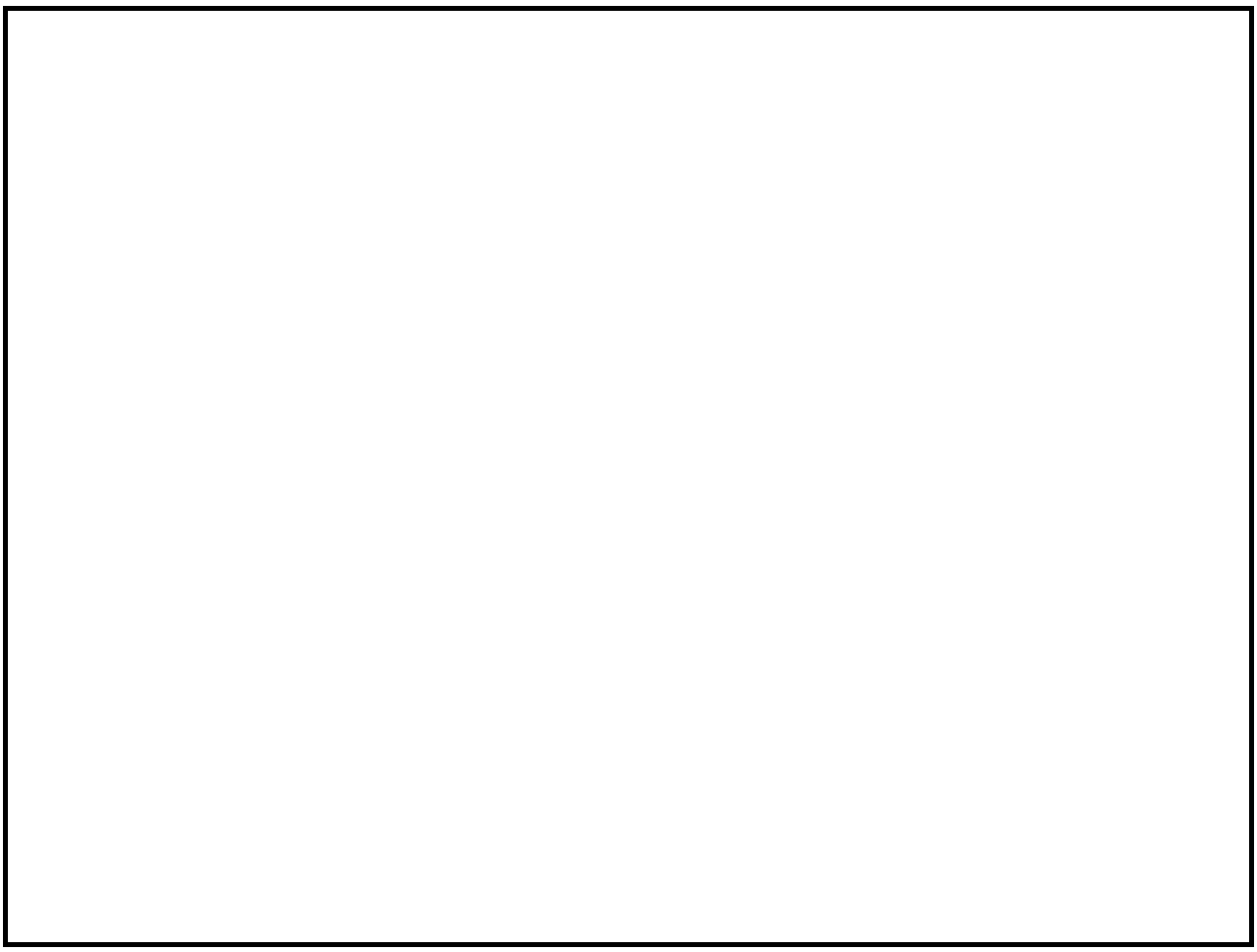
Joint Researchers: Doc. MUDr. Miroslav Červinka, CSc.
Doc. MUDr. Danuše Šubrtová, CSc.
MUDr. Halka Lotková
MUDr. Renata Svátková

Starting date: 1. 1. 1996 ***Duration:*** 3 years

Funds allocated for project - total: 328 000 Kč

S u m m a r y o f r e s u l t s :

The goal of our project is to characterise hepatocyte injury induced by administration of various hepatotoxic agents (tetrachlormethane, thioacetamide [TAA], galactosamine [GalN], endotoxin) in *in vivo* and *in vitro* conditions. Although we have been engaged for many years in experimental hepatology, we had no experience with *in vitro* methods. Therefore the first step was to adopt a technique of high-yield preparation of isolated hepatocytes. A two-step procedure using liver perfusion with collagenase containing medium (Seglen 1972) was used for preparation of hepatocyte suspension. We tested the influence of different parameters (time factor, temperature, oxygen saturation, collagenase concentration etc.) on the viability of isolated hepatocytes. Viability of hepatocytes in suspension was measured using the Trypan blue exclusion test. The next step was to introduce a method of hepatocyte primoculture, in this experiments we tested influence of cultivation media on viability of hepatocytes. To evaluate changes in cell viability during cultivation we developed a method of Trypan blue staining in monolayer. The most important source of information on cell viability and types of cells in primoculture was long lasting recording of cells in phase contrast using time-lapse video recorder (Mitsubishi). Metabolic activity of hepatocytes was evaluated by measuring mitochondrial activity (WST-1 test). Our *in vivo* experiments documented that TAA or GalN induced liver necrosis was accompanied by significant decrease of cytochrome c oxidase activity both in isolated liver mitochondria and in liver homogenates. This key enzyme is considered an indicator of cell oxidative capacity. Our data indicates that affected liver oxidative capacity could participate in TAA or GalN induced liver injury.



Name of the research project:

Determination of rational diagnostic and therapeutic procedures in purulent meningitis with simultaneous ear,nose, and paranasal sinuses inflammatory affection within the framework of interdisciplinary care.

Grant Agency: IGA MZ CR ***Project number:*** 3687-3

Researcher: Assoc. Prof. Václav Dostál, M.D.

Joint Researchers:

Prof. A. Pellant, M.D., V. Chrobok, M.D., S. Plíšek, M.D., K. Honegr, M.D., Z. Hermanová, M.D., A. Michl, M.D., M. Hartmann, Mgr.

Starting date: January 1, 1996 ***Duration:*** 3 years

Funds allocated for project - total: 1493150,- CZK

Summary of results :

Beside be well-know decrease in the number of the patients with inflammatory otogenic and rhinogenic intracranial complications and improved results of treatment due to antibiotics, patients have been also provided with more specialized and complex therapy recently.

The aim of this study:

1. The rational diagnosis and treatment of leptomeningitis, which are observed simultaneously with suppurative inflammation of the ear, nose and paranasal sinuses.

2. To compare the results of treatment in two periods, the retrospective study of period 1990-1994 and prospective study of period 1996-1998.

3. **To determine the role of ENT department and department of infectious diseases in diagnosis and treatment of these diseases.**

Close interdisciplinary cooperation in both diagnosis and treatment of these conditions, which is the main precondition of progress in this field, does not in any way decrease the importance and role of the otorhinolaryngologist.

Final results will be presented at the end of the study in the next year.

Name of the research project: **LOCALIZATION DIAGNOSTICS OF PANCREATIC ISLET-CELL TUMORS**	
Grant Agency: IGA MZ ČR	***Project number:*** 2949 - 3
Researcher: **Eliáš Pavel, MD, PhD**	
Joint Researchers:	**Michl Antonín, MD** **Krajina Antonín, MD, PhD,** **Vižďa Jaroslav, MD** **Lomský Radovan, MD,** **Čáp Jan, MD,** **Rejchrt Stanislav, MD,** **Bedrna Jan, MD, PhD**
Starting date: 1995	***Duration:*** 3 years
Funds allocated for project - total: **600 000,-**	

Summary of results:

PURPOSE: To assess the diagnostic value of various diagnostic methods (ultrasonography-US, dynamic incremental contrast enhanced CT - DICECT, intraarterial contrast enhanced CT - CTAG, conventional angiography - AG, arterial stimulation venous sampling - ASVS, endoscopic ultrasonography - EUS, somatostatin receptor scintigraphy - SRS) for detection of neuroendocrine tumors of pancreas (gastrinomas, insulinomas).

MATERIAL: We have found 21 surgically (13) and diagnostically (10) proved functioning islet-cell tumors in 14 patients during 3 year period.. There were 7 insulinomas in 4 patients and 14 gastrinomas in 10 patients. The average tumor size was 15 mm.

RESULTS : Accurate localizations were obtained in 3 of 8 (37 %) insulinomas and 5 of 14 (36 %) gastrinomas with US, in 2 of 8 (25 %) insulinomas and 5 of 14 (36 %) gastrinomas with DICECT, in 5 of 8 (63 %) insulinomas and 5 of 6 (83 %) gastrinomas with CTAG, in 1 of 8 (13 %) insulinomas and 3 of 7 (43 %) gastrinomas with AG, in 1 of 8 (13 %) insulinomas and 2 of 2 (100 %) gastrinomas with ASVS, 0 of 1 (0%) insulinoma and 8 of 8 (100 %) gastrinomas with SRS, and 1 of 1 (100 %) insulinoma and 7 of 8 (88 %) gastrinomas with EUS.

CONCLUSIONS : In cases of searching for insulinoma, the first step should be the EUS, followed by DICECT and CTAG. On the other hand, SRS is the most sensitive first step method for localization of gastrinomas. When SRS is positive, it should be followed by EUS, DICECT

or CTAG for more detailed topical information.

Name of the research project:

THE VALUE OF DOPPLER ULTRASONOGRAPHY FOR SCREENING OF RENOVASCULAR HYPERTENSION

Grant Agency: IGA MZ ČR	***Project number:*** 2965-3
Researcher: **Eliáš Pavel , MD, PhD.**	
Joint Researchers:	**Michl Antonín, MD** **Krajina Antonín, MD, PhD** **Žižka Jan, MD** **Vižďa Jaroslav, MD** **Ceral Jiří, MD** **Lukeš Antonín, MD** **Pintérová Eliška, MD** **Vortel Jiří, MD** **Ettlerová Eva, PhD**
Starting date: 1995	***Duration:*** 3 years
Funds allocated for project - total: 600 000,-	

Summary of results:

PURPOSE: To assess the diagnostic accuracy of Doppler ultrasonography (DUS) for the detection of renal artery stenosis (RAS).

MATERIALS AND METHODS: Between January 1995 and July 1997, 142 kidneys in 72 hypertensive patients were studied with DUS. Combined technique of the direct insonation of the renal artery (DDUS) and quantitative analysis of early systolic upstroke of distal Doppler waveform (IDUS) was performed in each kidney. Achieved feasibility for DDUS was 85 % and for IDUS 98 %. The diagnostic criteria for RAS (50 % or more) were as follows: PDUS - maximum systolic velocity more than 150 cm/s, IDUS (according to Stavros) - acceleration time more than 70 ms, acceleration less than 300 cm/s^{2}. The results were compared tho those from intraarterial digital subtraction angiography (DSA) in a prospective unbiased manner. Forty nine kidneys with RAS were found in DSA. Based on DSA as a gold standard, sensitivity, specificity, positive predictive value (PPH) and negative predictive value (NPH) were calculated for each kidney in order to estimate the diagnostic accuracy of DUS in detection of RAS. In a limited cohort (41 kidneys , 11 RAS), the results of captopril scintigraphy (CS) were also evaluated.

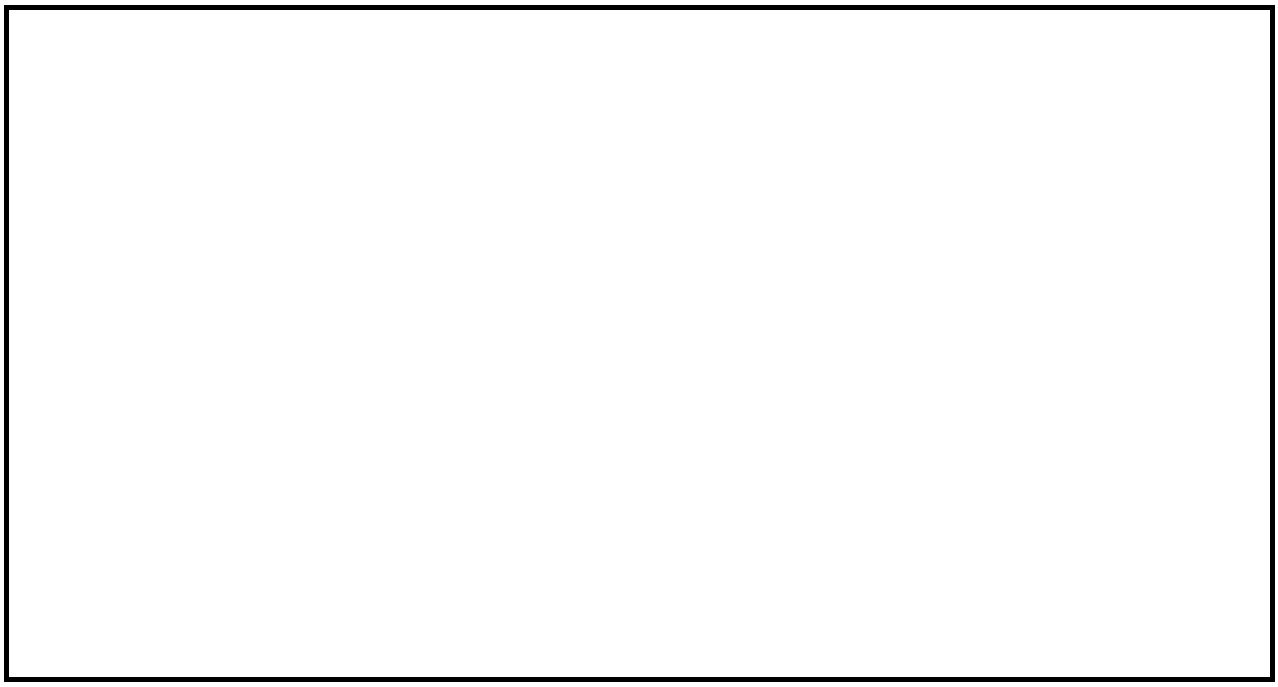
RESULTS: DUS yielded the sens. 94 %, spec. 75%, PPV 67 %, NPV 96%, the results of CS were less favorable : sens. 50 %, spec. 86 %, PPV 60 %, NPV 80 %.

CONCLUSION: According to our results, Doppler ultrasonography can be used for screning of renovascular hypertension.

Name of the research project:	Lyme Borreliosis: Development of Reccombinant DNA as an Internal Positive Control of Polymerase Chain Reaction for Molecular Detection of Borrelia
Grant Agency: IGA MZ ČR	***Project number:*** 3321-3
Researcher: dr. Zdeněk Fiedler	
Joint Researchers: dr. Radovan Haluza dr. Dagmar Hulínská	
Starting date: 1.9.1995	***Duration:*** 2 years
Funds allocated for project - total: 1 056 000 Kč	

S u m m a r y o f r e s u l t s :

At the very begining of PCR introduction into detection methods of pathogens, the false positivity caused by carry over contamination arrised as the problem. As soon as the UNG enzyme was introduced in the reaction, problem of the false negativity rested to be solved. Inhibitors of TAQ polymerase present in the biological sample even after the DNA purification decrease the detection limit of PCR dreadfully. The best way how one can be sure that the negativity of the sample is the real negativity is introduction of the internal positive control. Spiking the biological fluids and tissues with arteficially enlarged PCR target molecules makes all the detection more relliable. As amplicons arising from positive controle templates show quite longer DNA fragments than those obtained with speciemens, electrophoresis bands are easily distinquished. Making dicision to construct such a positive control, we have chosen chromosomal DNA target published by Rosa (Rosa P.A. et al., J Clin Microbiol,1991,29(3)524) for that purpose because of best detection limit achieved in our lab (35 fg DNA). Arteficial sequence 56 bp was synthetized in a common phosphoramidite manner carriing a HIND III cohesive end at the 3´site and the PST I restriction site at the 5´end. Amplicon 365 bp of Borrelia garinii M 192 was cleaved by HIND III into two fragments (210 bp + 155 bp). The smaller one was ligated with arteficial sequence and the molecule 211 bp was selected by PCR. By the means of site directed mutagenesis the new restriction site was introduced into the molecule 210 bp. Both molecules were cleaved by PST I, ligated and the new fragment 421 bp was selected by PCR. Ligation into the pUC 19 plasmid and subsequent cloning in E.coli followed. Recombinant plasmid DNA can be used as an internal positive control of PCR.



Familiar hypertrophic cardiomyopathy (FHC) is genetically and clinically heterogenic disease. From genetic viewpoint, FHC is an autosomal dominant inherited disease with various extent of penetrance. The most dangerous clinical consequence of FHC is heart failure and sudden cardiac death, also in otherwise asymptomatic individuals, often at young age. One third to one half of all cases of FHC is explained as a result of a mistake in the structure of gene coding for b-myosin heavy chain (MYH7) on chromosome 14 q11-q12. Most of all mutations on gene MYH7 is concentrated to several exons, especialy to xon 13. We performed DGGE (denaturing gel gradient electrophoresis) detection of PCR products comprising tested locus. Methods based on DGGE enable to investigate the whole exon and are convenient for primary search for mutations, especially at a larger number of samples tested parallelly. The method is based on enzymatic amplification (PCR) of tested DNA samples using primers with GC clamps, that enable to test melting behaviour of the whole chain of DNA with all domains including that not detectable without GC clamps.
As we did not have any confirmed positive DNA sample at disposal, we constructed positive controls using mutagenesis protocol. We constructed 3 positive samples with most frequent mutations of exon 13 described on codon 403: G->A: 403 $^{Arg->Glu}$, G->T: 403 $^{Arg->Leu}$, C->T: 403 $^{Arg->Trp}$. Melting behaviour of the constructs were studied on perpendicular DGGE and conditions convenient for testing all samples on parallel DGGE were established. The mutated constructs were loaded on gel parallely with samples of tested patient's amplified DNA.
Until now, we have investigated 54 DNA samples coming from patients with FHC well clinically diagnosed, especialy by ultrasonography. We have not found any positive DNA sample although elsewhere approximately one third of samples is described to be positive. We conclude that two explanations are possible: either Czech population differs from that investigated before and described in literature and most mutations are present on different site of MYH7 gene, or tested patients' phenotype does not result from mutation of MYH7 gene at all.

Name of the research project: Development of computer controlled multithermocouple unit for temperature measurement in hyperthermia

Grant Agency: IGA MH CR ***Project number:*** 3771-3

Researcher: Josef Hanuš

Joint Researchers:

Jiří Záhora

Karel Volenec

Starting date: 1996 ***Duration:*** 3 years

Funds allocated for project - total: 388 000 .- Kč

Summary of results :

In the second year of the project we solved these problems:

1- The electro-thermal model of the multithermocouple probe was defined on the basis of analysis of thermal properties of materials and the arrangement of the probe. We created an interactive program that enables the correction of errors of temperature measurements caused by the thermal interaction between successive thermocouples inside the probe. This program enables us to estimate the thermal properties of new designed probes.

2- We developed, tested, and realized the connection between the probe and measuring unit (card in the PC with A-D convertor). The common reference point of the probe, Pt sensor for its temperature measurement and the connectors are situated in the external shielded box with thermal isolation. It gives us an option to place the unit outside the room with HF generator to eliminate possible interactions.

3- We theoretically described the origin of parasitic thermovoltage which occurs in all points behind the measuring point lying in the sharp thermal interface. It was shown and verified that this parasitic thermovoltage can be partly eliminated by the appropriate choice of materials of common wire of the thermocouples. As a positive side effect of this phenomenon we develop a special “continuous” probe for the measurement of temperature maximum in given length of the thermocouple probe.

4- The experiments with HF generator (433.92 MHz, 140 W) on the phantom proved that it isn’t necessary to use only on/off mode of heating.

Name of the research project:

The development of antioxidant blood activity and the changes of lipoprotein subfractions during hypolipidemic therapy

Grant Agency: GA UK ***Project number:*** 153/95

Researcher: MUDr.Eduard Havel

Joint Researchers:

MUDr.Vladimír Bláha, RNDr. Dagmar Solichová, RNDr. Miluše Brátová, prof.MUDr.Zdeněk Zadák, CSc.

Starting date: 1995 ***Duration:*** 3 years

Funds allocated for project - total: 962.000 CZK

S u m m a r y o f r e s u l t s :

The extralipid mechanism whereby hypolipidemic therapy reduces coronary disease risk is not completely known. The changes of lipoprotein subclasses and changes of antioxidant activity of blood during hypolipidemic therapy where studied in connection to this question.

Cholesterol and triacylglyceroles were examined by enzymatic method (kits Lachema, Brno) in lipoprotein classes after ultracentrifugation (Beckman TL 100, Palo Alto, CA). Vitamin E was analysed by HPLC (Hewlett Packard 1084 A, Palo Alto) and fluorescence detector (Perkin Elmer MPF-3, Norwalk, CT). The whole antioxidant status of serum was measured using thiobarbituric acid reacting substances activity (TBARS) by a fluorescence method. The software Solo 4.0 was used for statistical analysis (paring T-test, mean level and standard deviation, criterion of significance $p<0,05$). **I.** Fifteen patients were treated by 40mg of **pravastatin** during the period of 8 weeks. We found favourable significant changes in whole lipoprotein classes: total cholesterol 9,85±2,35, 6,81±1,63, 7,92±2,15 mmol/l, VLDL cholesterol 1,88±0,86, 1,28±0,68, 1,61±0,87 mmol/l, LDL1 cholesterol 4,56±1,58, 3,11±1,07, 3,36±1,43 mmol/l, LDL2 cholesterol (dense subfraction of LDL) 1,86±0,84, 1,42±0,52, 1,26±0,33 mmol/l. The vitamin E /cholesterol ratio significantly increased. **II.** Nineteen diabetic hypercholesterolemic patients were treated by 300mg of **fenofibrate** for more than one year long period. In three control investigations during this period we found favourable hypolipidemic effect, which was associated with improving of diabetic hard exsudates of retina. The main decline of cholesterol was also in subfraction of dense LDL 2. Total cholesterol 7,50±1,64, 6,60±1,43, 5,80±1,04, 5,80±1,24 mmol/l, HDL cholesterol 1,40±0,51, 1,26±0,41, 1,20±0,37, 1,07± 0,23 mmol/l, a LDL2 cholesterol 2,15±1,38, 1,77±0,84, 1,52±0,96, 1,37±0,77 mmol/l. Vitamin E/cholesterol ratio in subfractions of lipoproteins was not afected. The total/HDL cholesterol ratio decline and the level of apoprotein A increased significantly 1,33±0,38, 1,57±0,28, 1,47±0,35, 1,54±0,27 g/l.**III.** The hypolipidemic effect of 20mg **simvastatin** daily treatment was studied in the group 14 hypercholesterolemic patients during eight weeks long period.The favourable effects was found in whole mesured parametres. Total cholesterol 9,28± 0,56, 6,64± 0,35 mmol/l, IDL cholesterol 1,76± 0,15, 1,08± 0,09 mmol/l and LDL cholesterol 3,8±0,35, 2,63± 0,23 mmol/l. The changes in HDL cholesterol level 1,77± 0,28, 1,17± 0,41 mmol/l and vitamin E 44,54± 3,62, 36,85± 1,72 umol/l were not significant. The decline of TBARS was significant 6,97± 0,69, 4,72± 0,48 umol/l.

Conclusion: The changes of lipoprotein fractions and antioxidant serum activity during hypolipidemic therapy (by simvastatin, pravastatin, fenofibrate) are favourable. It may be part of explanation of non LDL hypolipidemic drugs effect on improving of coronary heart disease risk.

Name of the research project:

Contrast sensitivity and visual acuity after photorefractive keratectomy

Grant Agency: Charles University ***Project number:*** 59/97/ LF HK

Researcher: Doc. MUDr. Dagmar Hejcmanová, CSc.

Joint Researchers: Langrová Hana, MUDr.

Peregrin Jaroslav, Prof. MUDr. DrSc.

Kvasnička Josef, Ing

Dvořáková Hana

Starting date: 08. 04. 97 ***Duration:*** 1 year

Funds allocated for project - total: 80 000 Kč

Summary of results :

1. 45 myopes (-3.0 to -6.0 D) were examined before and 1 and 6 months after photorefractive keratectomy (PRK). Visual acuity (VA) was tested using Snellen and logMAR charts. Contrast sensitivity (CS) was measured using a compuerized system.
2. Preoperative best corrected VA (BCVA) in myopes was significantly lower in comparison with a control group using logMAR chart only. A reduction of BCVA by both methods at 1. month and its return after 6 months nearly to original values was noted.
3. Significantly lower values of CS were found in patients before PRK compared to the control group. After 1 and 6 months stayed the values on preoperative level.

Name of the research project: **Amino acid metabolism in different forms of liver injury.**	
Grant Agency: **Charles University**	***Project number:*** **152/95**
Researcher: Doc. MUDr. Milan Holeček, CSc.	
Joint Researchers: MUDr. Ivan Tilšer, CSc. RNDr. Ing. František Skopec, CSc. MUDr. Josef Mráz, CSc. Ing. Luděk Šprongl	
Starting date: 1995	***Duration:*** 3 years
Funds allocated for project - total: 420 000,-- Kč	

Summary of results:

Four separate experimental studies were performed within this project:

Study 1: To assess the effect of pathogenesis of liver injury on the plasma amino acid pattern four models of hepatic injury were studied (partial hepatectomy, liver ischemia, carbon tetrachloride induced acute liver damage and carbon-tetrachloride induced liver cirrhosis. The results were published in *Amino Acids 10: 229-241, 1996.*

Study 2 and 3: In order to investigate the pathogenesis of reduced plasma levels of branched-chain amino acids (BCAA) in liver cirrhosis, we have evaluated the rates of leucine turnover, oxidation and incorporation into proteins in cirrhotic rats and in partially hepatectomized rats *in vivo* and in the isolated perfused liver. The results were published in *Journal of Hepatology 24: 209-216, 1996* and in *Journal of Hepatology 26: 1141-1147, 1997.*

Study 4: In this study the changes of individual plasma amino acid levels were assessed in relation (1) to the severity of liver damage and (2) to the process of liver recovery. The results are accepted for publication in *Amino Acids.*

Name of the research project: **Regulation of synthesis and breakdown of proteins.**	
Grant Agency: ***IGA MH CR***	***Project number:*** **3772-3**
Researcher: Doc. MUDr. Milan Holeček, CSc.	
Joint Researchers: Ing. Luděk Šprongl RNDr. Ing. František Skopec, CSc.	
Starting date: 1996	***Duration:*** 3 years
Funds allocated for project - total: 752 000,-- Kč	

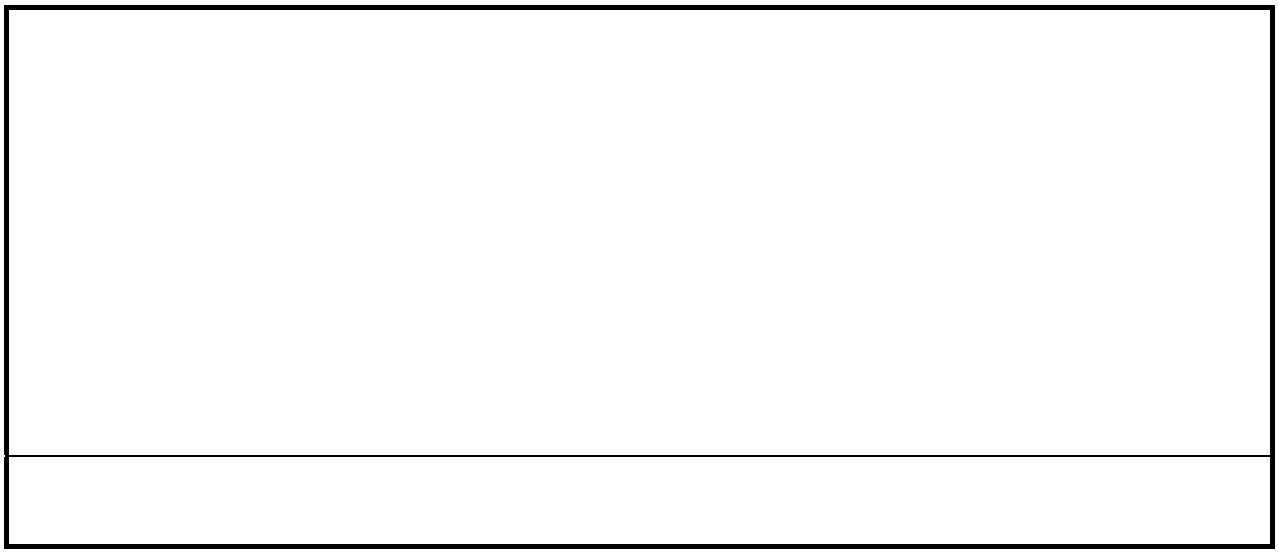
Summary of results:

The aim of the project is to study the effect of starvation, malnutrition and of humoral factors on metabolism of proteins (turnover, protein synthesis, proteolysis, oxidation of amino acids) in the whole body and in specific tissues.

Two studies were performed in 1997:

1. The effect of alanyl-glutamine (AlaGln) on protein metabolism in endotoxemic rats.
   The results demonstrate that the beneficial effect of administration of AlaGln on protein metabolism observed in systemic inflammatory response syndrome is associated with a decrease in proteolysis and not with an increase in protein synthesis.
2. The effect of AlaGln on leucine oxidation by perfused skeletal muscle of endotoxemic rats.
   The results demonstrate that the administration of endotoxin of *Salmonella enteritidis* induced a significant increase in leucine oxidation in perfused rat hindlimbs. This increased leucine oxidation was not affected by administration of AlaGln.

The results obtained in previous studies were accepted for publication in Am. J. Physiol. *(Endocrinol. Metab. 36)*: E..., 1997.



Name of the research project: Determination of the algorithm for the complex Lyme diseases diagnosis and the humoral and cellular immunity factors exercising influence on the Borrelia persistence in the host organism.

Grant Agency: ***GA MZ ÈR*** ***Project number:*** 2962-3

Researcher: Honegr K.

Joint Researchers: Dostál V., Plíšek S., Palièka V., Hulínská D., Fiedler Z., Hozák A., Kopecký O., Bašta J., Plíšková L., Gebouský P.,

Starting date: 1995 ***Duration:*** 3

Funds allocated for project - total: 1 732 000 Kè

S u m m a r y o f r e s u l t s :

Parameters of cells mediated immunity were evaluated (population and subpopulation of T cells, activated T cells and naive and memory T cells.) Lymphocytes were stimulated in vitro and the presence of membrane activation markers was measured by flow cytometry. The polymerase chain reaction was used to detect DNA of Borrelia burgdorferi sensu lato in synovial fluid , CSF or in plasma . In spite of the fact that detection limit achieved with primers by hybridizing within 16S rRNA gene was 50 bacteria per sample, the sensitivity of the whole reaction based on the retrospective study in patients was not sufficient. Manifestations of Lyme borreliosis is described in 120 patients with direct proof of Borrelia burgdorferi sensu lato (immunoelectron microscopy). Using a commercial kit for the examination of recombinant immunoblot the authors examined serum of 85 patients with direct evidence of Borrelia burgdorferi sensu lato in serum or cerebrospinal fluid or patients with typical dermal form of borreliosis. The results were compared with the results of assessment of specific antibodies by the ELISA test. The specificity and sensitivity of the investigated test were not sufficient. Examination of the immunoblot was negative in 8.8% of patients with direct evidence of the causal agent of Lyme borreliosis.

Name of the research project:

HBV DNA Quantification and Determination of HCV Serotype Extension of Selection Criteria in

Patients with Chron. VHB, VHC. Indicated for Inferon Therapy

Grant Agency: IGA MH CR	***Project number:*** 3692 - 3

Researcher: Horáček Jiří, Doc. MUDr. CSc.

University Hosp., Hradec Králové, Czech. Republic

Joint Researchers: *:* V. Štěpánová, L. Plíšková, V. Palička

University Hosp., Hradec Králové, Czech. republic

Starting date: 1.1.1996	***Duration:*** 31.12.1998 - 3 years

Funds allocated for project - total: *2 445 000,- Kč*

Summary of results:

Objective : The aim of the study is to compare the results of HCV typing by serotypes and genotyping in patients with HCV infection in Eastern Bohemia region of Czech Republic.

Methods : Serum samples of 44 anti-HCV positive patients (28 males, 14 females, age range 10 - 87 years, all anti - HIV negative, none from hemodiallyzing unit). Anti- HCV were tested by 3 rd generation Elisa (Murex Diagnostics). Serotypes were determined by HCV Serotyping 1 - 6 Assay (Murex Diagnostics). HCV RNA was tested by multiplex PCR with genotype specific primers from core region of HCV and by Inno-LiPA HCV II kit (Innogenetics).

Results : Of 44 anti-HCV positive patients serotype 1 was determined in 33 patients (75 %), genotypes 1a in 5 (11 %) and 1b in 30 patients (68 %). Serotype 2 in 2 patients and genotype 2b in 1 of them, serotype 3 and genotype 3a in 1 patient. Multiple serotype reactivity, 1 + 4, was detected in 1 and mixed infection with 2 genotypes (1a + 1b) also in 1 patient. Genotypes 1a and 3a were mostly found in young patients and related to drug addiction. 5 sera were not reactive and 2 were untypable in serotyping assay. HCV RNA was not present in 6 sera.

Conclusion : Good correlation between serotyping and genotyping methods was seen. Distribution of HCV serotypes and genotypes in Eastern Bohemia was similar to that in southern and central European countries.

Name of the research project: The endoscopic adenoidectomy	
Grant Agency: IGA MZ CR	***Project number:*** 2946-3
Researcher: Chrobok Viktor	
Joint Researchers: Vokurka Jan Hybášek Ivan Růžička Jaroslav Čelakovský Petr	
Starting date: January 1995	***Duration:*** 3 years
Funds allocated for project - total: 1 400 000 Kč	

Summary of results:

Contrary to classical adenoidectomy where the operation is blind, endoscopic adenoidectomy is an operation checked as a rule optically on a screen. This led to a qualitative change as regards the accuracy of indication as well as the operation proper. The operation must be performed in a standard manner under general anesthesia which substantially reduces the patient´s stress, the number of complications and the number of necessary re-adenoidectomies.

We can use two methods: transoral approach with 70^{o} degree optic and transnasal approach with 25^{o} degree optic. Both aproaches enable us to check the whole curettage visually.

In the long term follow up it is expected that it will improve the results of treatment of common chronic inflammations of the nasal cavity and paranasal sinuses and otitis media with effusion in children.

Name of the research project:
The histopathology of the hearing organ and temporal bone of newborn infants

Grant Agency: IGA MZ CR	***Project number:*** 3682-3
Researcher: Chrobok Viktor	
Joint Researchers: Šimáková Eva Hybášek Ivan Pellant Arnošt J(ttnerová Věra	
Starting date: January 1996	***Duration:*** 3 years
Funds allocated for project - total: 900 000 Kč	

Summary of results:

We examined 146 temporal bones from 76 fetuses. Fetal age dated from the 13-th to the 42-nd week of pregnancy.

1. The influence of the autosomal trisomy syndromes on histopathologic changes of the temporal bones. Temporal bones from fetuses with trisomy 21 had more abnormalities of the external and middle ears than of the inner ear. One half of the bones from the fetuses with trisomy 18 showed abnormalities of the external ear or middle ear and retarded development of the inner ear.

2. Haemorrhage in the middle and inner ear. Protracted asphyxia, delivery trauma, abortion, neonatal icterus and controlled ventilation belong to danger factors making the neonatal auditory organ liable to haemorrhage. The most frequent places of haemorrhage were middle ear, perilymphatic spaces of the cochlea and internal acoustic meatus.

3. 3-D reconstruction of the cochlea with Mondini dysplasia in trisomy 21. Flat cochlea, which consisted of one and a half coils only, was present in the both temporal bones from male fetus with trisomy 21.

4. The histopathology of temporal bones is helpful for undersrtanding of hearing organ pathology, hearing loss and deafness in newborn infants.

Name of the research project:

1) ***Significance of serum interleukin - beta levels in hairy cell leukemia and correlation with tumor mass and s IL-2R levels.***
2) ***Follow up of RdW values and dyserythropoiesis in HCL.***

Grant Agency: IGA MZ ČR ***Project number:*** 3690-2

Researcher: Prof.MUDr.Ladislav Chrobák,CSc.

Joint Researchers: MUDr.Karel Podzimek, MUDr.Pavel Žák, MUDr.Jaroslava Voglová, MUDr.Lenka Plíšková, Doc.MUDr.Josef Špaček,DrSc., Doc.MUDr.Vladimír Palička,CSc.

Starting date: 1.1.1997 ***Duration:*** 2 years

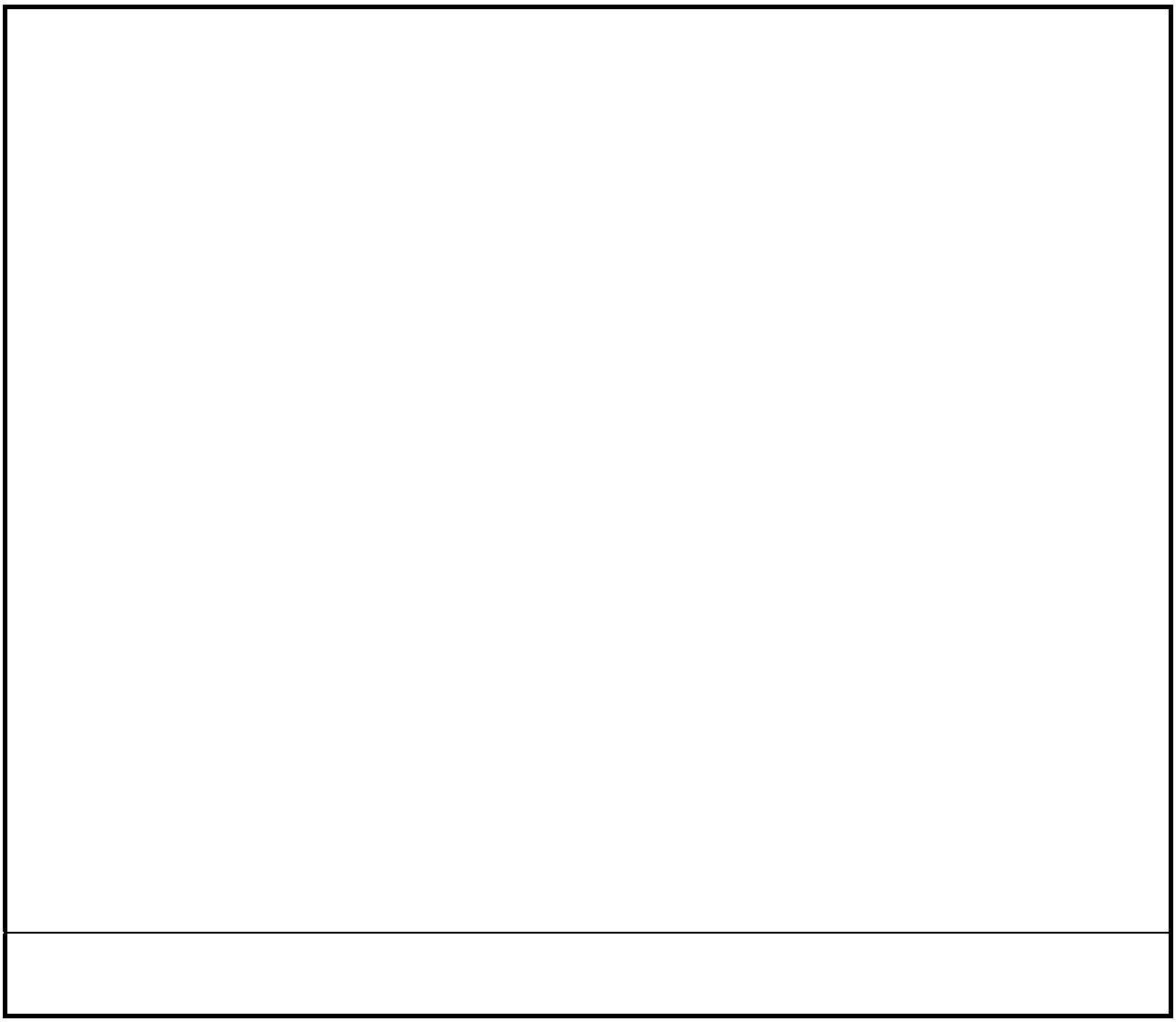
Funds allocated for project - total: 268.000,- Kč

S u m m a r y o f r e s u l t s :

Serum interleukin-beta levels do not reflect the tumor mass in hairy cell leukemia (HCL). In contrary serum interleukin 2 receptor (sIL-2R) levels were found to be a reliable non-invasive marker of HCL tumor burden. The sIL-2R levels were increased in all 15 patients before the iniciation of the therapy with 2-chlorodeoxyadenosine (median 1350 pM/ml, range 188 to 9000 pM/ml) and decreased after the successful therapy (median 84,3 pM/l range 37,0 to 382 pM/ml).

RdW values which reflect anisocytosis and dyserythropoiesis were avaluated in 18 patients with HCL treated with 2-chlorodeoxyadenosine (2-CdA) and in 5 patients treated with interferon-alpha (IFN-alpha). The mean value of RdW in the group of patients treated with 2CdA was 18,2% before therapy, 14% after 6-12 months and 14,2% after 18 months, with corresponding levels of hemoglobin 119,6 g/l, 145,2 g/l and 143,3 g/l respectively. In 5 patients treated with IFN-alpha the RdW value dropped from the median of 21,3% (range 19,2 to 28,7%) before therapy to the median of 15,3% (range 12,4 to 16,7%).

Bone marrow findings in respect to dyserythropoiesis and incorporation of iron into the erythroblasts were avaluated in 16 patients treated with 2-CdA. Definite dyserythropoietic changes were found in 3 patients, disturbed incorporation of iron with coarse granules or increased number of granules was found in 7 patients. These changes disappeared after successful therapy. In one patient association of HCL with sideroblastic anemia was encountered. These findings show that dyserythropoiesis may participate on the anemia in HCL.



Name of the research project: Relationship of women hormonal treatment to blood flow of leg-venous with used of duplex sonography.

Grant Agency: IGA MZ Czech Republic ***Project number:*** 2975-3

Researcher: Ivo Kalousek, M.D.

Joint Researchers:

Starting date: 1.1.1995 ***Duration:*** 31.12.1997

Funds allocated for project - total: 279 000 Kč

Summary of results :

Introduction

The research explores the relationship between two groups of women with hormonal treatment. We examined 76 women with HRT (hormonal repleace treatment) and 75 women with OC (oral contraceptive) older than 3O year. The examination was made before the treatment, and 3, 6 and 12 months after beginning of the treatment.

We examined biochemical parametres (bilirubin, ALT, AST, GMT, cholesterol, HDLC, TAG and LDLV), electrophoresis of the blood protein (total protein, A1G, A2G, BG, GG and A/G), hemocoagulation´s parametres (APTT, APTT patient/control, fibrinogen, d-dimer, antithombin III., APC/APTT, APC, protein C and protein S), Leiden mutation of the factor V. by the women with APC resistance. Next we investigated complete angiologic examination (Trendelenburg, Perthes, circuit of the leg, duplex sonography of the leg-venous, D-PPG, pulsation, murmers, ankle pressure, ultrasonography of the leg-arteries, family history, history, smoke, varix of the leg, job).

Results

We compared two groups of the women with hormonal treatment by use of the pair and unpair statistic test. Some of the results : in the bichemical parametres has the OC group higher level of ALT and AST after 12 months of the treatment than HRT group. The OC group has the changes in the HDLC, TAG a LDL. The results in electophoresis are the same in both groups. We dicovered 8 women with APC resistance in the HRT group and 8 women with APC resistance in the OC group. We found 4 women with Leiden mutation of the factor V in the OC group. In the same group are changes in the angiologic examination.

Conclusion

It is essential to addict the attention to women with OC after 30. We are projecting the questionnaire before starting of the OC by the older women with questions about tromboembolism and leg-venous problems in family history and history and select the high risk group of women. By this group will be urgent to examine APC resistance and leg-venous system by angiology examination and think about prescription of the OC.

Name of the research project:

Study of intestinal permeability in dependence on small bowel damage, possibilities of therapeutical influence

Grant Agency: IGA MZ ČR ***Project number:*** 2951-3

Researcher: MUDr. Pavel **KOHOUT**

Joint Researchers: Miluše Brátová, Zdeněk Zadák, Jan Maňák

Starting date: 1995 ***Duration:*** 3 years

Funds allocated for project - total: 1,7 mil. Kč

S u m m a r y o f r e s u l t s :

The damage of small bowel mucosa in the course of various diseases is possible to investigate using the measurement of small bowel permeability.

The aim of research project was to introduce test of small bowel permeability into clinical practise. The permeability test with lactulose and mannitol was used, the concentration of both sugars in 5 hours collected urine was measured using cappilary gas chromatography.

Index of small bowel permeability was increased in patients with untreated coeliac disease, after gluten-free diet it returned to the normal value, the same results we found during examination of children. On the contrary this index was not changed in patients with food allergy. The increased value of small bowel permeability was found (in the agreement with literature) in critically ill patients on ICU, in patients with active Crohns disease and ulcerative colitis, in children with cystic fibrosis, especially in homozygotes with alela delta F508.

In patients with Crohns disease and ulcerative colitis the value of small bowel permeability correlated with the other parameters of activity of these diseases, in patients with tumours of gastrointestinal tract is the permeability increased 7 days after the end of the cytostatic therapy.

Name of the research project:

Algorithm of food allergy examination, introducing of DBPCFC (double-blind placebo-controlled food challenge)

Grant Agency: IGA MZ ČR ***Project number:*** 2952-3

Researcher: MUDr. Pavel **KOHOUT**

Joint Researchers: Zdeněk Zadák, Otakar Kopecký, Květuše Ettlerová, Vladimír Bláha

Starting date: 1995 ***Duration:*** 3 years

Funds allocated for project - total: 1,3 mil. Kč

Summary of results :

Food allergy is unadequate reaction of the organism on the ingestion of food caused by immunological mechanisms. Its diagnosis and diferential diagnosis is very complicated. Double-blind placebo-controlled food challenge is in the literature described as the gold standart.

The aim of the research project was to elaborate the algorithm of the examination of the food allergy and introduce the double-blind placebo-controlled food challenge (DBPCFC) into the clinical practise.

50 examination in 17 patients with the suspicion on the food allergy was performed. In 22 examinations (44%) was the reaction positive and the elimination diet in these patients was succesfull. In the rest of the examinations (56%) DBPCFC was negative, in 50% of them the reintroduction of the suspected food was possible and remained without reaction. 50% of the false negative give a reason for a necessity of open exposition of the food, if the DBPCFC is negative.

If the food allergy is suspected, the following algorithm of the examination is recommended - anamnesis, physical examination, prick tests, total and specific IgE in the blood, DBPCFC and open exposition of the suspected food, if the previous tests are negative.

Name of the research project: Troponin T in neonatology

Grant Agency: UK ***Project number:*** 158/95

Researcher: Doc.MUDr. Zdeněk Kokštein,Csc.

Joint Researchers: MUDr.Michaela Adamcová,CSc., Doc.MUDr.Vladimír Palička,CSc., MUDr.Milan Košťál,CSc.

Starting date: 1.1.1995 ***Duration:*** 3 years

Funds allocated for project - total: 205.000,- Kč

Summary of results :

In our study 25 pregnant women were assessed with the respect to potential drug-induced cardiotoxic effects. We used the determination of cardiac troponin T (cTnT) that represents one of the most sensitive and specific method for detection of myocardial damage.

The cTnT concentration was measured in maternal venous blood obtained 24 hours after the beginning of therapy. In case that the therapy was not successful and did not last more than 4 days we tried to compare the levels of cTnT in the maternal venous blood just before labour and cTnT in the cord blood of neonates.

The cTnT activity in 15 healthy pregnant women (control group) without any therapy who were matched for week of gestation (33.5 ± 0.5) was (0.01 $\pm$ 0.00 ug/l). The cTnT activity in the pregnant women during the first day of treatment was in the physiological range (0.09 ± 0.03 ug/l) and sigificantly increased during the next 2-3 days of the tocolytic therapy (0.27 ± 0.13 ug/l). The cTnT levels in the cord blood of neonates (0.13 $\pm$ 0.03 ug/l) did not correspond with cTnT concentration in their mothers.

We tried to verify transport of cTnT across the in situ perfused rat term placenta. Troponin T was found to cross the rat placenta from the maternal-to-foetal direction easily. Time to reach equilibrium was within 30 minutes, when foeto-maternal concentration ratio (FMCR) reached maximum value 1.2. Then FMCR exceeded mildly the value 1 and both concentration curves descended with the slope 0.0020.

Conclusion:

1) Our results should give evidence that cTnT can cross through placental barrier both in materno-foetal and foeto-maternal direction in experimental rats.
2) The infusion tocolytic therapy can increase venous blood concentration of cTnT in the pregnant women.
3) The fact that cTnT levels in maternal venous blood and cord blood of neonates are not the similar can reflect the limited transfer of cTnT through the human placenta.

Name of the research project: Cardiovascular system in healthy and pathological neonates

Grant Agency: MZ ČR ***Project number:*** 2938-3

Researcher: Doc.MUDr. Zdeněk Kokštein,Csc.

Joint Researchers: MUDr.Michaela Adamcová,CSc., Doc.MUDr.Vladimír Palička,CSc., MUDr.Milan Košťál,CSc., MUDr.Miroslava Podholová

Starting date: 1.1.1995 ***Duration:*** 3 years

Funds allocated for project - total: 954.000,- Kč

S u m m a r y o f r e s u l t s :

1. The level of serum TnT in the cord blood of term healthy neonates was determined. Normal value=0.05±0.03 ug/l is comparable with dates published in the literature. These observed dates served as a control group.
2. The level of serum TnT in venous blood was determined in 10 neonates after severe perinatal asphyxia. The mean value of TnT was significantly elevated. That result confirmed presumed myocardial damage during severe hypoxia. The similar results have been already published in the literature.
3. We monitored influence of tocolytic therapy (beta-mimetics), used as a routine method to prevent premature labour, on the foetal myocardium. 70 neonates after tocolytic therapy was included to our study. Three various beta-sympatomimetics were used to prevent labour – fenoterol, ritodrine, terbutalin. Results received in this study:

a) The mean level of serum TnT in the cord blood was significantly elevated after acute infusion tocolysis. TnT values were the most frequently elevated when pregnancy were finished 2-3 days after beginning of therapy. This result corresponds with knowledge that maximum side effects of tocolytic therapy can be observed during the first 3-4 days of tocolysis.
b) The mean level of serum TnT in the cord blood was not significantly elevated after preventive long term orally given tocolytics drugs. Nevertheless TnT level was elevated above 2 sigma of normal value in 9 neonates.
c) We did not found any difference in TnT level when various tocolytic drugs were compared.

Conclusion: troponin T could become suitable marker of myocardium damage also in neonatology. It seems that known negative side effect of beta-mimetics tocolytic was proofed in our study with the use of troponin T. Elevated level of troponin T also proofed myocardial damage after severe birth asphyxia in our study. Next studies will be necessary to confirm our result.

Name of the research project:

Flow cytometry and double colour immunofluorescence in diagnosis of leukemias

Grant Agency: Ministry of Health	***Project number:*** 2944-3
Researcher: Otakar Kopecký, M.D., Ph.D.	
Joint Researchers: Jan Krejsek, Ph.D., Miroslava Toušková, MSc.	
Starting date: January 1st, 1995	***Duration:*** 3 years
Funds allocated for project - total: 31,730 US Dollars	

Summary of results:

Flow cytometry was established for the immunophenotypic analysis of malignancies of hematopo- ietic origin. Double and triple immunofluorescence analysis of either unseparated or separated samples of peripheral blood, bone marrow, liquor, pleural and pericardial fluids was performed. Method for the measurment of cytoplasmic and nuclear molecules was developed. Initially, flow cytometry was run simultaneously with standard UV microscopy to compare the results. Totally samples of bone marrow and (or) peripheral blood obtained from 983 individuals were analysed. According to our results following panels of monoclonal antibodies for diagnosis of acute leukemias, lymphoproliferative diseases, hairy cells leukemias, multiple myelomas and myelodysplastic (myeloproliferative) diseases were proposed.

Acute leukemias: isotypic control, CD45/CD14, CD3/HLA DR, CD5/CD19, CD10/CD19, CD38/CD13, CD14/CD33, CD2/CD7, CD15, CD34, CD41, CD65, cTdT, cMPO, cCD3, cμ.
T-ALL: (extended panel) CD7/CD2, CD3/CD4, CD3/CD8, TcRαβ, TcRγδ, CD8/CD38, CD1a.
B-ALL: (extended panel) CD79a, CD79b, cCD22.

Multiple myeloma: isotypic control, CD45/CD14, CD3/HLA DR, CD5/CD19, CD13/CD38, CD14/CD33, CD34, CD15, CD38/CD138/CD56, CD38/CD138/CD54, cκ/CD138/CD56, cλ/CD138/CD56.
Chronic leukemias (lymphomas): isotypic control, CD45/CD14, CD3/HLA DR, CD5/CD19, CD10/CD19, CD2/CD7, CD20, CD21, CD22, CD23, CD37, CD15, CD34, κ/λ, μ
Hairy cell leukemias: isotypic control, CD45/CD14, CD3/HLA DR, CD5/CD19, CD10/CD19, CD2/CD7, CD3/CD4, CD3/CD8, CD20/CD25, CD19/CD25, CD20/CD11c, CD103, κ/λ, μ

We conclude that immunophenotyping is the best approach to diagnose blood malignancies but re- sults have to be interpreted in context with clinical evaluation and other laboratory tests. Extended panels of monoclonal antibodies together with double- and triple staining and determination of intracellular molecules are useful in the delineating of unusual cases of leukemias. Clinical relevan- ce of certain immunophenotypes was sought in our study.

Name of the research project:

Adoptive immunotherapy - ex vivo generation of lymphokines activated cytotoxic cells and their clinical applications

Grant Agency: Ministry of Health	***Project number:*** 2945-3

Researcher: Otakar Kopecký, M.D., PhD.

Joint Researchers: Jan Krejsek, Ph.D., Petr Jílek, Ph.D., Jan Bureš, M.D., Ph.D.
Pavel Jandík, M.D., Miroslava Toušková, MSc.

Starting date: January 1st, 1995	***Duration:*** 3 years

Funds allocated for project - total: 82,000 US Dollars

Summary of results:

Samples of ascitic fluids and pleural effusions were obtained from 15 patients suffered from metastatic form of breast cancer, ovarial carcinoma and colon carcinoma. Tumor cells and adherent cells were removed from the suspension by nylon wool. Tumor infiltrating cells (TIL) were than expanded in AIM-V medium, therapeutic grade (GIBCO) supplemented by rGM-CSF and rIL-2. Duration of incubation was individual, up to 1 month. Different number of samples were processed from various individuals (up to 10 samples). Cytotoxic activity of TIL as well as their phenotypes were measured at the begining and at the end of cultivation using above mensched methods and panels of monoclonal antibodies.

Clinical aplications of The procedure for the generation of lymphokine-activated killer cells was developed. Briefly, density gradient separated mononuclear cells were incubated in RPMI-1640 supplemented with hu- man albumin up to 14 days. Optimal concentration of rIL-2 also in combination with rGM-CSF was used for the induction of LAK cells. Cytotoxic activity was determined by standard ^{51}Cr release test at the beginning and than at finishing of cultivation. Changes in the expression of membrane molecules during cultivation were measured by flow cytometry using following panel of monoclonal antibodies: isotypic control, CD45/CD14, CD3/HLA DR, CD3/CD4, CD3/CD8, CD3/CD16+CD56, CD3/CD25, CD5/CD19, CD57/CD8, CD8/CD28, CD3/CD45RA, CD3/CD45RO, CD3/CD80, CD95/CD45, CD3+CD14/CD4, CD19/CD86, CD3/CD152 at days 0, 4, 7, 11, and 14. Correlations between particular phenotype and cytotoxic activity of LAK cells were sought.

TIL cells were performed in 1 patient. There were no significant side-effects. Therapeutic efficiency of this adoptive immunotherapy is under study now.

Guidlines for the complex care including immunotherapy (rIL-2, rINFα) or chemoimmunotherapy were developed for patients suffering from metastatic form renal carcinoma and malignant melanoma. Totally 32 patients were treated according to these guidlines. Efficiency of this complex approach to the treatment of cancer is now evaluated.

Name of the research project: Prediction of vancomycin and aminoglykosides dosage regimen at the preterm neonates in the first week of postnatal age. Part - Pharmacokinetics of vancomycin at neonates.

Grant Agency: IGA MZ ***Project number:*** 2950 - 3

Researcher: MUDr.Zítek Martin (1995,1996), PharmDr.Kopecká Jana (1997)

Joint Researchers:

MUDr.Pokorná Pavla
MUDr.Buriánková Božena
doc.MUDr.Pařízková Eva, CSc.
prof.MUDr.Martínková Jiřina, CSc.
Ing.Chládek Jaroslav
Krupičková Hana
Kokštein Zdeněk
MUDr.Vortel Jiří

Starting date: 1.1.1995 ***Duration:*** 3 years

Funds allocated for project - total: 1712 000 Kč

Summary of results:

The purpose of this study was:
- to examine the relationship between vancomycin pharmacokinetics and various indices of maturation in a population of critically ill neonates.
- to investigate a dynamic pharmacokinetic model based on Bayesian algorithm.

Pharmacokinetic population parameter estimates were determined in the 1st Group of neonates (15 preterm and 6 full term neonates,27-41 wks of postconceptional age).After the first dose of vancomycin hydrochloride (30 mg/kg/day over a 60 min infusion) 4-5 serum vancomycin concentrations were fitted by nonlinear least-squares regression analysis to a one-compartment infusion model. Linear regression was used to determine significant relationships ($p < 0.05$) between pharmacokinetic parameters and patient characteristics.

The mean for the apparent volume of distribution Vd was 0.699 L/kg (SD 0.366 l/kg), for vancomycin clearance CL 0.077 L/h/kg (SD 0.054 L/h/kg). The mean population parameter estimate for Vd was set at 0.699 L/kg . We entered the equation that explained the relationship between vancomycin clearance and creatinine clearance Clcr into the model ($r=0.61$, $p < 0.01$):

$$CL = (0.527 \times Clcr) + 0.0206$$

The Vd and CL were allowed to be fitted during the Bayesian estimation (ABBOTTBASE Pharmacokinetic Systems, Abbott Laboratories). Inputs of initial peak and trough concentrations of vancomycin in Group 2 of neonates (17 preterm and 3 full term, 25-41 wks of postconceptional age) were used as feedback information for the Bayesian estimation of subsequent concentrations. Using population-based parameters and creatinine clearance the mean error ME and the mean absolute error MAE for predicting concentrations were 0.453 mg/L and 2.75 mg/L.

Conclusions: The vancomycin clearance and volume of distribution was directly related to postconceptional age by linear regression analysis.The study found a significant correlation between vancomycin clearance and creatinine clearance. Vancomycin dosage should be prescribed based on postconceptional age and weight. In view of the interpatient variability in pharmacokinetic parameters observed in the neonates, monitoring of vancomycin concentration is necessary to attain safe and effective therapy. The use of population-specific pharmacokinetic parameters and Bayesian forecasting insure an accurate dosage regimen design to achieve steady-state peak concentrations of 20-40 mg/L and trough concentrations of 5-10 mg/L.

Name of the research project:

Model of visual motion perception

Grant Agency:	**Grant Agency of Charles University**	***Project number:***	56/97/C LF HK
Researcher:	Jan Kremláček		
Joint Researchers:	Miroslav Kuba, Zuzana Kubová, František Vít		
Starting date:	1997	***Duration:***	3 Years
Funds allocated for project - total:	150, 000.- CZK		

S u m m a r y o f r e s u l t s :

The aim of the project is to create a model of electrophysiological properties of the magnocellular visual system (dealing with motion perception). Recordings of the EEG activity related to the onset of structure movement - *motion-onset visual evoked potentials* (M-VEPs) were used for the model construction which was based on the idea of independent harmonics oscillators synchronised and forced by an external pulse.

The Fourier analysis and the principal component analysis (PCA) were applied to the pre-stimulus spontaneous EEG and to the post-stimulus M-VEPs recorded from occipital and parietal locations. The comparison of pre- and pos-tstimulus parts brought the following findings about the M-VEPs generation:

1. There is a strong forcing and synchronisation phenomenon in the delta and theta band (2-7 Hz), small decrease of the alfa band power density and small changes in the beta and gama bands in power and synchronisation.
2. Also the PCA has shown a higher organisation in the postimuls part - the first two independent principal components described about 80% of the spontaneous EEG variabilitys and about 90% of M-VEPs variability. The Varimax rotation was used and four independent oscillators were found.

The project will continue by an analysis of the M-VEPs obtained under different stimulus conditions (1) to prove the set of independent oscillators and find their dependency on stimulus parameters.

A side product of the project solution is an universal, cheap and flexible visual stimulator (2) based on animation technique available free of charge on the e-mail address: jan.kremlacek@lfhk.cuni.cz

Publication:

1. Kremláček, J., Kuba, M., Kubová, Z.: Dependence of motion-onset VEP parameters on stimulus localisation. Proceedings of the XXXVth ISCEV Symposium, Asilomar (USA), July 20 - 24, 1997.
2. Kremláček, J., Kuba, M., Kubová, Z., Vít,J.: Simple and powerful visual stimulus generator. Computer Methods and Programs in Biomed., 1997 - submitted to publication

Name of the research project:

COMPUTER ASSESSMENT OF BRAIN ELECTRIC ACTIVITY FOR EVALUATION OF CENTRAL NERVOUS SYTEM FUNCTION

Grant Agency: Grant Agency of Czech Republic	***Grant Number:*** 309/960959
Researcher: Miroslav Kuba, M.D., Ph.D.	
Joint Researchers:	Zuzana Kubová, M.D., Ph.D. Ing. Jan Kremláček Ing. František Vít
Starting date: 1996	***Duration:*** 3 years
Funds allocated for project - total: 554,000.- CZK	

Summary of results: (Year 1997)

In the second year our research was oriented towards event related potentials (ERP) methods. For the time being, ERP(cognitive evoked potentials - P300) are commonly generated by either auditory stimulation or by pattern appearance (both using oddball paradigm). We tried to obtain cognitive components of visually evoked potentials when changes in motion direction, motion velocity or motion coherence were recognized by a subject (using our own method of stimulation (1)).

P300-like responses were examined in frontal, central and parietal leads and their dependence on parameters of the simultaneously recorded primary motion-onset VEPs from the temporo-occipital leads was tested.

Achieved P300 were comparable with the standard P300 to pattern-onset stimuli. Quite large interindividual variability of amplitudes and latencies of P300 did not correlate with the motion-onset VEPs parameters. First clinical findings demonstrated that mainly dominating magnocellular system dysfunction influences significantly the described variant of P300.

Our results implicate an idea of possible use of the new cognitive responses for more complex examination of visual processing of motion and visual perception disorders.

Publications:

1. Kremláček, J., Kuba, M., Kubová, Z., Vít,J.: Simple and powerful visual stimulus generator. Computer Methods and Programs in Biomed., 1997 - submitted to publication
2. Kuba, M., Kremláček, J., Kubová, Z.:Cognitive potentials evoked by variations in visually perceived motion. Proceedings of the XXXVth ISCEV Symposium, Asilomar (USA), July 20 - 24, 1997.

Name of the research project:

NEW ELECTROPHYSIOLOGICAL EXAMINATIONS FOR NEURO-OPHTHALMOLOGICAL DIAGNOSTICS

Grant Agency: Grant Agency of Ministry of Health (IGA MZ)	***Project number:*** 3230-3
Researcher: Miroslav Kuba, M.D., Ph.D.	
Joint Researchers:	Zuzana Kubová, M.D., Ph.D. Ing. Jan Kremláček Ing. František Vít
Starting date: 1995	***Duration:*** 3 years
Funds allocated for project - total: 459,000.- CZK	

Summary of results:

- **Generation of new visual stimuli for visual evoked potentials (VEPs) -** animation technique was used for stimuli generation on a PC monitor with special synchronisation pulses (1)
- **Increase of VEP examination sensitivity in Multiple Sclerosis and differentiation of Neuritis Retrobulbaris (2,3)**
- **Dynamic objective perimetry via motion-onset VEPs (4,5)**
- **Early detection of glaucomatous changes in the visual pathway (6)**
- **Localisation of reference electrode in secondary (associate) visual centra examination (7) -** activation of associate cortex excludes a use of any cephalic reference and either zygoma or ear lobes are recommendable
- **Research results were verified in 700 neuro-ophthalmological patients** and we were entitled by International Society for Clinical Electrophysiology of Vision to organise the ISCEV Symposium in 1998 on the topic "Electrophysiological and related measures of motion detection" (see http://www.lfhk.cuni.cz./udalosti/iscev98).

Publications:

1. Kremláček, J., Kuba, M., Kubová, Z., Vít,J.: Simple and powerful visual stimulus generator. Computer Methods and Programs in Biomed., 1997 - submitted to publication
2. Kubová, Z., Kuba, M.: Motion-onset VEPs improve the diagnostics of multiple sclerosis and optic neuritis. Sbor. věd. prací LF UK v Hradci Král., 38, 1995, pp. 89 - 93.
3. Waberžinek, G., Kremláček, J., Kuba, M., Kubová, Z.: Comparative study of electrophysiological methods in diagnostics of multiple sclerosis. European J. Neurology, 4, Suppl. 1, 1997, p. S 158.
4. Kuba, M., Kubová, Z., Kremláček, J., Svěrák, J.: Visual field examination via motion-onset VEPs. Vision Res. 35, Suppl., 1995, S166.
5. Kremláček, J., Kuba, M., Kubová Z.: Objective perimetry based on motion-onset VEP examination. Proceeedings of the XXXIVth Symposium ISCEV, Tübingen, July 20-24, 1996, Poster Nr. 70.
6. Kubová, Z., Kuba, M., Svěrák, J., Hrochová, J.: Motion-onset visual evoked potentials improve the diagnostics of glaucoma. Doc. Ophthalmol. 92, 1996, pp. 211 - 221.
7. Kuba, M., Kubová, Z., Kremláček, J.: Relevant reference electrode for motion-onset VEP recordings. Proceeedings of the XXXIVth ISCEV Symposium, Tübingen, July 20-24, 1996, Poster Nr. 71.

Name of the research project:

MOTION RELATED VISUAL EVOKED POTENTIALS (VEPs) AND THEIR DIAGNOSTIC APPLICATION

Grant Agency: EC Brusel - program COST	***Project number:*** CIPACT 93 0220
Researcher: Miroslav Kuba, M.D., Ph.D.	
Joint Researchers:	Zuzana Kubová, M.D., Ph.D. Ing. Jan Kremláček Ing. František Vít
Starting date: March 1, 1994	***Duration:*** 39 months
Funds allocated for project - total: 63,000.- ECU	

Summary of results:

- **Specification of the pattern and motion specific components in VEPs (1)** - The motion-onset related VEPs were characterised in 94% of the population by a dominant negative peak with latency in the range of 135 - 180 ms.and maximum amplitude in lateral temporo-occipital leads. Stimulation of the lower half of the visual field gave larger reponses in comparison with the upper half (1).
- **Contrast sensitivity of motion-onset VEPs and their dependence on spatial frequency of a moving pattern** - Large check sizes (> 30') provide detectable responses up to the minimum contrast (0.3 %), which gives evidence chat the motion related negative peak of the VEPs represents a magnocellular system activity (2, 3).
- **Diagnostic applications of motion-related VEPs based on their specific properties** (4,5,6,7) - Motion specific VEPs provide new diagnostic possibilities because in comparison with any other VEPs they predominantly test the magnocellular system. The following profits can be achieved from their examination: higher sensitivity for visual pathway demyelination; better differential diagnosis of retrobulbar neuritis; early detection of subclinical optic nerve changes in glaucoma; peripheral visual field defects verification; objective testing of amblyopic eyes.

Publications:

1. Kremláček, J., Kuba, M., Kubová, Z.: Dependence of motion-onset VEP parameters on stimulus localisation. Proceedings of the XXXVth ISCEV Symposium, Asilomar (USA), July 20 - 24, 1997.
2. Kubová, Z., Kuba, M., Spekreijse, H., Blakemore, C.: Contrast dependence of motion-onset and pattern-reversal evoked potentials. Vision Research, 35, 1995, pp. 197 - 205.
3. Kremláček, J., Holčík, J., Kuba, M., Kubová, Z., Vít, F.: Model of contrast sensitivity in visual perception of motion. Bridging Disciplines for Biomedicine - Conf. Proc., IEEE Engineering in Medicine, ISBN 90-9010009-9 (CD-ROM), 1996.
4. Kubová, Z., Kuba, M., Spekreijse, H.: VEP evidence for predominant parvocellular pathway impairment in retrobulbar neuritis. Invest. Ophthalmol. Visual Sci., 35, 1995, p. 197.
5. Kubová, Z., Kuba, M., Juran, J., Blakemore, C.: Is the motion system relatively spared in amblyopia? - Evidence from cortical evoked responses. Vision Res., 36, 1996, pp. 181 - 190.
6. Kubová, Z., Kuba, M., Svěrák, J., Hrochová, J.: Motion-onset visual evoked potentials improve the diagnosis of glaucoma. Doc. Ophthalmol., 92, 1996, pp. 211 - 221.
7. Kuba, M., Kubová, Z., Kremláček, J., Svěrák, J.: Visual field examination via motion-onset VEPs. Vision Res. 35, Suppl., 1995, S166.

Název výzkumného projektu:

Screening poruch metabolismu tryptofanu (Screening for defects in the metabolism of tryptophan)

Grantová agentura: **IGA MZ ČR** *Číslo úkolu:* **4097-3**

Řešitel: **MUDr. Eliška Marklová, CSc.**

Spoluřešitelé: **prof. MUDr. Jaroslav Dršata, CSc.**
MUDr. Leo Klein
doc. MUDr. Marie Nožičková, CSc.
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Doba řešení: **1.1.1997 - 31.12.1999** *Přidělené fin. prostředky:* **497 tis. Kč / rok (včetně investice)**

Souhrn dosažených výsledků:

- Vypracovali jsme **algoritmus vyšetřování poruch metabolismu tryptofanu** (tenkovrstvou chromatografii v 1. etapě vyšetřování doplňujeme kapalinovou chromatografií (HPLC) na obrácených fázích, *Chromatographia 1997, 45: 195-198, Acta Medica (Hradec Králové) Suppl. 1997, 1:67-73*), definovali jsme soubor příznaků, které indikují zahájení tohoto vyšetření.
- Uvedli jsme **do provozu nový detektor** (zakoupený z finančních prostředků tohoto výzkumného grantu), umožňující skenování píků během analýzy, a seznámili se s ovládacím softwarem.
- Vypracovali jsme **postup předčišťování vzorků před HPLC** na kolonce Sep-Pak (postupné vymývání nečistot gradientem dodecylsulfátu v metanolu, eluce směsí amoniak - metanol), zmodifikovali jsme manuální postup zpracování použitím vakuové jednotky „manufold“ pro 12 vzorků.
- Ke zmapování situace u zdravých, a zvláště u „nemocných“ kontrol provádíme souběžně **vyšetřování** materiálu (především moče) **většího souboru dětí různého věku, s různými diagnózami a symptomy**, které podle dosavadních znalostí přímo neindikují vyšetření metabolismu tryptofanu (plánujeme takto postupně vyšetřit všechny vzorky moče, které jsou zasílány do naší laboratoře v rámci screeningu dědičných poruch metabolismu). V současné době je zpracováno 115 vzorků, vyhodnocení bude provedeno později.
- Pokračujeme ve vyšetřování nemocných **s popáleninami a některými chorobami kůže (**u těchto diagnóz jsme již dříve pozorovali změny v exkreci metabolitů tryptofanu)..
- Obnovili jsme **spolupráci** s unikátním tuzemským pracovištěm (**Gnotobiologická laboratoř** Mikrobiologického ústavu ČAV v Novém Hrádku) a získali větší soubor (72) pro nás velmi cenných vzorků moče a krve od jedinců (minisele, typ Minnesota) s přesně definovanou bakteriální střevní flórou a jedinců zcela bezmikrobních. Tento materiál umožní pokračovat v již dříve zahájeném zkoumání původu některých metabolitů Trp v moči savců, a pátrat tak po příčinných souvislostech jejich patologické exkrece u člověka. Všechny analýzy dosud nebyly dokončeny.

Předkládáme posterové sdělení, prezentované na: *5th Internat. Congress on Amino Acids, Kalithea, Chalkidiki, Greece 25. - 29. 8. 1997,* Abstracts in *Amino Acids 1997, 13 (1) 38*; práce byla zaslána k publikaci in extenso.

Name of the research project:

New Cytostatic and Immunosupressive Drugs

Grant Agency: GA UK	***Project number:*** 69/96

Researcher: Doc. MUDr. RNDr. Mělka Milan

Joint Researchers:

Doc. MUDr. V. Geršl, CSc., doc. MUDr. Y. Mazurová, CSc., PharmDr. M. Niang,

RNDr. V. Panajotova, CSc., Ing. L. Šišpera, CSc.

Starting date: 1996	***Duration:*** 1996 - 1998

Funds allocated for project - total: 150 000,-- Kč

Summary of results:

The cytotoxic activities of mitoxantron and some deoxysaccharides were measured by derermining the incorporation rate of [6-^{3}H]thymidine and uniformly labelled [U-^{14}C]amino acid mixture into trichloroacetic acid-insoluble fraction of L1210 leukemia cells during a short-term incubation with the drugs.

L1210 leukemia was used also for testing of mitoxantron in combinations with L-carnitine and its derivatives in vivo.

Total energies of compounds were calculated after optimalization of structure by MM+ and/or AM1 methods using program HyperChem 2.0 Autodesk, Inc. and interactions of molecules were simulated.

From the results obtained it follows that some combinations are more effective in comparion with mitoxantron alone.

Potential therapeutic synergism of mitoxantron is discussed.

Name of the research project:

Composite Dermoepidermal Graft for Burn Treatment

Grant Agency: IGA, Ministry of Health ,Czech Republic	***Project number:*** 3696-3

Researcher: Pavel Mìøièka, M.D.

Joint Researchers:

1. Hana Straková,RN.D.
2. Ass.Prof. Danuše, Šubrtová,Ph.D.
3. Jaroslav Mokrý,M.D.,Ph.D.
4. Leo Klein,M.D.,Ph.D.
5. Ass.Prof. Miroslav Èervinka,M.D.,RN.D.,Ph.D.

Starting date: 1.1 1996	***Duration:*** 3 years

Funds allocated for project - total: CZK 600 000

Summary of results :

The application of the composite dermo-epidermal graft prepared in vitro from allogeneic acellular deepidermized dermis (DED) and autologous keratinocytes represents one of the possible approaches to treatment of full-thickness burns. In our experiments, we focused on in vitro preparation and the morphological examination of two types of the composite dermo-epidermal graft: 1) using the dermal component and keratinocytes harvested from one dead donor-i.e. "autologous" model of the composite graft, 2) using the dermis and keratinocytes taken from different dead donors - i.e. "allogeneic" model. The dermal component, the DED, with destroyed cellular elements was prepared by the method of Krejci et al. (J. Invest. Dermatol. 1991, 97, 843-848) and stored at -80 ^{o}C. The primary culture keratinocytes were seeded in suspension on the papillary surface of the DED and were growing as submerged culture in presence of 3T3 cells for 10-13 days. In both in vitro models, confluent epithelial sheets gradually adopting the character of a stratified squamous epithelium were obtained. In sites of increased layering of the epithelium, keratohyalin was present in keratinocytes of the upper cell strata showing differentiation of cells. Nevertheless ,some defects in the cultivated epithelia were also detectable (regressive changes in some cells, probably reflecting worsening nutrition conditions in multicellular cultures). The dermal border line showed irregularities (i.e. occasional dermal "papilae" interdigitating with epithelial pegs) and ultrastructurally, various patterns of the attachment of basal keratinocytes to the dermis including that, mimicking the normal dermal-epidermal junction were detected.

Beside the above cultivation procedure the cocultivation of cadaveric keratinocytes and the deep-frozen DED without the presence of 3T3 fibroblast feeders was performed. We also made preliminary trials with cultivation of keratinocytes on the DED stored at +4 ^{o}C and keratinocytes cultured on a hyaluronic ester membrane.

Our hitherto experience with in vitro preparation of the composite grafts shows that these models, with their structural appearance of the dermal-epidermal junction, to a certain extent

recapitulate the in vivo situation. This represents the phenomenon favourable for maintaining cohesion between the epithelium and the underlying tissue bed. On the contrary, formation of the flat dermal-epidermal junction after transplantation of the only keratinocyte sheets on deeply excised burn wounds is described as major disadvantage leading to increased fragility of the restored skin cover. The results we have obtained are encouraging for further experimental and prospective preclinical studies.

The study was presented at scientific meetings of the European Tissue Culture Society (Mainz, FRG, October 1997) and at the international symposium "Current Therapy in Burns" (Bydgoszcz, Poland, October 1997).

Name of the research project:

Influence of metabolic polymorphism of cytochrome CYP3A on electropharmacological and pharmacokinetic properties of amiodarone.

Grant Agency: Charles University	***Project number:*** 57/97/C
Researcher: MUDr. Stanislav Mičuda	
Joint Researchers:	MUDr. Petr Pařízek prof. MUDr. Jiřina Martínková, CSc. Ing. Luděk Šišpera, CSc. prof. MUDr. Vladimír Pidrman, DrSc. MUDr. Martin Hodač
Starting date: January 1997	***Duration:*** 3 years
Funds allocated for project - total: -250.000,-	

Summary of results:

Amiodarone (AM) has a critical role in controlling both of ventricular tachycardia or fibrillation (VT/VF) and supraventricular tachyarrhythmias. The drug appears to exhibit all mechanisms typical for four main antiarrhythmic classes. Nevertheless, early and delayed actions of AM on the heart are quite different. Changes in electrophysiological parameters (prolongation of the AH, HV and QT_c intervals, increase in the effective refractory periods in most cardiac tissues), as criteria of antiarrhythmic action, are significantly greater during long-term therapy. In part, this difference is caused by the major metabolite of AM, N-desethylamiodarone (DEA). It is proved, that the major metabolic pathway leading to conversion of AM to DEA is catalysed by a cytochrome CYP3A. The activity of CYP3A is known to be polymorphic (interindividually extremely different). The objective of our work is to determine the relationship between DEA formation and changes of delayed electrophysiological parameters. In this way, the antiarrhythmic action of AM in patients in risk of sudden death due to severe ventricular arrhythmias may be predicted.

Methods: As a model drug serves endogenous cortisol and its metabolite, 6-b-hydroxycortisol. The ratio of the hydrophilic metabolite to its parent compound (6-b-OHF/UFC) found in the urine have been used as means of measuring hepatic CYP3A catalytic activity. We have developed a sensitive high-performance liquid chromatographic method for quantification of 6-b-hydroxycortisol using 6-b-hydroxycortisone as internal standard. The urine samples was purified on Sep-Pak C_{18} cartridge. After elution with ethylacetate, the eluent was washed with 1 M NaOH in 20% Na_2SO_4 and 1% CH_3COOH in 20% Na_2SO_4 and evaporated to dryness. The redissolved residue was injected into the liquid chromatograph. The column was Nova-Pak C_{18} operated at 1 ml/min using the following stepwise gradient: to = 0-32 min $\rightarrow$ 100% A, t = 32-42 min $\rightarrow$ 100% B, to = 42-52 min $\rightarrow$ 100% A. Mobile phase A consisted of 10 mM KH_2PO_4 at pH = 3,5 in water – methanol – acetonitrile (87:9:4). Mobile phase B is the same mixture in the volume ratio (67.5 : 22.5 : 10). The column effluent was monitored at 244 nm. The level of UFC was determined by a radioimmunoassay.

Preliminary results: Preliminary data suggest marked inhibitory effect of amiodarone on cytochrome CYP3A activity.

Name of the research project:

Cultivation of neural precursor cells and modification of their further development in vitro.

Grant Agency: **GA**	*Project number:* **304/97/1117**
Researcher: **MUDr. Jaroslav Mokrý, CSc.**	
Joint Researchers: **Prof. MUDr. Stanislav Němeček, DrSc** **Doc. MUDr.Danuše Šubrtová, CSc.** **MUDr. Viktor Bartanusz, CSc.**	
Starting date: **January 1997**	*Duration:* **3 years**
Funds allocated for project - total: **1,293,000 CZK**	

Summary of results:

The inverted microscope NIKON ECLIPSE TE 300 that was purchased from instrument investments provided by GA CR enabled a detailed study of formation of multicellular spheroids by neural precursor cells (NPCs) in vitro. Dissociated NPCs isolated from the fetal rat brain were cultivated in serum-free medium supplemented with epidermal growth factor (EGF) or basic fibroblast growth factor (bFGF) or a mixture containing EGF plus bFGF. Phase contrast microscopy revealed that mitogenic growth factors caused proliferation of NPCs and formation of multicellular spheroids under all the above specified conditions. The inverted microscope enabled a taking of tiny samples of spheroids from cultures in the course of the earliest stages of their cultivation. The spheroids were processed for histology at light and electron microscopical levels. Peroxidase immunohistochemistry utilizing a broad set of primary antibodies provided information on expression of characteristic antigens in EGF-responsive spheroids yielded from primary cultures at different time-points and confirmed that composition of spheres changed over time in that aspect that EGF-responsive NPCs tended to differentiate inside of spheroids. Although the tissue blocks of bFGF-responsive NPCs embedded in paraffin await for processing, a preliminary observation of different adhesive properties of cultured bFGF- and EGF-responsive spheroids suggests that both subsets of NPCs may reveal different properties.

The study of the inner structure of spheroids composed of EGF-responsive NPCs on semithin resin-embedded sections stained with toluidine blue and paraffin-embedded sections stained with haematoxylin and eosin or propidium iodide confirmed the presence of degenerating cells with pycnotic nuclei. These were abundant even in the tiny early spheroids formed by immature proliferating NPCs. Our finding of segmented nuclei with condensed chromatin is considered to be a criterion of apoptotic cell death; nevertheless, we were unable to identify the DNA fragmentation in these cells using the TUNEL method. The floating spheres can adhere together and form large structures of bizarre shapes. This property is more pronounced in cultures with high density of plated NPCs and in media with reduced concentrations of growth factors. Fusion

of neurospheres containing NPCs derived from different regions of the CNS makes this model attractive for confrontation studies. Prior to a further study of interactions between neurospheres and brain tumours we performed preliminary cultivations of explants of human brain tumours. To ensure standard results in experiments it proved necessary to introduce cultures of stable experimental cell lines (C6 glioma and NG108 mixed astroglioma/neuroblastoma). At present we carried out immunophenotypization of these cell lines grown in monolayers.

Name of the research project:

Cultivation of neural stem cells and their transplantation into the donor's brain.

Grant Agency: **IGA**	*Project number:* **3233-3/95**
Researcher: **MUDr. Jaroslav Mokrý, CSc.**	
Joint Researchers: **Prof. MUDr. Stanislav Němeček, DrSc.** **Doc. MUDr. Danuše Šubrtová, CSc.** **Doc. MUDr. Jiří Náhlovský, CSc.**	
Starting date: **January 1995**	*Duration:* **3 years**
Funds allocated for project - total: **1,271,000 CZK**	

Summary of results:

In this project, IGA MZ enabled us to purchase a basic equipment for cultivation of fetal mammalian cells. We adopted the method for isolation and cultivation of neural precursor cells (NPCs) in serum-free medium supplemented with epidermal growth factor (EGF) originally developed by Reynolds et al. (J. Neurosci. Res. 1992, 12, 4565-4574). In the course of this 3-year grant project, we modified the original procedure for processing the fetal rat brain and cultivation of neural cells which enabled us to increase significantly the numbers of EGF-responsive NPCs harvested from cultures.

Under a mitogenic effect of a human recombinant analogue of EGF, Long-EGF, NPCs expressing appropriate receptors were stimulated to proliferate and formed multicellular structures of spheroidal shape, the so called neurospheres. We successfully reproduced experiments with neurospheres plated on polylysine-coated surface in medium supplemented with serum that confirmed the ability of NPCs to emigrate from the core of neurospheres and differentiate. Experiments with passaging dissociated spheroid cells confirmed that some spheroid cells retained their ability to form secondary neurospheres.

Our histological study of inner structure of neurospheres brought original results on changes showing in composition of the neurospheres during the extended cultivation periods. Immunophenotypization of spheroid cells confirmed a gradual maturation of NPCs inside of neurospheres: while the early neurospheres were composed of cells that expressed markers specific for immature neural cells like nestin or vimentin and proliferating cell nuclear antigen (PCNA), the neurospheres yielded after the prolonged cultivation contained only sporadic mitotic cells and many cells expressing antigens characteristic of mature neural cell lines, i.e. glial fibrillary acidic protein (GFAP)-immunoreactive astroglia, myelin basic protein-immunopositive (MBP^+) oligodendroglia and neurofilament$^+$ (NF^+), synaptophysin$^+$ and microtubule associated protein 2^+ ($MAP\text{-}2^+$) neurons. Light and transmission electron microscopy of paraffin- and resin-embedded neurospheres gave evidence that these solid three-dimensional formations consisted of heterogeneous population of neural cells that were able to undergo gradual differentiation; the neurospheres yielded 115 days after establishment of primary culture revealed, besides chemical synapses, sporadic myelinated fibres suggesting maturation of neuronal and oligodendroglial cell lines.

Parallel to the histological study of neurospheres we processed the intact brains of fetal and

postnatal rats for histology including immunohistochemistry. To identify low levels of antigens in the developing CNS we embedded the brains and spheres in low-melting paraffin (Polyester wax) and applied the Catalyzed signal amplification (CSA) method which enhances the sensitivity of the immunodetection. These techniques enabled to detect specific markers of mature neural cell lines at earlier stages of development than routine procedures (i.e. formalin-fixed and paraffin-embedded tissue). Gradual appearance of characteristic markers and structures in EGF-responsive neurospheres over extended periods of time mimicked corresponding phenomena occurring in the development of mammalian brain. Our original results of phenotypic changes occurring inside of neurospheres proved that spheroids of EGF-responsive NPCs represent a novel three-dimensional model for the study of neurogenesis in vitro.

Preliminary trials with grafting NPCs into the forebrain of adult rats suggest the cells cease to proliferate early after transplantation that prevents to increace their numbers. Nevertheless, the applied cultivation method enables to increase the amount of native NPCs in vitro prior grafting. The process of gradual differentiation of NPCs that occurs in primary cultures can be eliminated or interrupted by dissociation of neurospheres and subsequent passaging dissociated spheroid cells in EGF-supplemented serum-free medium.

Name of the research project: Interleukin IL-6 in humor		
Grant Agency:	***IGA MH CR***	***Project number:*** 4096-2
Researcher:	MUDr. Jan Novák, CSc.	
Joint Researchers:	Mgr. Miroslava Toušková Doc. RNDr. Jan Krejsek, CSc.	
Starting date:	1. 1. 1997	***Duration:*** 2 years
Funds allocated for project - total: 648 000 Kč		

Summary of results:

Introduction. Interleukin 6 (IL-6) is a multifunctional protein that plays an important role in host defence, acute phase reactions, immune responses, and hematopoiesis. IL-6 exerts multiple functions on numerous target cells. IL-6 plays an important role in immune functions. It has effects on B cell differentiation. IL-6 is known as a multifunctional cytokine of inflammatory condition. Very high IL-6 levels were found in endophthalmitis, uveitis and vitreoretinopathy. The function of the IL-6 level in the aqueous humor (AH) of relative normal eye is not clear.

Methods. The AH samples from 197 patients were collected at the start of surgery using AH aspiration through the cornea. IL-6 level were measured in 81 AH samples of myopic eyes (refractive surgery), patients with incipient senile cataract and patients with damaged hemato-ocular barrier (glaucoma surgery, early reoperation, diabetic cataract, uveitis). CK levels were determined in one part of the AH samples with the use of the ELISA method (Quantikine, R&D Systems).

Results. IL-6 levels among patients (n = 81, average 135,9 pg/ml, range 3,67 - 1130 pg/ml) varied greatly individually. The higher IL-6 levels were observed in a small group of 4 mature cataracts.

Conclusions. A theoretical explanation of the high IL-6 level in AH of relatively normal eyes is absent. TheAH compartment is shown to be one with an active secretion of IL-6. The diagnostic possibilities of IL-6 level in AH for determination of the eye diseases are not very clear. The specificity of the IL-6 level examination in the aqueous humor is very low. The enlargement of the group and a more detailed analysis of the clinical file is being planned for the next year (1998).

Name of the research project: Children and Parents (Děti a rodiče - DAR)

Grant Agency: Projekt podpory zdraví MZ ČR ***Project number:*** 28/97

Researcher: Doc MUDr Eva Pařízková

Joint Researchers:
MUDr Vladimír Němec

Starting date: 01/01/97 ***Duration:*** 1 year

Funds allocated for project - total: 785 000 Kč

Summary of results :

The aim of the project was in improvement of the co-operation of children and parents with health services professionals in both - preventive and curative health care. There was the attempt to achieve the higher effectivity of the children care together with decrease of children stress due to contact with healt service. The picture booklet LUCY IS ILL was published and is suitable for the at school as well as at home. It is capable to prepare the child for adequate co-operation with physicians during the preventive examinations, vaccinations, and treatment of illnesses.

A videoprogramme was recorded to describe it is possible to make good conditions for small patients respecting The Chart of Richts of the Ill Child with very small amount of financial support. The prexence of parents during the treatment and examination of children in the departments and professionaly organised games casuse that the psychical state of children and knowledge of parents are improved. There are also enriched the skills and habits of parents to provide optimal home care, rehabilitation, and prevention. Due to them the whole life style of the family is well changed. The video mentioned earlier can become a useful instrument of motivation and education.

Name of the research project: Antibodies and Cytokines in Serum and Cell Cultures in Children Suffering from Primary Immunodeficiencies

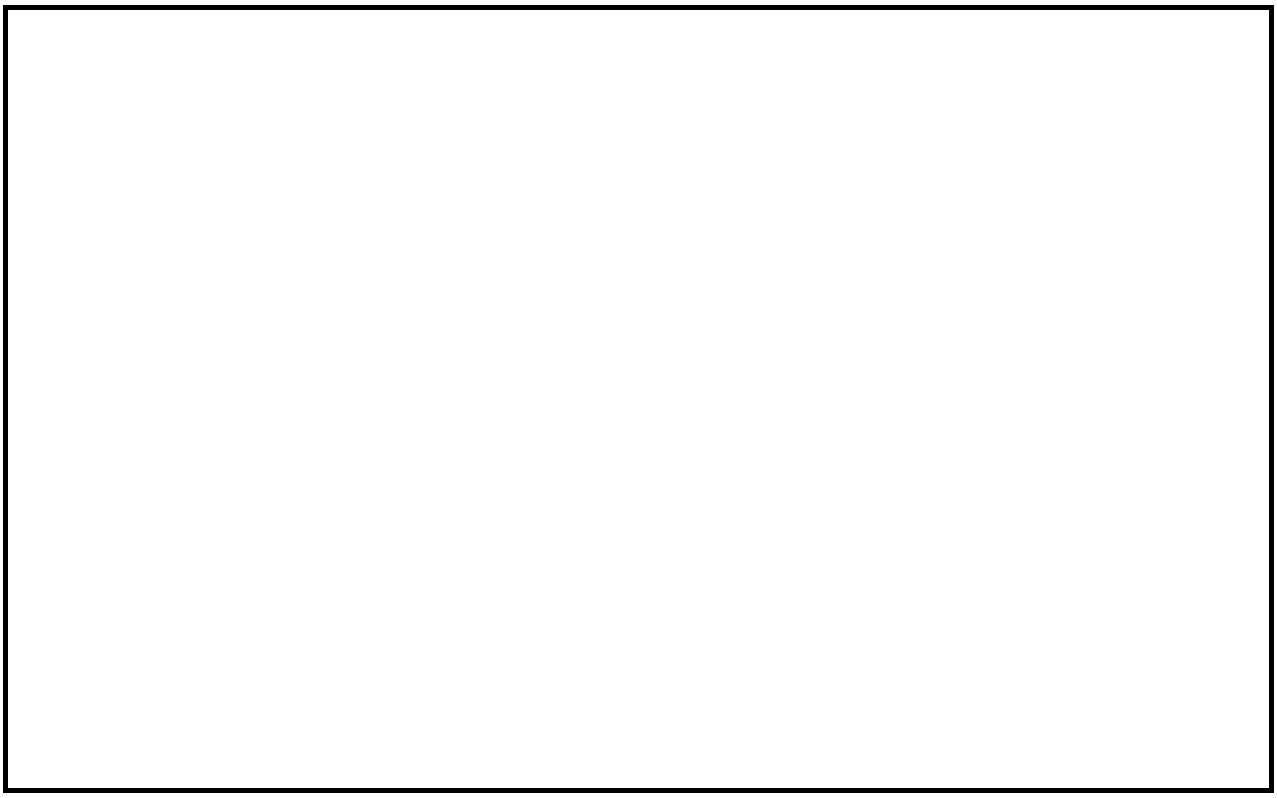
Grant Agency: IGA MZ ČR	***Project number:*** 4098-3
Researcher: Doc MUDr Eva Pařízková, CSc	
Joint Researchers: MUDr David Komárek RNDr Ctirad Andrýs PharmDr Doris Vokurková	
Starting date: 1997	***Duration:*** 3 years
Funds allocated for project - total: 999 000,- Kč	

Summary of results:

There were prepared protocols to examine the cellular immunity in children. The panels were designed to make the diagnosis of the child on the base of investigated surface markers on the surface of the immunocompetent cells and on the base of the answer to the stimulation of the cells by mitogens, antigens, and specific antibodies to the surface receptors. Indications for the examination are clinical or clinical and laboratory signs of the disease. Therefore the panels are used for clinical praxis as well as for the reserch purpouses. By this time it is required to interpretate the rusults individualy because there are no reference ranges for most of the examined surface markers (and those which are available are not age corrected). There are also prepared the clinical protocols to reduce the duplication of laboratory tests to reach the diagnosis and to reduce the cost of the examination.

There were selected children with severe immunodeficiencies which were diagnosed clinicaly and laboratory and more precious diagnosis took place using the previously mentioned panels. There are patients suffering from both types (humoral and cellular) of the immunity defects (X-linked hypogammaglobulinemia, Common variable immunodeficiency, Wiskott-Aldrich syndrome, DiGeorge syndrome etc). There are measured serum levels of immunoglobulins in patients who have IVIG substitution and there are studied the changes with predicted and actual serum levels and the influence of acute infections to the followed up parametres. The age of patients varies from the infancy up to early adulthood.

The laboratory approach to reach optimal results of cytokine production in supernatant of separated lymphocytes is investigated. There is used the stimulation by mitogens (PWM, PHA) to prepare the laboratory protocols. Some of the wery common and well known cytokines are used to prepare the laboratory approach (IL2 etc).



Name of the research project: **Diagnosis of proarrhythmic effect of antiarrhythmic drugs**	
Grant Agency: **IGA MZ ČR**	***Project number:*** **2948-3**
Researcher: **Prof. MUDr. Vladimír Pidrman, DrSc.**	
Joint Researchers:	**MUDr. Petr Pařízek** **MUDr. Martin Hodač**
Starting date: 1.1.1995	***Duration:*** **3 years**
Funds allocated for project - total: **750.000,- Kč**	

Summary of results:

Proarrhythmia can be a life-threating complication of antiarrhythmic therapy. The aim of this study was to determine if programmed stimulation of ventricles (PVS) is useful in recognizing proarrhythmia that may not be noted using noninvasive techniques.

The patients (52 men, 6 women, mean age 58+11,5) underwent in summary 123 PVS. The cause of antiarrhythmic therapy was CPR (21 pts), syncopy (15), preasyncopy (6), sustained VT (21). The drug administered was amiodaron (33 pts) and propapheron (12), the other used some of combination of antiarrhythmics. All patients underwent initial PVS and in 51 of them a subsequent PVS was repeated after an interval at least of 6 weeks after the treatment was started.

Criteria for proarrhythmia during PVS were present in 47% of pts (15 pts conversion of induced non-sustained VT to sustained VT or increase of the frequency of VT, 5 pts induction of arrhythmias with less agressive stimulation protocol, 8 pts new requirement for electrical cardioversion to terminate tachycardia - 7 pts showed combinations of these criteria). Ambulatory Holter ECG was positive in 9 pts (only in 12% in association with positive PVS). There were no significant correlation between PVS and late potentials, ejection fraction or ergometry. We observed proarrhythmias in 33% of pts after amiodaron treatment and in 66% after propaphenon treatment. During follow up (mean 35+27 month) the pharmacotherapy was succesfull in 77%. There were 3 sudden seaths and 3 implantations of ICD - without any difference between pts with positive or negative PVS.

Conclusions: This study confirms that PVS is a usefull method for recognition of early patterns of proarrhytmia. The patients with higher cardiovascular risk should undergo this examination during antiarrhythmic treatment. Follow up data show that early diagnosis of proarrhythmia markers may be of very important value in guiding therapy.

Name of the research project:

OPTIC NERVE SHEATHS DECOMPRESSION

Grant Agency: GA ČR	***Project number:*** 308/96/0756
Researcher: Prof.Pavel ROZSÍVAL,MD ,PhD	
Joint Researchers: Naďa JIRÁSKOVÁ ,MD	
Starting date: 1996	***Duration:*** 3 years
Funds allocated for project - total: ***333 OOO Kč***	

Summary of results:

The autors present results of optic nerve sheaths decompression (ONSD) in 55 eyes. The decompression surgery was performed for progressive form of anterior ischemic optic neuropathy
(AION) in 21 eyes, AION combined with central retinal vein occlussion (CRVO) in 2 eyes, low-tension glaucoma in 7 eyes (+ l reoperation), CRVO in 2 eyes, optic nerve head drusen in 16 eyes, chronic optic disc edema in pseudotumor cerebri in 5 eyes, amiodaron optic neuropathy in 1 eye. All our patients underwent thorough preoperative and postoperative ocular evaluations**.**

Our results of 55 surgical procedures indicate the best beneficial effect in cases of pseudotumor cerebri (all 5 eyes improved) . Also succesful ONSD was for other diagnosis: progressive AION (12/21 eyes improved), optic nerve head drusen (11/16 eyes improved) and low-tension glaucoma (3/7 eyes improved). Patients satisfaction was high. After surgery a transient double vision developed in 5 patients. We have not seen any other complications.

We advocate this metod of treatment for vision - threatening optic neuropathies after thoroughly balanced assessment of possible risks and benefits.

PhD was finished during this research project.

Name of the research project:

Development of programs for learning of medical chemistry and biochemistry.

Grant Agency: FR VŠ	***Project number:*** 1420/97
Researcher: Ing. Pavel Šiman, CSc.	
Joint Researchers: Doc. MUDr. Alena Stoklasová, CSc.	
Starting date: January, 1997	***Duration:*** 1 year
Funds allocated for project - total: 60 000,- Kč	

Summary of results:

Computer programs of three types for teaching medical chemistry and biochemistry are in development. The first type of such programs is graphic and interactive information from selected areas of chemistry and biochemistry. At present time the "Titration" is finished. This program gives basic information about acid-base titration and makes possible to try "virtual" titration of three model solutions (HCl, acetic acid or acetate buffer) or two "unknown" samples (HCl and HAc). Students can also try to calibrate interactively the pH-meter. The representative of the second type of teaching software is the interactive computer aid for statistical and visual evaluation of experiments using photometry. This program is in development now. Computer program for testing of chemical knowledge is of the third type of teaching software. This aid makes possible computer testing according to practice in the department of medical biochemistry of our school.

Remark:

In consequence of the decision of the Ministry of Finance about allocation of money the work on development of educational software could start in october 1997. So, the planned time for programming was dramaticaly shortened.

Name of the research project:

Pharmacokinetics of low doses of methotrexate in patients with psoriasis over the early period of treatment

Grant Agency: IGA MZ ČR	***Project number:*** 2960-3

Researcher: MUDr. Marie Šimková

Joint Researchers: MUDr. Jaroslava Vaněčková
MUDr. Věra Koudelková
doc. MUDr. Marie Nožičková,CSc.
prof. MUDr. Jiřina Martínková,CSc.
Ing. Jaroslav Chládek

Starting date: January 1995	***Duration:*** 3 years

Funds allocated for project - total: 2-127 tis. Kč

Summary of results:

Abstract.

Objective: to study the relationship between pharmacokinetics and pharmacodynamics of low-dose methotrexate in the early phase (3 months) after the start of antipsoriatic therapy.

Methods: 10 male and female psoriatic patients who failed to respond to previous conventional therapy were treated with 15 mg oral methotrexate administered once a week. Methotrexate (MTX) and 7- hydroxymethotrexate (7-OH MTX) pharmacokinetics in plasma and urinary excretion were investigated after doses 1, 5 and 13. On the same occasions, MTX accumulation was followed in erythrocytes obtained before the MTX administration. Pharmacodynamics of MTX were evaluated using PASI-score reflecting skin disorders.

Results: There were marked intersubject differences (range of CV%: 34.9 - 76.3) in MTX AUC, Cmax and Cl_{tot} . Only part of this variability could be predicted using the linear relationship between Cl_{tot} and creatinine clearance ($r^2 = 0.541$, $p<0.0001$). Cl_{tot} of MTX was proportional to Cl_{ren} ($r^2 = 0.735$, $p<0.0001$) which accounted for 73 $\pm$ 19 % of the former. There was a strong linear relationship ($r^2 = 0.819$, $p<0.0001$) between Cl_{ren} of MTX and creatinine clearance. Within 48 hours after drug administration, the urinary excretion of MTX was 46 - 99 % of the dose and that of 7-OH MTX 1.5 - 8.6 %, respectively. In 8/10 patients, more than 70% of MTX dose was recovered. No intraindividual variations of the kinetic parameters during treatment were observed. Pharmacodynamic measurement versus pharmacokinetics revealed a significant inverse relationship between PASI-score and MTX AUC ($r_s = -0.912$, $p < 0.002$) and between PASI-score and erythrocytic MTX ($r_s = -0.988$, $p < 0.002$).

Conclusions: The relationship between MTX pharmacokinetics (AUC or erythrocytic MTX) and pharmacodynamics (PASI-score) may exist. It is likely that the efficacy of psoriasis therapy with MTX could be improved by adjusting the dose according to plasma concentrations obtained after first MTX administration.

Key words: Methotrexate, psoriasis, PASI-score, pharmacokinetics of low-dose MTX

Name of the research project:

THE CHANGES OF ENERGY METABOLISM AND BODY COMPARTMENTS DURING ABSOLUTE FASTING

Grant Agency: IGA MZ ČR	***Project number:*** 2963-2

Researcher: Doc. MUDr. Luboš SOBOTKA, CSc.

Joint Researchers:

doc. MUDr. Jiří Chaloupka, CSc., prof. MUDr. Zdeněk Zadák, CSc., doc. MUDr. Vladimír Palička, CSc., MUDr. Eduard Havel

Starting date: 1.1.1995	***Duration:*** 3 years

Funds allocated for project - total: 547 tis. Kč

Summary of results:

The aim of present study was to assess the influence of prolonged total fasting on resting energy expenditure (REE), thermic effect of nutrition (TEN), body compartments and plasma protein and lipid parameters.

Nine patients accepted to metabolic care unit because of obesity were studied before and after fourteen days of absolute fasting. During the period of fasting they received noncaloric fluids (water, tea, coffee), vitamins and potassium. Before and after fasting period their REE and TEN after mixed liquid meal (4 kcal/kg body weight; 55% CHO, 30% fat, 15% protein) were measured by indirect calorimetry (MMC Horizon, Sensor Medics). At the same time intervals serum samples were analysed for protein and lipid levels. During the fasting period balance trends were also followed.

The patients lost 9.98 ± 1.6 kg of body weight during the fasting period. The total loss of 570.6 ± 118.8mmol Na corresponded to loss of 3.96 ± 0.83 kg of extracellular fluid. Protein loss reached to 0.89 ± 0.20 grams which corresponded 3.54 ± 0.80 kg of muscle tissue. Urinary sodium and nitrogen outputs decreased exponentially during the fasting period. Calculated reduction in fat stores reached 3.64 ± 2.34 kg of fat tissue. This results corresponded to energy expenditure measured by the indirect calorimetry. The relationship of protein loss to fat tissue loss was higher during the first week of fasting.

REE decreased from 2334.4 ± 171.7 kcal/d to 2000.5 ± 88.26 kcal/d, however TEN to mixed meal (4 kcal/kg b.w.) was not influenced by the fasting period.

These data shows us that during the first week of absolute fasting the body weight decrease is associated predominantly with water, mineral and muscle tissue loses. Adipose tissue decline can reach maximally 200 - 300 g per day. Energy expenditure decreases during fasting period without any changes in thermic effect of nutrition.

Name of the research project:

The Influence of Antioxidant Balance on Clinical Outcome and Biochemical Parameters in the Aged - Open Prospective Clinical Trial

Grant Agency: IGA MZ CR ***Project number:*** 2953-3

Researcher: RNDr. Dagmar Solichová

Joint Researchers:

MUDr. Vladimír Bláha, CSc., prof. MUDr. Zdeněk Zadák, CSc,
RNDr. Miluše Brátová , RNDr. Petr Žďánský,
MUDr. Miloš Klejna, MUDr. Bohuslav Melichar

Starting date: 1.1.1995 ***Duration:*** 3 years

Funds allocated for project - total: 1 414 thousand crowns

S u m m a r y o f r e s u l t s :

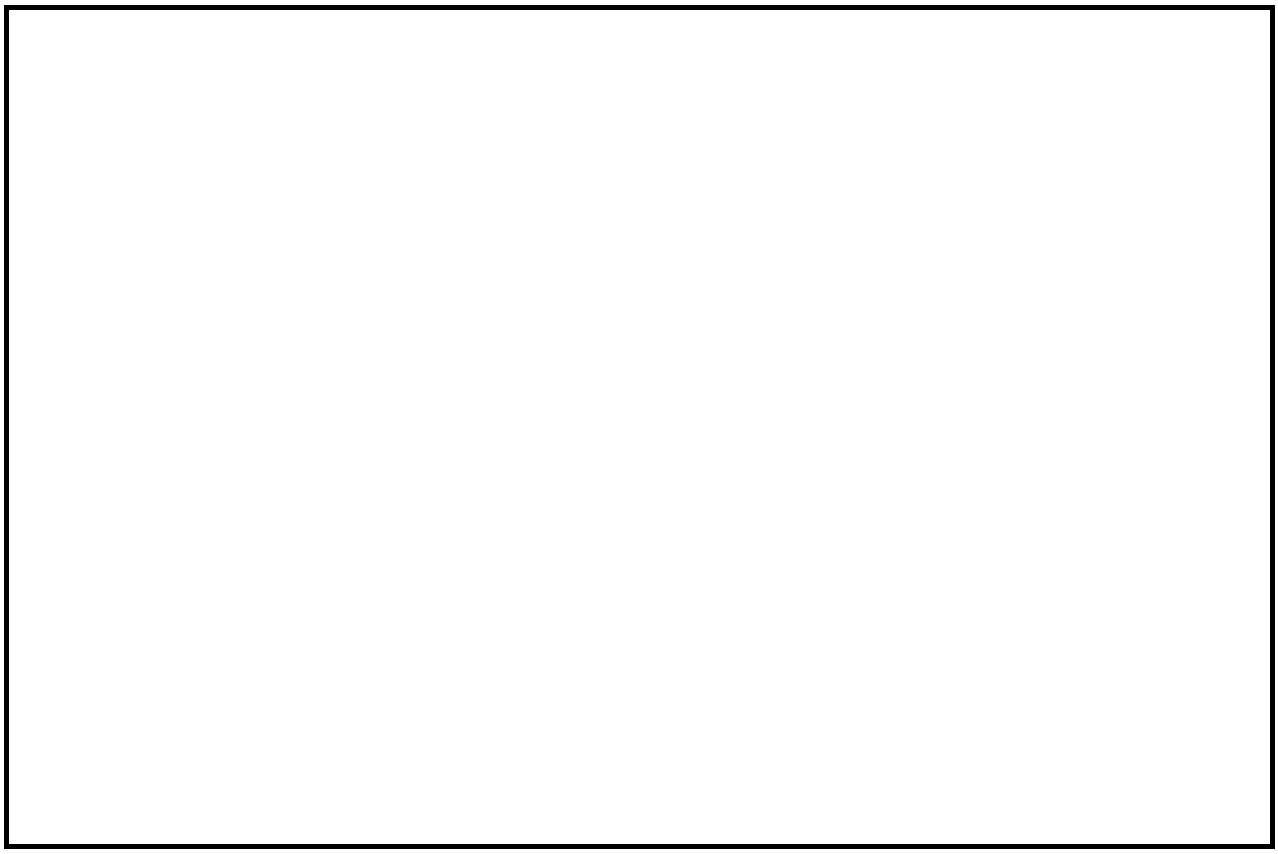
The aim of the project was to evaluate the role of overall biochemical parameters in predicting mortality and morbidity in aged population. Thirty-eight nonagenarians aged 92 $\pm$ 2 (range 90 - 100) years, followed at the Department of Gerontology and Metabolism entered the study. At the start of the study, a sample of peripheral blood and urine were obtained for analysis of 50 basic biochemical, hematological and biological parameters. The samples of urine and blood were then obtained in 6 - 12 months intervals.

The significance of difference between surviving subjects and those who died was examined by Mann-Whitney U test. Correlation was studied by Spearman rank correlation coefficient. The decision on significance was based on P = 0.05 level.

During the period of observation, 21 subjects died, leaving 17 persons still alive at the end of the study. The mean time from the first measurement to the death was 12 $\pm$ 10 (range 0 - 33) months. The mean follow-up time in surviving subjects was 31 $\pm$ 12 (range 4 - 45). The summary of results of the project can be seen in the table. Serum vitamin E and calcium were significantly higher, and serum ALT and urinary neopterin were significantly lower in survivors compared to the subjects who died. No other parameters were significantly different in survivors and in persons who died. Urinary neopterin exhibited a significant correlation with serum sodium concentration (rs = - 0.50, P < 0.01), but the other parameters did not correlate significantly.

Summary of statistically important biochemical parameters based on survival

Variable	Unit of measurement	Survivors			Died			P
		n	Mean ($\pm$ SD)	Range	n	Mean ($\pm$ SD)	Range	
Age	years	17	92 (2)	90 - 100	21	93 (2)	90 - 98	N.S.
serum vit. E	μmol/l	17	27.9 (8.6)	10.7 - 41.2	21	21.0 (5.6)	8.3 - 30	0.02
ur. neopterin	μmol/mol crea	16	370 (131)	159 - 633	20	701 (552)	203 - 2479	0.02
serum calcium	mmol/l	17	2.28 (0.13)	1.93 - 2.46	17	2.21 (0.15)	2.09 - 2.71	0.02
serum ALT	μkat/l	17	0.30 (0.11)	0.11 - 0.55	21	0.42 (0.20)	0.20 - 1.07	0.03



Name of the research project: RECURRENT AND CHRONIC MYCOTIC VULVOVAGINITIS. IMPROVEMENT OF DIAGNOSIS, THERAPY AND PROPHYLAXIS.	
Grant Agency: IGA MZ ČR	***Project number:*** 3694-3
Researcher: Š P A Č E K Jiří, MD	
Joint Researchers: Buchta Vladimír, RN.D, Ph.D.Krejsek Jan, Assoc.Prof., RN.D, Ph.D.Jílek Petr, Pharm.D, Ph.D.Tošner Jindřich, Assoc.Prof.,MD, Ph.D.	
Starting date: 1996	***Duration:*** 2 years
Funds allocated for project - total: 287 000 Kč	

Summary of results:

Recurrent vulvovaginal candidiasis particularly its chronic form (four or more episodes per annum) is an unsolved problem in gynecology. Risk factors are usually absent in these women and there is often a remarkable discrepancy between symptoms (itching, burning, discharge) which are usually intensive and clinical findings which are mostly poor (absence of inflammation). In women with idiopathic RVVC, antifungal therapy is highly effective for eliminating topical clinical symptoms but not mycological eradication. This is the reason for failing to prevent next attack. Vaginal Candida infection is distinctly hormone dependent, occurring rarely in premenarcheal girls as well as in postmenopausal women. In our set of patients (n=50) Candida albicans was prevalent in etiology of RVVC because it accounted for 86,9 % of strains isolated from vagina. The remaining species were represented Candida glabrata (10 % isolates), C. tropicalis, C. parapsilosis and Saccharomyces cerevisiae. It seems that the microscopic examination has little use for diagnosis of RVVC in contrast to acute episodes. Culture is more sensitive method which in addition enables identification of a isolate at species level. Identification of etiologic agent and evaluation of its antifungal susceptibility in vitro is an important part of therapeutic management of RVVC. The decreased susceptibility of some non-albicans Candida especially to azole derivatives (fluconazole, ketoconazole) can contribute to failure of treatment in some cases of RVVC. Our experience suggests that for successful management of RVVC is necessary elimination of underlying factors. Gestagen administration (Depo-Provera) is one of the possibility as a alternative approach to management of women with

RVVC. Our study is based on a group of 12 patients with chronic vulvovaginal candidiasis treated with Depo-Provera (administration 150 mg every 3 months by intramuscular injection). 6 patients (50%) are successfully treated = no symptoms, normal clinical and microbiological findings; 2 patients (16,66%) = improvement of symptoms, normal clinical and microbiological findings; 1 patient (8,33%) = unsuccessful treatment; 3 patients (25%) = beginning of the treatment The immunological examination of peripheral blood was performed. Among 27 parameters, 22 were normal, the other were slightly changed. Only IgE levels and CRP concentrations as well as monocyte count were very low, while neutrophile counts were often elevated as well as IgM. No remarkable changes between period of recrudescence and remission in individual patients were found. No patognomic changes in immunological parameters appeared. The parameters of local immunity are now under investigation.

Name of the research project:

THREE-DIMENSIONAL ORGANIZATION OF SYNAPSES AND ENDOPLASMIC RETICULUM OF DENDRITIC SPINES

Grant Agency: NIH BETHESDA (Fogarty)	***Project number:*** TW 00178

Researcher:

J. Špaček, Dept. of Pathology, Charles University Faculty of Medicine, Hradec Králové

Joint Researchers:

K.M. Harris, Div. of Neuroscience, Harvard Medical School, Children´s Hospital

Starting date: 1.7. 1994	***Duration:*** 31.1. 1997

Funds allocated for project - total: 60 000 USD for the host laboratory

Summary of results:

Dendritic spines of cerebral cortex, synapses placed on them and changes of their number, shape and size (so called synaptic plasticity) are supposed to represent a structural basis for processes of learning and memory. That is why these structures have been in the centre of interest of many neurobiologists during last two decades. Results of our new observations on the spines and synapses were as follows:

A new model of morphologically inhomogeneous synapse is presented in which apart from an active zone associated with synaptic vesicles, an adherent zone free of vesicles appears. 45 % of synapses localized on dendritic spines (rat, hippocampal area CA1) contains this punctum adhaerens-like zone or vesicle free transitional zone.

64 % of all remaining puncta adhaerentia (i.e. of those not associated with synapses) are localized between large dendritic spines and astrocyte processes in their vicinity. A close functional relationship between synapses and astrocytes is thus morphologically fixed.

A smooth endoplasmic reticulum is associated with puncta adhaerentia and synapses. The amount of endoplasmic reticulum in parent dendrites is proportional to a number of spines and synapses originating along their lengths. On the small spines only small macular synapses are placed and only a small amount or no reticulum is present. The large spines contain a cisternal spine apparatus derived from reticulum and directed to the extensive „perforated“ synapse.

The results strongly support the role of smooth reticulum in regulation of ionic microenvironment of synapse and adherent zones. The spine apparatus also appears to produce a building material for puncta adhaerentia.

References:
Špaček J, Harris KM (1997) Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat. J. Neuroscience
17: 190 - 203
Špaček J, Harris KM (1997) Three-dimensional organization of cell adhesion junctions at synapses and dendritic spines in area CA1 of the rat hippocampus. J. Comp. Neurol. (In print.)

Name of the research project: Prediction of cytostatic treatment induced bleeding using an artificial neural network.

Grant Agency: IGA MZ ***Project number:*** 17052

Researcher: Libor Straka, M.D.

Joint Researchers:
Miloslav Kmoníček, M.D.
Václav Šebesta, DrSc.

Starting date: 1.1.1996 ***Duration:*** 3 years

Funds allocated for project - total: 777 000,- Kč

Summary of results :

Thrombocytopenia and bleeding is a dangerous complication in the treatment of hematologic malignancies. Therapy and prophylaxis of bleeding is based only on administration of platelet transfusion.

The main adverse effect of this therapy is refractoriness and lowered effect of the subsequent transfusion. Physician's decision whether to administer platelet transfusion or not is based on two facts:

1. Estimation of bleeding risks (80 % of decision).
2. Estimation of refractoriness development risk (20 % of decision).

In our decision support system we are solving estimation of bleeding risk (item 1). In the beginning we have been searching for significant factors influencing bleeding.

Method:

We have completed database of 22 patients, and 1810 hospitalising days.

By means of method GUHA, which generates hypothesis type: “assuming factor A, B, C ….is (is not) present then bleeding occurs (does not occur)” and by statistical evaluation of statistical significance of number “bleeding” and “non-bleeding” days by means of 2x2 table and a χ^2 test. The null hypothesis was: "There is no difference in the number of bleeding and non-bleeding days with and without presence of a selected factor."

Results

We can suppose that following factors are significant for bleeding: sepsis on the evaluated day and on the day before, blood pressure on the evaluated day, hypertension and diabetes mellitus in anamnesis, number of cytostatic cures, transfusion of erythrocytes on the evaluated day and on the day before.

There are factors that are not significant for risk of bleeding: decreased level of ALT and AST, administration of growth factors, previous bleeding (on the evaluated day and on the day before).

Name of the research project:
HIGH DOSE CHEMOTHERAPY WITH SUPPORTIVE CARE BY WHOLE BLOOD RICH IN STEM CELLS

Grant Agency: IGA ČR ***Project number: 3679-3***

Researcher: MUDr.Jaroslav Vaňásek, CSc.

Joint Researchers:
MUDr.Stanislav Filip
MUDr.Vlasta Medková
doc.MUDr.Milan Bláha,CSc.
MUDr.Pavel Měřička
doc.RNDr.Jiřina Vávrová,CSc.

Starting date: l996 ***Duration: 1997***

Funds allocated for project - total:160 000,- CZK

S u m m a r y o f r e s u l t s :

To support multicyclic, dose-intensive chemotherapy in breast cancer, we assessed the effects of reinfusing hematopoietic progenitors either as a leukapheresis product or as mobilized unprocessed whole blood. We tested three mobilisation regimens, standard FAC (fluorouracil 400 mg/m2, cyclophosphamide 400 mg/m2 and adriamycin 40 mg/m2), HD-FAC (fluorouracil 400 mg/m2 , cyclophosphamide 1200 mg/m2 , adriamycin 40 mg/m2) and EC regimen (epirubicin 150 mg/m^2 and cyclophosphamide 1250 mg/m^2). In regimens FAC and HD-FAC were yields of PBPC in whole blood insufficient for clinical use. In our study, 16 consecutive female breast cancer patients were given six cycles of chemotherapy regimen EC (epirubicin 150 mg/m2 and cyclophosphamide 1250 mg/m2 on day 1). In the firs cycle 24h after chemotherapy, mobilization of the peripheral blood progenitor cells (PBPC) was started with growth factor G-CSF (Neupogen, Amgen Roche) at a dose of 5 μg/kg/day for 13 days. In all other cycles G-CSF had been given at the same dose from day 7. On days 11,12 and 13 the leukaphereses were performed and their products cryopreserved. On day 14 whole blood was collected. The median peak incidence of CFU-GM (granulocyte-macrophage colony-forming unit) in peripheral blood was approximately 50 times the baseline level. The leukaphereses PBPC were divided into portion and reinfused after the fourth, fifth and sixth chemotherapy courses. The support with mobilized whole blood was given after the second and third cycles. the best yields of leukaphereses were achieved on day 13 after initiation of chemotherapy. The mean number of CD34+ cells was 4.93x10^6 /kg (range 0.36-10.54x10^6/kg) the amount of CFU-GM was 2.18x10^5/kg (range 0.07-4.2x10^5/kg). The yields of CFU-GM in 450 ml whole blood collected on day 14 reached 0.51x10^6/kg (range 0.05-1.5x10^6/kg and CD34+ cells were 1.3x10^6/kg (range 0.18-2.58x10^6/kg). PBPC yields in 450 ml of unprocessed whole blood were in some cases not sufficient for good hematopoietic recovery after EC cycles. Grade 4 leukopenias and thrombocytopenias were two times higher in cycles with whole blood support than in cycles with cryopreserved PBPC support. An increase of PBPC harvest can be simply achieved by collecting larger amount of unprocessed blood, as used by some authors. Hematologic effects of G-CSF and EPO combination after priming intensive chemotherapy in the treatment of female breast carcinoma were tested. We found that the administration of G-CSF and EPO combination following intensive chemotherapy reduces hematologic toxicity and induces large amount of hemopoietic progenitros suitable for autologous transplantation in women with breast carcinoma.

Keywords: peripheral stem cells, autologous transplantation, G-CSF, CFU-GM, CD34+ cells, breast carcinoma

Name of the research project:

Evoked Potentials in Early Diagnosis of Multiple Sclerosis

Grant Agency: Grant Agency of Ministry of Health (IGA MZ) ***Project number:*** 2980-3

Researcher:
Gerhard Waberžinek

Joint Researchers: Jan Kremláèek
Zuzana Kubová

Starting date: 1995 ***Duration:*** ***3 years***

Funds allocated for project - total: ***614.000,- CZK***

S u m m a r y o f r e s u l t s :

A scheme for multiple sclerosis (MS) examination was designed on the basis of results obtained from a group of more then four hundred patients investigated by various techniques of evoked potentials. The observations were completed with CT or MRI findings and cerebrospinal fluid analysis results.

The entire group of patients was examined for the parvocellular subsystem of the visual pathway by means of pattern-reversal visual evoked potentials (P-VEPs) and about 20% of the group underwent motion-onset VEPs (M-VEPs) examination of magnocellular subsystem.

Next to the visual pathway we inspected auditory pathway in about three hundred patients through brain stem evoked potentials (BAEPs) and in ninety patients we recorded somatosensory evoked potentials (SSEPs).

A single type of the evoked potentials yielded diagnostic sensitivity up to 70%. The evaluation already two types of evoked potentials increased the sensitivity up to 90% (1). The study confirmed generally accepted recommendation to combine various kinds of evoked potentials and suggest combine the P-VEPs and the M-VEPs as the first choice. In special questions concerning brain stem lesions the BAEPs should to be examined. Brain and spinal cord white matter lesions (demyelinisation) can be detected by SSEPs.

The electrophysiological methods seemed to be very important when the imaging techniques brought no positive findings especially in the early stages of MS.

Publication:

(1) Waberžinek G,Kremláèek J, Kuba M, Kubová Z: Comparative study of electrophysiological methods in diagnostics of multiple sclerosis, European Journal of Neurology, v.4 Supplement I, June 1997.

Name of the research project:

The Degree of Cytokine Network Activation and Quality of Renal Allograft

Grant Agency: IGA MH CR ***Project number:*** 3689-3/96

Researcher: Živná Helena

Joint Researchers: Živný Pavel, Černý Vladimír, Navrátil Pavel, Palička Vladimír, Dostál Pavel

Starting date: January 1996 ***Duration:*** 3 years

Funds allocated for project - total: 560 000 CZK

S u m m a r y o f r e s u l t s :

The aim of this project is to study the role of selected laboratory tests in prediction of renal graft quality. The study had two parts: methodological and experimental.

1) Methodological part: The possibilities of cytokine estimation in urine samples.

Patients and methods: This part of study included 11 children (6 boys, 5 girls) without renal disease. TNF-α, IL-1α, IL-6, IL-2 and sIL-2R in plasma and urine were measured using EIA kits (Immunotech). Estimations were performed without previous concentration of urine samples, except TNF-α (samples concentrated 5times with Minicon concentrators). Results: TNF-α was not detected in urine of healthy children, IL-6 concentration was very low (0.2±0.1 pg/ml), IL-1α, IL-2 and sIL-2R were detected in urine of healthy children.

2) Experimental part: Comparison of blood and urine cytokine levels in kidney transplant donors and recipients. Patients and methods: 5 renal graft donors and relevant recipients were enrolled in this study. Blood and urine samples were taken in donors at the time of brain death diagnosis and then every 4 hours. Samples from recipients were taken approximately 6,24 and 72 hours after transplantation. Blood and urine concentrations of sIL-2R, IL-6, IL-1β were measured using ELISA kits (R&D Systems,USA). Selected results are presented as mean±SEM in pg/ml. Statistical analysis was made using Jandel Scientific software. Results: Blood levels of IL-6 in donors (245.1±40.5) were higher than in recipients (37.9±7.7, $p<0.05$). IL-6 in urine was 11.1±2.4 in donors and 100.8±27.3 in recipients. Blood levels of sIL-2R in donors (60.4±30.0) were lower than in recipients (122.8±10.1, $p<0.05$). Conclusion: These preliminary results and comparison with clinical findings in renal transplant recipients suggest that blood IL-6 concentrations could predict graft quality.