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ABSTRACTS

ABSTRACTS FROM "THE SIXTH SCIENTIFIC CONFERENCE OF THE CHARLES UNIVERSITY FACULTY OF MEDICINE AND UNIVERSITY HOSPITAL", 23 JANUARY 2002, HRADEC KRÁLOVÉ

Abstracts of papers of "The Sixth Scientific Conference of the Charles University Faculty of Medicine and University Hospital" contain summaries of research projects completed through different grant agencies.

Cardiac troponin T in children

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The aim of this study was to compare the protein profiling of the regulatory proteins in the samples of myocardium obtained during surgery on children with congenital heart diseases. Two methods (1, 2) were used for the isolation of cardiac troponin T (cTnT) from both the right ventricular and right atrial musculatures. SDS - PAGE (12.5%) was carried out; in some cases, proteins were immunoblotted and analysed using JLT-12 MAb (Sigma). It could be concluded that (a) the first method enables the determination of the intracellular compartmentation of cTnT, the second one is better for the study of cTnT isoforms expression, (b) the cytosolic pool of cTnT represents 12.5 %, the myofibrillar pool of cTnT was 87.5 %; this proportion was not affected by hypoxaemia, (c) the expression of regulatory proteins may be functionally important after a surgical repair (3). Furthermore, we tried to evaluate the diagnostic performance of cTnT as a marker of myocardial damage during therapy with high doses of betasympathomimetics. The plasma concentration of cTnT was measured using Elecsys Troponin T STAT Immunoassay (Roche). cTnT in neonates after the infusion of betasympathomimetics ($0.24 \pm 0.05 \mu\text{g/l}$) was significantly higher ($p < 0.05$) in comparison with the control group ($0.05 \pm 0.01 \mu\text{g/l}$). The maximal values were reached about the 3rd day of therapy ($0.39 \pm 0.11 \mu\text{g/l}$). It is possible to conclude that minor myocardial lesions caused by betasympathomimetics can be detected by the measurement of cTnT, which is very important from the clinical point of view. Reference: 1. Potter JD et al. *Methods Enzymol* 1982;85:241-63, 2. Bleir J et al. *Clin Chem* 1998; 44 (9): 1912-8, 3. Adamcová M, Pelouch V. *Physiol Res* 1999;48:235-47.

Supported by Grant: GA ČR No. 305/98/P261.

Assessment of accessible methods application of local anaesthetics in dentistry

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Students, the project leaders, applied local anaesthesia to fellow volunteer students in the oral cavity using all methods available. First, they applied the oldest and most commonly used syringe to needle method. Then, they chose the cartridge syringe cylindrical ampoule application, and they also tested a new computer guided method (Wand) with controlled anaesthesia dosage. The last anaesthesia application was performed by the needleless injector (SyriJet). The students chose three types of local anaesthesia: Infiltration injection anaesthesia, block and periodontal ligament injection anaesthesia. Using the standardised psychological tests, the students evaluated both qualitative and quantitative pain perception and other unpleasant feelings during the application. At the same time, the students tried to keep the same conditions of application (identical anaesthesia, place of application, non-inflamed area, age, health conditions, one-man application). The obtained data were statistically analysed and put into graphic form. In pain comparison of the methods, statistically, the needleless injector application was the best one (according to the Friedman test). The same result was obtained while comparing the qualitative perception (Bonferroni mutability test). The factual output of the project was to obtain the overall view of possible anaesthesia application as well as hands-on training and recognition of the advantages and disadvantages of the various methods. In addition, the University must provide students with the latest information and methods, to give them both theoretical and practical knowledge.

Supported by Grant: FRVŠ No. 1719/G3/01.

Practical material problems in the teaching of prosthetic technology

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According to the new dental curriculum at the Medical Faculty of the Charles University in Hradec Králové, the fourth term of the dental syllabus of lectures and practical classes is devoted to prosthetic technology. During the lectures the students familiarize themselves theoretically with both the materials used in the fabrication of the dental prostheses and the working procedures necessary for the manufacturing of the basic kinds of dentures. During the practical classes they proceed to the basic types of prosthetic materials and to the fabrication of some simple restorations and dentures on their own. So far the practical classes have taken place in the clinical prosthetic laboratory, which did not meet all the demands of the big students groups. In the newly reconstructed building of the stomatological clinic, the student teaching prosthetic laboratory will be part and parcel of the preclinical department. The equipment of this laboratory was mostly financed from the funds of this grant. We bought: gypsum casts cutter; mechanical press; gypsum vacuum mixer; polymerization device; polishing device; thermostatic reservoir for dental wax. This laboratory will be consequently completed so that it will be possible to fabricate all the basic kinds of all-resin and metallic fixed restorations, complete removable dentures and both partial removable dentures with simple wire clasps and rather complex metallic cast framework.

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The importance and mutual correlation between the indicators of atheromatosis activity after LDL-apheresis

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The project aims to find out the importance and mutual correlation between the indicators of atheromatic activity (lipoproteins level, soluble adhesive molecules expression and the indicators of antioxidant balance) in patients with severe familial hyperlipoproteinemia after LDL-apheresis (where the cholesterol level decreases greatly – usually to 2 mmol/l). The first part of study (which began a year ago) aimed at developing suitable (technically and economically acceptable) treatment regime, without dangerous

side-effects. In 98 procedures (long-term treatment of 8 patients) the LDL-apheresis with Pokard absorber and absorption-desorption automat (Medicap, Germany) was shown to be effective, relatively very safe (6 % of side-effects, the most frequent short episodes of citrate toxicity). The treatment was successful to stop the progress of atherosclerosis in 6 patients. With the aim of finding an early indicator of atherosclerotic process activity we tested the aggregation activity of platelets after the various stimulators (ADP, epinephrin, collagen, ristocetin, propylgalat) and the early coagulation phase (apparatus: PF-100). The results after 88 examinations demonstrated that the increased thrombocyte activity dropped in homozygous patients (there is a big drop in the cholesterol level) after the procedure. References: Bláha M et al. Trombosa a hemostáza, Eds.: Credit, Hr. Kralove 2001, p. 62–70. ISBN: 80-902753-4-6.

Supported by Grant:

IGA MZ ČR No. NB6549-3, 6822-3, 5205-3.

Metabolism of saturated and polyunsaturated fatty acids in hyperlipidemic subjects associated with coronary atherosclerosis

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Abnormal metabolism of fatty acids has been associated with coronary artery disease in hyperlipidemic subjects. The influence of fat quantity and saturation on atherosclerosis and thus progress of cardiovascular disease is mediated in part by effects on plasma cholesterol, low density lipoprotein-cholesterol (LDL-C) concentrations, alterations in cellular functions, changes in cellular membrane fatty acid composition, delivery of LDL cholesterol to arterial cells, and ability of HDL to remove cholesterol from arterial cells. To analyze the association of coronary atherosclerosis and fatty acid composition of plasma, erythrocyte membrane and serum lipoproteins, a group of hyperlipidemic subjects undergoing coronary angiography was studied. The role of individual fatty acids of serum, lipoprotein fractions and erythrocyte membrane in coronary arterial disease (CAD) has been studied in patients who underwent an elective coronary angiography for CAD (n=75, age 33-73 years) in Group A (luminal narrowing <50%) and Group B (>50% stenosis). Severe CAD in Group B significantly correlated with higher age, serum LDL/HDL cholesterol ratio, plasma C18:3n6, IDL C18:1n7, HDL C22:6n3, and erythrocyte membrane C22:6n3 (p<0.05). IDL C18:1n7 was significantly higher in Group A (p<0.05). Plasma C18:3n6, HDL C22:6n3, and erythrocyte membrane C22:6n3 fatty acids

were significantly higher in Group B ($p < 0.05$). We conclude that an altered metabolism of gamma-linolenic (C18:3n6) and docosahexaenoic (C22:6n3) fatty acids is associated with coronary atherosclerosis, indicating enhanced transport of plasma C18:3n6 and removal of HDL C22:6n3 from arterial wall cells. References: Hornstra G, Barth CA, Galli C et al. Verschuren. Functional food science and the cardiovascular system. Brit J Nutr 1998;80:S113-S146.

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Functional importance of cholesterol biosynthesis and fatty acid metabolism in atherogenesis of hypercholesterolemic subjects treated by LDL-apheresis

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LDL (low-density lipoprotein) apheresis is a procedure for the selective removal of LDL cholesterol that is used in patients affected by severe familial hypercholesterolemia (FH) resistant to drug therapy. The mutual interaction between fatty acids, cholesterol synthesis and lipoperoxidation in atherogenesis and, in particular, the evidence that these mechanisms are associated with increased LDL cholesterol levels in vitro and in experimental animals, prompt us to test the hypothesis that LDL-apheresis could reduce such atherogenic lipid-related factors, and therefore positively interfere with key mechanisms in atheroma formation. The aim of this study is to evaluate metabolism of saturated and polyunsaturated fatty acids, cholesterol biosynthetic pathway and lipoperoxidation before and after LDL-apheresis in a group of FH patients. Bioanalysis of blood and urine samples is performed using gas chromatography/mass spectrometry, reversed-phase high-performance liquid chromatography and spectrofluorometry. Preliminary results from nine patients (5 men, 4 women, age 14 - 56y) had shown that the favorable effect of LDL-apheresis on the atherosclerotic process depends not only on mechanical removal of LDL cholesterol, but also on its influence on the other above-mentioned mechanisms of atheroma formation. The results might improve our knowledge and thus treatment of atherosclerosis, which are the leading causes of morbidity and mortality in the Czech Republic. References: Bláha V, Havel E, Bláha M, Solichová D, Brátová M, Zadák Z. Atherosclerosis 1999;144(1):189. Kempen HJM, Geversw Leuven JA, van der Voort HA, de Knijff P, Havekes L. Metabolism 1991;40:231-235.

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Low-molecular weight heparin vs. unfractionated heparin during percutaneous coronary interventions

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To prevent thromboembolic complications during percutaneous coronary interventions (PCI) unfractionated heparin (UFH) is used as a standard therapy. UFH is an indirect thrombin inhibitor. Heterogeneity of its molecules is the reason for great variability in its efficacy. UFH is insufficient to inhibit thrombin within the clot. UFH is inhibited by platelet factor 4 (PF-4). The effect of UFH on platelets is antagonistic - it restricts the initiation of platelet aggregation, but raises thromboxan synthesis in thrombocytes. UFH interferes with thrombocyte adhesion by von Willebrand factor inhibition. Low-molecular weight heparin (LMWH) overcomes the above mentioned disadvantages of UFH. It has a relatively constant dose-related anticoagulation effect and is not inhibited by PF-4. It is able to inhibit the activated Xa factor even within the clot. It does not inhibit coupling of von Willebrand factor with thrombocytes. In 2001 we evaluated the influence of UFH and LMWH on platelet functions and clinical course in 120 patients undergoing elective PCI. A significant increase of thrombocyte aggregation induced by adrenalin, ADP and arachidonic acid was seen after administration of UFH. LMWH increased only platelet aggregation induced by arachidonic acid. A significant decrease in platelet count was observed after administration of both UFH and LMWH. The application of LMWH insured the satisfactory anticoagulation effect throughout the whole procedure. There were no statistical significant differences in the occurrence of ischemic and haemorrhagic complications between UFH and LMWH groups.

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Inhibition of growth hormone secretion by somatostatin analogues in acromegalic adenoma tissue cultures

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Somatostatin (SST) exerts its biological effects via five distinct receptor (SSTR) subtypes. The aim of our study was to investigate the inhibition of GH secretion by somatostatin analogues preferentially binding SSTR 2 (BIM 23197,

BIM 23014), and SSTR 5 (BIM 23268), with special focus on the possibility that their combination might have a potentiating effect. Adenoma tissue has been mechanically dissociated on single-cell suspension and cultivated in multiple-well plate for 3–5 days. After the cells had adhered, the medium was evacuated and replaced with serum-free medium with somatostatins in appropriate concentration. After a six hours' incubation the concentration of GH was measured by immunoassay. As in the previous study we were unable to demonstrate any difference between individual analogues and their combinations using concentrations of $1\text{E-}7$ and $5\text{E-}8$ mol/l, a wider concentration range ($1\text{E-}9$ to $1\text{E-}12$ mol/l) has been used. This approach clearly demonstrated individual differences. In one case the inhibition was minimal, in three cases it was the same with analogue BIM 23197 and BIM 23268, and in another three cases it proved to be much higher with BIM 23197 preferentially binding SSTR 2. In one of them the potentiating effect of the combination BIM 23197 + BIM 23268 could have been demonstrated. The analogue with the preference for SSTR 5 (BIM 23268) is in some adenomas as effective as clinically used analogues with the preference for SSTR 2 and in exceptional cases their combination seems to have a potentiating effect.

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Neurovascular compression of the rostral ventrolateral medulla in hypertensive patients compared to normotensive subjects – preliminary results

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Compression of the left rostral ventrolateral medulla oblongata (RVLM) by an abnormally located artery may be one of the possible causes of essential hypertension, as some experimental studies, post-mortems, and neurosurgical observations suggest. At present the only imaging method capable of detailed evaluation of the relations between posterior fossa vessels and brain stem structures is magnetic resonance imaging (MR). Design and Methods: We prospectively examined 32 hypertensive patients and 40 BMI and age matched normotensive subjects by MR. Patients with severe arterial hypertension in whom renal, renovascular and endocrine hypertension was ruled out were included. MR imaging protocol consisted of transverse and coronal T2 TSE, transverse 3D TOF MRA and 3D CISS

imaging sequences positioned over the brain stem. MR images were assessed in a blind fashion. Results: In the essential hypertension group, 24 (75%) of 32 cases showed neurovascular compression of the medulla oblongata at any level, 10 (31%) of 32 patients demonstrated neurovascular compression at the RVLM, out of which (19% of total) were at the left RVLM. In the control group of normotensive subjects (N = 40), 32 cases (80%) showed compression of the medulla oblongata, 20 cases (50%) showed neurovascular compression at the RVLM, out of which 13 subjects (32% of total) showed neurovascular compression at the left RVLM. Conclusions: Neurovascular compression of medulla oblongata is a very frequent finding in both hypertensive patients and normotensive subjects. In hypertensive patients, higher prevalence of neurovascular compressions at the left RVLM was not confirmed. Our results do not support the hypothesis of neurovascular compression at the left RVLM as an etiological factor of essential hypertension.

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Installation of personal computers in biochemical practical classes

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With respect to the lack of computerization of biochemical practicals up to now we have established a new instrumental laboratory equipped with three personal computers (PC) with appropriate hardware. Besides that, some spectrophotometers have been attached to communicate with the PCs via the RS 232 port. Using Visual Basic 6.0 we have created "user-friendly" program applications based on Excel spreadsheet for the processing of experimental data: 1. Graphic presentation of various modes of spectral measurement. 2. Fitting standard curves and various mathematical functions with experimental points. 3. Statistical evaluation of analytic parameters of the methods used including calculation of uncertainty of measurement and confidence interval of results. 4. Evaluation of reaction kinetics especially in enzymology; determination of velocity of enzyme-catalyzed reactions and K_m ; linear transformation of the Michaelis-Menten plot. 5. Collection and processing of the data obtained from measuring instruments which on-line communicate with the PCs and evaluation of the individual students data at seminars. 6. Computer-aided training in biochemistry using commercial educational programs on CDs.

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Experimental and clinical models for serious organ failures

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Successful therapeutic management of serious organ failures requires co-operation of several theoretical and clinical departments. The following topics were studied during 2001: a) Effects of pyridoxal isonicotinoyl hydrazone (Fe-chelating agent, potentially active in pathological states resulting from both iron-overload and free radicals formation) on troponin T was measured in rabbits. b) Induction of apoptosis in several in vitro and in vivo models (pituitary adenoma cells, leukemic cell line, epithelial cells) was measured by several immunocytochemical apoptotic markers. c) Comparison of periodontal reparation by subgingival curettage and by Vector treatment was done in 30 patients. d) Development of new electrophysiological methods enables early diagnosis of functional disorders of CNS. e) Reparation of (ibotenic acid-) lesioned striatum by transplantation of rat neostriatum was reported (as a model of neurodegenerative process), grafts survive for 9 months. f) Incidence of secondary cataract after implantation of several types of intraocular lens was analyzed. g) Bacterial complications after organ (renal) and cell (bone marrow) transplantations were monitored. h) Concentration of leptin in patients with polytraumata and colorectal carcinoma was measured. i) Cytotoxicity and biocompatibility assessment of materials (artificial blood vessels) used in organ failures treatment was performed. j) Glucose, urea, lactate and amino acid concentration in skeletal muscle interstitium in septic patients were analysed in a microdialysis study. In experiments with hepatotemised rats amino acids were compared in muscle and liver interstitium.

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Proteome centre for the study of intracellular parasitism

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The main goal of this project is the study of intracellular parasitism in model infection with the bacteria

Francisella tularensis. In the first step, cultivation conditions were optimised for three model cell lines – B2, B10, and J774, and a protocol for experimental infection was developed. In 2002 new technology for proteomic analysis and mass spectrophotometry was introduced in our laboratory. With the help of these technologies, analysis of microbial markers of *F. tularensis* was performed, and a proteomic map was constructed. Together it was identified 125 new bacterial proteins, and data was transferred to the international proteomic database. The protein map of *F. tularensis* with 176 identified proteins is part of a new 2-DE database “PMMA-2DPAGE”, which is available on internet address www.pmma.pmfhk.cz. Comparative genomic analysis of protein spectra enables classification of proteins typical for particular stages of infection. In another part of the project, immunoreactive components of the *F. tularensis* were studied and proteomic analysis of the phagosome was performed utilising the DAGE method. The subcellular fractionalisation of the phagosomes from infected cells was done in two lines of mouse macrophages (congenic in Bcg locus, different in allele Nramp). The study of gene expression in host cells after interaction with pathogen and the analysis of intercellular signals related to this interaction began. Monitoring of the infection process was performed with the help of a genetically modified strain of *F. tularensis* with a new gene for green fluorescence protein. The co-localisation of specific markers of *F. tularensis* and specific markers of macrophages phagosomes was performed by confocal laser scanning microscopy. Phenotypisation of selected surface molecules and basic characterisation of expression of cytokine genes was done.

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Experimental biliary cirrhosis in rats – impact on small intestine

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The aim of our experiments was: a) to introduce a model of biliary cirrhosis in rats by ligation of the bile duct; b) to study the impact of bile duct ligation on biochemical markers of liver injury and liver DNA synthesis; c) to evaluate the impact of bile duct ligation on the functional and morphological parameters of the small intestine. The experiments were performed on 40 male albino Wistar rats with an initial body mass of 210-225 g. In the first group secondary biliary cirrhosis (BC) was induced by ligation of the bile duct, the second control group (C) underwent median laparotomy. The rats were sacrificed by exsanguination from the abdominal aorta three weeks after the operations. The extent of liver damage and liver regeneration was de-

terminated by assessment of serum activities of AST, ALT, GMP and AP, serum concentrations of albumin and CRP, liver DNA synthesis (measured by incorporation of 3H thymidine), and mitotic activity of hepatocytes. Intestine permeability was assessed by lactulose-mannitol test, morphological changes were evaluated using histological estimation, gut reparative process was determined by intestine DNA synthesis. A significant increase ($p < 0.05$) of all recorded biochemical parameters of liver injury together with liver morphological changes clearly documented development of BC in rats with bile duct ligation. Lactulose-mannitol test and intestine DNA synthesis were significantly increased ($p < 0.05$) in BC rats to compare with control rats. Secondary biliary cirrhosis induced injury of the small intestine is shown by histological changes (confluence and irregularity of villi) and functional changes (impairment of intestinal barrier). These changes are probably to some extent related to the presence of portal hypertension. Intestine injury was followed by the induction of reparative process judging by the increased DNA synthesis in the small intestine.

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Acute and chronic liver injury in in vivo and in vitro animal experiments and clinical practice

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The research project is divided into several mutually interconnected branches. Twenty-two researchers from seven departments of our faculty participated on the project. The following main topics were studied during the last year: a) Activation of mitochondrial glycerophosphate cytochrome c reductase by triiodothyronine in rat liver regenerating after partial hepatectomy (70 %). b) Hepatotoxic effect of galactosamine in vivo and protective role of gastric administration of lipid emulsion enriched by medium chain fatty acids. c) Effect of s-adenosylmethionine (protective role against glutathione depletion) on hepatocyte primary culture exposed to t-butylhydroperoxide (non-specific peroxidating agent). d) Differences in selected biochemical markers and histological findings after bile duct ligation in male and female rats. e) Role of leptin in initiation of liver regeneration in male and female rats of various strains. f) In situ hybridisation in effort to study fibrillin-2 (component of elastic fiber) which plays an important role in elastin storage. g) Impact of TIPS on insulin resistance in patients with and without diabetes mellitus. h) Markers of autoimmune insulinitis in patients with autoimmune liver disease (primary biliary cirrhosis, autoimmune hepatitis). i) Incidence of hemochromatosis among patients with severe hepatopathy by estimation of iron metabolism and

incidence of gene mutations associated with hemochromatosis.

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Residual mesenchyme in the middle ear cavity of newborns with malformation of the kidney

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The role of mesenchymal tissue in the development of the middle ear is controversial. It has been shown that in the human middle ear the resorption of mesenchymal tissue begins normally during the 12th gestational week and is completed between the 32nd gestational week and the 13th month postpartum. Diminished quantity of amniotic fluid surrounding the foetus (oligohydramnios) is associated with an increased amount of mesenchyme in the middle ear cavity. Oligohydramnios characteristically occurs in combination with other findings: renal and urinary tract malformations, facial malformations, and hypoplastic lungs. Such findings are known as Potter's sequence. Microscopic sections of 37 temporal bones from 22 foetuses were examined, five with renal agenesis, six with Potter's sequence without renal agenesis, and two with mild kidney pathology. Every tenth section containing the stapes were used for measuring the volume of mesenchymal tissue in the middle ear cavity. Image analysis by PC computer (LUCIA -M) was used. At 5% significance level, foetuses with serious renal pathology had more mesenchymal tissue in the middle ear cavity than a control group of nine foetuses with no renal or urinary tract abnormality. Pneumatization (luminization) of the middle ear cavity starts from eustachian tube and continues along the tympanic membrane to the lateral part of mesotympanum. The mesenchyme remains longer in the medial part of mesotympanum around the stapes. Conclusion : The foetuses with renal pathology had no typical malformations of the hearing organ. However, there was bigger volume of mesenchymal tissue in the middle ear cavity in cases with serious pathology of kidney than in the control group.

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Long-term follow up of patients with functional dyspepsia. Dependence of dyspeptic complaints on Helicobacter pylori infection?

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The importance of H. pylori infection in etiology of functional dyspepsia (FD) remains open. The problem has

been studied mainly in association with the prevalence of H.pylori in FD and with the effect of H.pylori eradication in FD on dyspeptic complaints. The results were controversial. The short period of the follow up of published studies has been criticized. The aim of our study was to compare the prevalence of main dyspeptic complaints and the quality of life in FD-patients with and without H.pylori infection during the long-term follow up. 1742 patients with the diagnosis of FD were examined for H.pylori and were asked by questionnaire for dyspeptic complaints (epigastric pain and fullness, nausea, vomiting) and quality of life. 563 patients answered the questions, but only answers of 253 patients were reliable. H.pylori was found in 86 patients (27 m, 59 w, mean age 43.1), in 167 patients (53 m, 114 w, mean age 40.5) the examination was negative. The mean follow-up period in the H.pylori+ group was 9.6 years, in the H.pylori- group 6.6 years. Dyspeptic complaints and quality of life during the first examination, one year later, and in the year of the questionnaire, were evaluated. No significant differences have been found in the prevalence of any complaints and of the quality of life during the entire follow-up. Conclusions: Long-term follow-up of dyspeptic complaints and quality of life in FD-patients did not show any dependence on H.pylori infection. H.pylori should not be taken as a factor in the etiology of FD-symptoms.

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Pharmacokinetics (PK) and pharmacodynamics (PD) of low-dose methotrexate (LDMTX) in the treatment of psoriasis

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The aim of this prospective study was to investigate and compare the PK and PD of oral LDMTX as a bolus dose or in a divided schedule. The drug was administered to patients with resistant forms of psoriasis. 24 men and 17 women (mean age 50.2 ± 11.5 yr) were divided into two groups. The first group was treated with LDMTX of 7.5 mg (N=6) or 15 mg (N=11) a week in a single bolus dose (Study A). The second group was taken either 2.5 mg (N=12) or 5 mg (N=12) MTX given in 12-h intervals 3-times a week (Study B). Blood was sampled at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 10, 14 and 24 h (Study A) and at 0, 1, 2, 4, 8, 12, 14, 24, 25, 26, 28, 32 and 36h h (Study B) after the initiation of the drug dosage. The PK analysis was provided in week 1 and week 13 in each patient. The plasma concentrations were determined by HPLC. AUCMTX was calculated using trapezoidal rule (i.e AUC0-24h, Study A) or by MW-pharm programme (AUC0-36h, Study B). PD were assessed continuously at weeks 1, 5, 9 and 13 using the Psoriasis Area and Severity Index Score system (PASI score). PK/PD analysis revealed a significant inverse relationship between PASI score and

AUCMTX ($\rho=-0.65$, $p<0.0001$). 20 subjects achieved higher than 50 % drop in PASI score in week 13 and were considered responders. 17 out of 20 responders had AUCMTX higher than 2500 nmol.h/L compared with only 1 out of 21 non-responders ($p<0.001$, Fisher's exact test). Nevertheless, patients achieving AUCMTX more than 3300 nmol.h/L had a significantly shorter time to reach ALT and AST activity higher than the upper limit of the physiologic range (ANOVA; $p<0.001$). Moreover, patients with a maximal drug plasma concentration higher than 500 nmol/L had significantly higher incidence of adverse effects like nausea, headache and fatigue (F-test, $p<0.01$).

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Modelling of low dose methotrexate pharmacokinetics (PK) and pharmacodynamics (PD) in therapy of severe psoriasis. Population pharmacokinetics of high-dose busulfan in bone marrow transplant patients

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Two studies investigated PK and PD of LDMTX given orally as a bolus dose (Study A) or as a triple dose regimen (Study B) to 41 patients (24 men and 17 women) with resistant forms of psoriasis. PK of MTX was investigated at weeks 1 and 13 and PD was assessed monthly using the Psoriasis Area and Severity Index Score system (PASI score). PK/PD analysis revealed a significant indirect relationship between PASI score (skin clearing effect) and AUC MTX ($\rho=-0.65$, $p<0.0001$). The AUC of MTX higher than 3000 nmol.h/L was found in 91% responders (11 subjects with higher than 80% drop in PASI) and only 15% nonresponders and was therefore associated with a significantly better outcome ($p<0.001$, Fisher's test). Moreover, PK/PD relationships were defined which allow us to predict acute adverse effects (headache) and long-term toxicity (hepatotoxicity). Between 1998 and 2001, we have monitored plasma levels of busulfan in high-dose (HDBUS) settings in 46 patients (aged 44 ± 10 yr, 24 females and 22 males) who underwent peripheral blood stem cell transplantation at the University Hospital in Brno. Busulfan was given orally every 6 hours at days -7 to -4 prior to the day of transplantation. The standard single dose of busulfan 1 mg/kg of ideal body weight was flexibly adjusted with the help of therapeutic drug monitoring in order to achieve a target range of 300-600 microg.h/ml for the AUC. A very low incidence of adverse effects (8.3 %) was observed. Venooclusive liver

disease occurred in 1 patient. Early posttransplant mortality was 10.4 %. Pharmacokinetically guided dosing of HDBUS resulted in an improved outcome of transplantations.

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Effect of acidosis on protein and amino acid metabolism

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The aim of the project is to analyze the effect of acidosis on protein and amino acid metabolism. The main aim of the current studies is to estimate the effect of acidosis on the metabolism of branched-chain amino acids (BCAA) in liver and muscle tissue of white rats and the effect of administration of ketoanalogues of amino acids on protein metabolism in patients with chronic renal insufficiency (CRI). The preliminary results of in vitro studies demonstrate a decreased rate of leucine oxidation in m. soleus of Wistar rat incubated in medium of pH 7.2 against control (pH 7.4). We did not find the effect of decreased pH on protein synthesis. The changes in proteolysis are being analyzed at the present time. The effect of acidosis of hepatic leucine metabolism was evaluated in the isolated perfused liver (IPL) of Wistar rat using the single pass technique with L-[1-14C]leucine and α -keto[1-14C]isocaproate (KIC) as a tracer. Perfusate solution was bicarbonate buffer pH 7.2 or 7.4 (control) containing glucose (10 mM) and amino acids. The results demonstrated a significantly higher rate of KIC oxidation in hepatic tissue perfused with a medium pH of 7.2 against control group (90.0 ± 5.3 vs. 65.1 ± 5.7 ; $p < 0.05$). The results of a clinical study evaluating the effect of acidosis on BCAA and protein metabolism in CRI patients demonstrated a beneficial effect of administration of ketoanalogues of essential amino acids on protein balance (increased albumin concentration in blood, decreased loss of proteins by urine). In CRI patients treated by ketoanalogues of amino acids, we also observed increased concentrations of BCAA and decreased concentrations of cholesterol and triglycerides in blood.

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Effect of glutamine on BCAA metabolism in hepatic tissue

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The aim of the project is to study the pathogenesis of muscle wasting in severe illness and the effect of humoral

factors on protein and amino acid metabolism in the whole-body and in specific tissues. In our previous studies (1, 2) we demonstrated activated resynthesis of branched-chain amino acids (BCAA) in hepatic tissue of endotoxemic and TNF-treated rats. The aim of the current study was to estimate if the observed changes in the hepatic BCAA metabolism can be induced by increased availability of glutamine. Parameters of hepatic leucine metabolism were evaluated in isolated perfused liver (IPL) of Wistar rats using the single pass technique with L-[1-14C]leucine and keto[1-14C]isocaproate (KIC) as a tracer. Perfusate solution was bicarbonate buffer (pH 7.4) containing glucose (10 mM) and amino acids. Glutamine concentration was 1 mM in glutamine group (n=9) and 0 mM in controls (n=8). Statistical analysis was performed using the Mann-Whitney test. The addition of glutamine to perfusion solution caused a significant increase in leucine oxidation (13.67 ± 2.38 vs. 6.66 ± 1.04 , mmol/g dry liver/h, $P < 0.05$) and a decrease in KIC oxidation (92.0 ± 12.9 vs. 163.7 ± 16.5 mml/g dry liver/h, $P < 0.05$). These results demonstrate a marked increase in hepatic BCAA aminotransferase activity and a decrease in hepatic branched-chain keto acid dehydrogenase activity. The results indicate decreased BCAA oxidation and increased conversion of branched-chain keto acids to BCAA. We concluded that increased delivery of glutamine to hepatic tissue enhances resynthesis of essential BCAA from their ketoanalogues. This metabolic alteration enables the preservation of BCAA for the body's requirements. References: 1. Holeček M et al. Am J Physiol 1997; 273:E1052-8. 2. Holeček M et al. Metabolism 1998; 47:681-5.

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Multimedial computer treatment and presence of forensic-medicine expertises

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At the department of Forensic Medicine (Faculty of Medicine in Hradec Kralove, Charles University in Prague) the educational collection of 450 digital photographs was produced. The documentation from dissections resolved during the year 2001 was used in this project. The photographs were taken directly during the dissections and were processed by the computer programs: Paint Shop Pro, Adobe Photo shop, Photo Impact; than gradually filed in accordance with the type of apposite case to the database, which was adjusted to the final form along with graphic editing of the background image, the didactic highlighting of our findings, and the final detailed graphic layout of the program. The database was used for the creation of an interactive computer program which covers differential diagnostic overview, a comprehensive overview about forensic medicine problems and also enables an individual

approach to the single cases, all in the necessary multi factorial perspective determined by particularity of the discipline (forensic medicine). The individual pictures from the database were also used for the compilation of ad hoc consecutive sets, used in practical classes or lectures. The program was also copied on the CD-ROM, which is a very suitable tool in all forms of teaching and studying, serving as an atlas for self-study purposes. A professionally-paced program using a modern audiovisual technique is an irreplaceable tool, especially in a discipline like forensic medicine, where the digital documentation partly replaces otherwise difficult practicable demonstration of some cases.

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The influence of covering of stents on the occurrence of stenoses in patients treated with transjugular intrahepatic portosystemic shunt - TIPS - part II Prospective randomized control study

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Introduction: In our previous grant-supported study, which we conducted from 1996 to 1999, we proved that coating of the stents in the parenchymal part (supporting the channel in the TIPS) by polytetrafluorethylen (ePTFE) reduces occurrence of stenoses inside the stent. Aim of the study: The aim of this proposal is to prove in a similar, prospective randomized controlled study, that the use of longer all-over ePTFE coated stents will lead to a decrease of both types of stenosis. Results: During the year 2001 seven patients were treated by TIPS using ePTFE covered stents. Another ten patients were treated with non covered stents in the control group. Because of the short time of follow-up, we do not have enough results for analysis. During this next year we will continue in our work. Summary: 7 patients in the study group and 10 patients in the control group were enrolled in the above mentioned study during the year 2001.

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Breath isoprene as a measure of the depression in cholesterol synthesis in intensive care patients

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Analysis of breath isoprene brings important information about cholesterol synthesis rate due to its unique properties. The whole expired breath of ten ICU patients receiving total

parenteral nutrition and ten healthy controls was collected and analyses were performed by Solid Phase MicroExtraction followed by GC/MS. Breath sampling was performed at 3 am and 2 pm. The night levels were 2.33 (SD 0.87) nmol/l vs. 11.23 (SD 1.28) nmol/l in healthy controls. The day levels were 2.29 (SD 0.67) nmol/l vs. 3.38 (SD 0.88) nmol/l in healthy controls. During the following year the possible use of MicroFID in breath isoprene analysis will be evaluated. The aim is maximum simplification and automation of expired air sampling and analysis. Also, the isoprene formation mechanism will be examined in vitro. The proposed investigation represents a practical application of results obtained within the framework of a GAČR grant n. 203/99/1165. References: Hyšpler R, Crhová Š, Zadák Z, Gasparič J. Breath isoprene as a measure of the depression in cholesterol synthesis in intensive care patients. *Atherosclerosis* 2001; 2(2):102. Hyšpler R, Tichá A, Indrová M et al. A simple, optimized method for the determination of sulphide in whole blood by GC-MS as a marker of bowel fermentation processes. *J Chromatography B*, accepted for publication, in press.

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Dipeptidyl peptidase IV and thyroid peroxidase in thyroid neoplasia

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The aim of the study was to evaluate expression of dipeptidyl peptidase IV [DPP IV] and thyroid peroxidase [TPO] in thyroid tumors. DPP IV is a membrane-bound exopeptidase, which is not present in normal follicular cells. TPO is a crucial enzyme in the biosynthesis of the thyroid hormones and thyroid differentiation. It has been reported to be absent or faintly positive in malignant thyroid tumors. The study was carried out on 200 patients [172 females, 28 males, mean age 48]. The results were as follows: The sensitivity of DPP IV was 71%, and the specificity was 99 %, respectively, with a 50% threshold of positive follicular cells in cytochemistry. The sensitivity was 71 %, and the specificity was 94 %, respectively, with a 5 % threshold of positive follicular cells in histochemistry. DPP IV positivity was correlated with angioinvasion, capsuloinvasion, metastase, and oncocytes. The immunohistochemical detection of DPP IV/CD 26 using catalysed signal amplification has been introduced. The preliminary results were: the sensitivity was 65 %, and the specificity was 99 %, respectively. The sensitivity of TPO was 78 %, and the specificity was 92 %, respectively. In a series of 36 tumors, both markers were estimated: the sensitivity was 81 %, and the specificity was 96 %, respectively. The assessment of both markers increased statistical parameters in this series. The data suggest DPP IV and TPO to be helpful additional markers in differential diagnosis between malignant and benign thyroid tumors.

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Evaluation of the model of anthracycline-induced cardiotoxicity in rabbits in vivo

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The aim of the study was to evaluate a model for testing of potential cardioprotective agents against anthracycline toxicity in rabbits in vivo. For this purpose, selected cardiovascular (incl. echocardiographic), biochemical and hematological parameters were observed during repeated, 10-week long (once weekly) administration of daunorubicin (3 mg/kg i.v.) and a combination of daunorubicin with known cardioprotective iron-chelating agent dexrazoxane (60 mg/kg i.p., 30 min. before daunorubicin, 3 mg/kg i.v.). The control group received saline (1ml/kg i.v.). The repeated administration of daunorubicin induced cardiomyopathy with progressive impairment of cardiac function: at the end of the experiment, left ventricular ejection fraction was reduced to 73 % and PEP/LVET ratio increased up to 134 % of the initial values; LV dp/dtmax was reduced to 58 % and blood pressure to 76 % comparing with the control group. All these changes were statistically significant. Combination with dexrazoxane did not cause consistent and significant changes of any of these cardiovascular parameters. The observed significant decrease of proteins and albumins and increase of triglycerides and cholesterol could be caused by the nephrotoxic effect of daunorubicin. The combination with dexrazoxane prevented these changes and mostly also an increase in cTnT. It also improved survival of animals (daunorubicin - 73 %, combination with dexrazoxane - 100 %). Daunorubicin caused significant decrease in erythrocyte count, hemoglobine and hematocrite. These only changes, typical for aplastic anemia, were not affected by dexrazoxane. These results confirm suitability of the model for testing of other potential cardioprotective agents.

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Digitalization of photodocumentation in modernization of the biology course for dentistry

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Medical biology and genetics belong to the theoretical disciplines taught at the Faculty of Medicine in Hradec

Králové. To enhance the efficiency of its courses, it is recommended to complement them with extensive, easily and quickly accessible photodocumentation. The aim of this project, which is connected to the implementation of a new study curriculum for Dentistry, was to digitalize photodocumentation, with special focus on the characteristic features of some inheritable conditions and most frequent tumors in the orofacial area. The resulting photographs originated from a vast photoarchive owned by the Stomatology clinic at the Faculty teaching hospital in Hradec Králové. The digitalization has been effectuated using the digital camera, Nikon COOLPIX 950, or using the scanner, Hewlett Packard ScanJet 6200C. The resulting images were computer-processed using the graphics program Corel PHOTO-PAINT 8, equipped with descriptions and commentary, and stored in the compressed format jpeg. This photodocumentation served as a basis for the preparation of Power Point presentations used during dentistry lectures and practical classes in the 1st study year. Starting from the winter semester 2001/2002, the presentations are available to the students at the intranet computer LUCIA. Furthermore, we are planning on presenting some files on-line at the address: www.biologie-lfhk.cz/. This will enable all academics as well as students of our faculty to use them as educational and reference material.

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Lateralisation of the inferior alveolar nerve in combination with placement of dental implants

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The project is focused on choosing the optimal type of osseointegrated implant using the transposition of the inferior alveolar nerve from the bone defect point of view, which is created during finding and preparing of the nerve. For this study we use three types of fixtures (titanium self-taped and plasma-sprayed fixtures, titanium implants with chemically etched and titanium implants coated with hydroxy-apatite in the middle parts. We placed 10 implants in the lateral region of the lower jaw distally from mental foramen so that the lateralisation of the inferior alveolar nerve was necessary. We did lateralisation of the nerve in 7 cases and we transposed the whole nerve three times. In all cases we placed titanium uncoated implants "Impladent" (Lasak Ltd.). Out of 10 implants, 6 were chemically etched on 3,7 mm of the diameter (STI-Bio), the others were only plasma sprayed on 5,0 mm of the diameter. In four fixtures, we placed fixtures bicortically, the

others only monocortically. We evaluated the perception of the mental nerve one week after operation, one month and three months after the procedure. We were figuring out the sensitivity on sharp and blunt stimuli, the ability to differentiate two points and movement of the brush in 10 mm distances. We published "Lateralisation of the inferior alveolar nerve in combination with dental implant placement" which is published in Quintessence. Literature: Babbush, CA.: Transpositioning and repositioning the inferior alveolar and mental nerves in conjunction with endosteal implant reconstruction. *Perio* 2000, 1998, 17, 183-190.

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Cardiovascular and metabolic changes during physical activity (practical classes in Pathophysiology)

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During practical classes of medical students in Pathophysiology, dependence of the heart rate, blood pressure, glycemia, arterial pO₂ and body temperature on the level of physical load (during bicycle ergometry) was evaluated in 140 healthy subjects (age range of 19-23 years). "W170/kg" index (expressing the load in Watts/1kg of the body weight needed to achieve the heart rate 170/minute) was measured and compared with the "recovery index" (calculated from the decrease of the heart rate in the first minute after the physical load) provided by the ergometer ERGORACER (Kettler company, Germany). Interindividual differences were evaluated with respect to basic body parameters of subjects. A mathematical model explaining relationships between acquired data is in construction. It will be used for better recognition of pathological reactions to physical activity.

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Intraindividual variability of visually evoked cognitive potentials

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Intraindividual circadian variability of cognitive responses (P300) to moving stimuli (coherent versus non-coherent linear motion recognition) was tested in a group of

6 healthy subjects. Additionally, P300 changes due to glycemia variations were evaluated and compared with changes in reactions from primary and secondary (associated) visual cortical areas. Despite a larger interindividual variability (generally recognised feature), cognitive responses displayed no significant circadian changes in relatively constant glycemia. Controlled decrease of glycemia (to the lower border of a norm) was mostly detectable in cognitive responses only (prolonged latencies and amplitude decrease). Primary visual evoked potentials (pattern-reversal VEPs) displayed a tendency to increase amplitude (equivalent of hyperreflexia in hypoglycemic status?). Although these preliminary data must be verified in a larger group of subjects, they confirm the formerly reported higher sensitivity of the extrastriate visual cortex to changes in the internal environment of the body (Kuba M et al. *Acta Medica (Hradec Králové)* 1996;39:21-6).

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Transparence of intraocular lens and its influence on differential ability of the eye

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The aim of our study is: 1. to objectify and quantify subjective complaints in conditions of lower contrasts and under glare a) in patients with an early cataract before and after cataract surgery, b) in patients after YAG capsulotomy because of a secondary cataract, 2. to examine visual functions in myopes undergoing a) implantation of phakic intraocular lenses, b) photorefractive keratectomy (PRK), c) laser in situ keratomileusis (LASIK) for correction of the refractive error. The examinations of patients with early cataract preoperatively and after cataract surgery were continued (n=40) and evaluated. The significant improvement of the best corrected visual acuity and contrast sensitivity without glare and under glare in 20 arthepakic patients after YAG capsulotomy because of secondary cataract was observed and the manuscript was prepared. Myopes (n=20) undergoing implantation of phakic IOL were examined preoperatively and 1 and 3 months after surgery. The postoperative improvement in the majority of parameters tested will be presented next year. The comparison of visual functions in myopes after PRK (n=41) and LASIK (n=31) was presented and published.

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Pathogenesis, diagnosis and therapy of malignant diseases

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Work in the third year of the project concentrated in its experimental part on the following research areas: a. study of tumor cells' invasiveness and its possible influencing in in vitro conditions; b. cardiotoxicity, prediction of cardiotoxicity and potential cardioprotective effectiveness of new antineoplastic substances. In the clinical part were the following research areas: a. morphology of gene regulation disorders in relation to biological behavior of the pulmonal carcinoids; b. timely diagnosis of colorectal tumor, mutation of Ki-RAS oncogenes; c. timely diagnosis of recurrence and metastasis of colon tumor by immunoscintigraphic means; d. breathing tests with utilization of natural isotope of carbon 13C, complex functional GIT diagnosis during and after cytostatic treatment; e. treatment of hemoblastosis and some immunopathological diseases by allogeneous graft after nonmyeloablative regimen; f. acquired thrombophilic conditions in patients with hemoblastosis and tumor diseases and their treatment; g. monitoring and prophylaxis of multiorgan failure in patients with hematological malignities and septic complications of bone marrow transplant treatment. We evaluated the first results in the field of carcinoma cell lines, nephrotoxicity of cytostatics, haemostatic changes of the 211 patients with hemoblastosis were evaluated and we transplanted 72 patients with haematological malignancy under various conditions.

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Fluid balance and neuromuscular disorders in critical illness

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In severe sepsis, massive fluid resuscitation is needed to achieve haemodynamic stability. Due to the capillary leakage, fluid retention with oedema formation occurs. Severe

muscle weakness evolves frequently further in the course of the illness, delaying the weaning from mechanical ventilation and prolonging the length of stay in the hospital. The relationship between the fluid retention and neuromuscular condition has never been studied in the literature. Objective: to describe the relationship between fluid retention and the neuromuscular parameters. Setting: 10 bed medical ICU in a university hospital. Patients: 28 patients (mean 58 (42-78)years) with severe sepsis and consequent limb weakness (no movement against gravity). Parameters: Fluid retention: curves of cumulative fluid balance during the hospital stay were obtained. The slope of the fluid accumulation and oedema mobilisation, maximum fluid retention and area under the curve were calculated. Neuro-muscular condition: electrophysiologic examination of peripheral nerves (conduction velocities, action potentials of motor and sensitive nerves) and muscles (spontaneous electrical activity) was performed. Clinical: severity of organ failure was assessed (SOFA score), biochemical and haematologic laboratory examination was performed. Results: Out of the 28 patients, complete data were collected in 14. In electrophysiology, signs of axonal polyneuropathy were found in 13 patients, one patient had myopathic features. No correlation between electrophysiological findings and parameters of fluid balance was found. Significant correlation was found between the decrease in compound muscle action potential and maximum SOFA score ($r=0,7$; $p=0,002$). Conclusion: no correlation was found between the amount of fluid accumulated and electrophysiological changes. More likely, the neuromuscular changes can be regarded as a part of multiple organ failure.

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Biochemical monitoring of metabolic changes and tissue blood flow in skeletal muscle during cardiac operation and postoperative care by interstitial microdialysis (preliminary report)

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Purpose: Hypoperfusion of peripheral tissues and splanchnic organs can be a serious complications of cardiac surgery using cardiopulmonary bypass (CPB). The aim of this study is to monitor metabolism and interstitial blood flow in peripheral tissue during cardiac surgery and postoperative care by interstitial microdialysis. Methods: 20 patients operated on using CPB were investigated. Interstitial microdialysis was performed by 2 probes CMA 60 inserted into the patient's musculus deltoideus. Samples were collected at exact intervals. Out of the obtained dialysates,

glucose, urea and lactate were estimated. The tissue blood flow was monitored by dynamic microdialysis with gentamicin as a marker (a technique originally developed by us). Results: The concentrations of dialysates were significantly lower during CPB in comparison with the period after weaning CPB and postoperative care. Glucose 1.47 ± 1.3 (median 1.7) vs. 4.05 ± 1.52 (med.4.6), urea 1.65 ± 1.42 (med.1.8) vs. 3.76 ± 1.63 (med.3.3), lactate 0.93 ± 0.92 (med.1.0) vs. 2.32 ± 0.89 (med.2.1). The interpretation of these results could be modified by the significantly lower volumes, 3-5 times, of obtained microdialysis samples during CPB. Simultaneous tissue perfusion measurement using microdialysis perfusion solution supplemented with gentamicin indicated markedly lower perfusion of peripheral tissue during CPB (preliminary results). Conclusions: The lower concentrations of glucose, urea and lactate in skeletal muscle during CPB are influenced by dramatic changes in tissue perfusion, capillary fluid pressure and osmotic changes during CPB. Simultaneous measurement of tissue perfusion, sample volume and changes in microdialysis solution composition (Na^+ , K^+) seems to be necessary in microdialysis studies with expected rapid tissue flow and osmotic changes.

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Innovation of medical curriculum

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Solving of the research design in the year 2001 went on in agreement with the plan and took place on four different levels. Above faculty level: a focused gathering and scrutinizing the foreign experience - analytical and comparative activities were done concerning different approaches to curriculum reforms at some foreign medical faculties. California State University, Medical School at Los Angeles and Medical School at San Francisco were visited. Also pan-European cooperation in the field of pharmacology continued within the frame of the Ephar Net project. Our faculty belongs among 6 European places using the exchange of electronic lecture texts. Faculty level of study program: For the second year there went on the implementation of the new curriculum in stomatological studies. The basic strategy of curriculum changes in general medicine studies was agreed on: to reduce the obligatory part of teaching, open a wider space for optional subjects, not to limit the elected subjects to a specific year but to allow the students to make their choice during all the higher years, to reduce the number of lectures and the intensity of the practical and seminary forms of teaching, to coordinate both the contents and terminology of teaching within single years and even between separate years, to amplify the in-

tensity of practical and family medicine education. The whole faculty level: An inquiry among the students of all years, as well as among those graduated about their opinions on the teaching quality in different subjects and possible curricular changes was carried out. Two studies were performed: one concerning the preparation of students for their contact with patients - members of ethnic, religious and other minorities, and the second one concerning the hidden curriculum of the Medical faculty. There were also performed partial reforms in many departments at the faculty.

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Social support among children and adolescents in stressful situations (psychosocial perspective)

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The output of the year 2001 may be summarized into 6 areas. Survey studies: mapping the new approach in psychology orientated to surveying the positive aspects in individuals during functioning of both the small groups and big human communities, assessing the main theoretical approaches to the social support research analysis of social definitions, survey of concrete methods for diagnostics of social support in children, the survey study concerning the sources of social support: family in general, mothers, nurses, playing therapists, teachers and educators in hospital schools, summarizing studies of stumbling blocks and risks of the social support. Verification of the diagnostic methods: translation of selected diagnostic methods: SSQ6, SSS, SRI, MSPS, authentication of selected questionnaire methods on a sample of children and adolescents: SSQ6 on the 1176 pupils within the age span 10-18 years, verification of the "map" of social support and "thermometer" of this help in admitted children. Empirical research of social support in specific groups of children and adolescents: in chronically ill children, in paediatric hematological-oncological patients, in operated squint-eyed children, in adolescents accused of a criminal act. Intervention ways: using family therapy when working with clients and detailed description of risks of social support. Technical outcomes: a CD ROM with a file covering the identification data of 30 paediatric haematological-oncological patients and of their 30 drawn answers, a CD ROM with picture material concerning selected medical procedures - aimed at the work of playing specialists preparing the children for painful procedures. WWW pages of the research workers. Publication outcomes: 1 monography, 1 reader report, 8 magazine articles, 18 articles in reports, English-Czech dictionary of terminology from the sphere of social support.

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Isoelectric focusing of transferrin in the screening of congenital defects of protein glycosylation

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Carbohydrate-deficient glycoprotein syndromes are caused by a deficiency of various enzymes in the process of protein glycosylation. This recently recognised group of metabolic disorders is characterised by a disturbance of many physiological functions and a broad spectrum of symptoms. Diagnosis and differentiation into several types involves the demonstration of pathological glycosylation patterns of serum glycoproteins and decreased enzyme activities in leukocytes or fibroblasts. The aim of the first stage of the research project was to introduce a method for the screening of the congenital disorders of glycosylation (CDG), which has not been performed in our country so far. From the various methods referred to in the literature we have chosen the planar arrangement of isoelectric focusing of transferrin (TF), using rehydrated Immobiline dry gels (Pharmacia), anti-IgG-TF immuno-fixation, Coomassie-blue detection and densitometric evaluation. We have checked on conditions of the procedure with respect to the sample pre-treatment, gel hydration, volume and the manner of sample application, storage conditions when discontinuous analysis was used, and sensitivity and reproducibility of the method. Over 70 healthy persons of various ages and 170 children with a possible congenital metabolic defect have been examined. Serum of alcohol abusers served as a pathological reference sample. No patient with a specific defect of glycosylation (expected incidence 1:40000) has been screened out so far. References: Marklová E. Dědičné poruchy glykosylace (CDG). Čes-Slov Pediat 2001;56(3): 143-8.

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Our contribution to EPHARNET focused on treatment with antihypertensive drugs

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The aim of the project was to join the EPharNET project (EC) focused mainly on etiopathogenesis and treatment of hypertension. Within this framework, new software (on CD-ROM) devoted to hypertension was created and Prof. Martínková was named as one of 11 contributors to the contents. Moreover, Prof. Martínková and co-authors developed 9 case-reports (computer-assisted interactive studies in Czech and English), helpful for teaching both exper-

imental pharmacology (for students in years 3 and 4) and clinical pharmacology (for students in year 5).

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Connecting the student dormitory "Na kotli" to internet

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In the academic environment, access to the internet from student dormitories is quite a common way of satisfying students' demands for internet connection. There has been no such opportunity for Charles University students in Hradec Králové until now. We decided to build a Local Area Network (LAN) in the buildings of the student dormitory "Na kotli" and connect this LAN to the Faculty of Pharmacy, which is the closest to the hostel of all the nodes of the Metropolitan Area Network (MAN) HKNET, and connects all academic institutions in Hradec Králové to the internet. All five buildings of the dormitory were connected together by the optical backbone network, using a multi-mode fiber optic, while for connections within the buildings, Cat 5E metallic cabling was used. The network consists of the total of 147 end-user connections, all of which are connected to 100 Mb/s Cisco 2950 switches. The backbone network also uses the same speed. The connection to the Faculty of Pharmacy uses a single-mode fiber optic and was built in cooperation with the University of Hradec Králové. Its speed is 100 Mb/s. The network of the student hostel is using IP addresses from the intranet range, which are translated to one public address by Network Address Translation service (NAT), provided by a PC running Windows 2000 Server. We also built two small computer labs in the hostel, each with five computers. In case the students' demand for more internet connections increases, it should be possible to increase the number of end-user connections in the hostel quite easily and with minimal expenses.

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Neural transplantation into experimental model of Huntington's disease

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Transplantation (TR) of fetal neural tissue appears to be promising treatment also for Huntington's diseased (HD) patients. To better understand the role of fetal neural graft in alleviating HD pathology, we used an animal model

to characterize: 1) host/graft interaction; 2) changes in proliferative activity of the subependymal layer (SEL) of lateral brain ventricles in reaction to the neurotoxic lesion and following neural TR. Neurodegenerative changes within the striatum of the rat brain (Long-Evans strain, n = 58) were induced mostly unilaterally by ibotenic acid (IA). TR of mesencephalic tissue (ED 10, 13, 15) was performed 1 week later (n = 29). Rats were sacrificed 2 weeks, 1, 3, 6 and 9 months after TR. Immunohistochemical methods in particular were used for morphological evaluation. The grafts' structure was influenced rather by the age of donor/fetus than by the length of animals' survival. Host/graft interaction was also evident in long-term surviving rats. We were interested in reaction of SEL especially in long-term surviving animals with respect to the long-lasting degenerative process in the brains of HD patients. Increased proliferation in SEL (represented mostly by GFAP-positive astrocytes) was highest in IA-lesioned rats, less in sham-lesioned and sham-transplanted animals surviving 1 and 3 months after operation, in comparison with the intact brains. The intensity of SEL reaction markedly decreased in 6- and 9-month surviving animals. Surprisingly, in grafted rats the proliferative rate within SEL was always lower in comparison with the contralateral, non-lesioned side. It can be supposed that fetal intrastriatal graft diminishes the reaction of SEL owing to its overall positive influence on the degenerated striatum.

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Malignant ascites as a model to study the host-tumor relation

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Metastatic involvement of the peritoneum is the dominant form of metastatic spread in epithelial ovarian carcinoma, and is frequently accompanied by the formation of ascites. Ascitic fluid presents an opportunity to study the cellular populations in the peritoneal cavity and the tumor microenvironment (1). Activation of the population of leukocytes resident in the peritoneal cavity by biologic agents also represents one of the approaches to improve the cure rate in ovarian cancer patients with peritoneal carcinomatosis. Along with tumor cells and mesothelial cells, all major leukocyte populations are represented in the malignant ascites. Lymphocytes isolated from the malignant ascites are frequently called tumor infiltrating lymphocytes (TIL). Most of the TIL are T-cells. We found that the percentage of NK cells in the peritoneal cavity is similar to the values reported for peripheral blood of cancer patients. Interestingly, the percentage of NK cells has been found to be elevated in ascitic fluid of patients with liver metastases

(2). B-lymphocytes are represented in a smaller portion than in peripheral blood. Monocytes/macrophages represent a major, sometimes predominant population of leukocytes in malignant ascites. The presence of all 5 monocyte populations defined in the peripheral blood of normal subjects by the expression of CD14, CD16 and CD56 was evident in the malignant ascites. Significant cytotoxic and cytostatic activity could be elicited by treating ascitic monocytes with interferon-gamma or interleukin-2. References: 1. Melichar B, Freedman RS. Immunology of the peritoneal cavity: Relevance for host-tumor relation. *Int J Gynecol Cancer* 2002, in press 2. Melichar B, Toušková M, Tošner J, Kopecký O. The phenotype of ascitic fluid lymphocytes in patients with ovarian carcinoma and other primaries. *Onkologie* 2001;24:156-60.

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Impact of the atrial contribution on pacing mode preferences

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Atrial contribution (AC) to ventricular filling is a parameter which is not commonly used in pacemaker practice as it is difficult to estimate it noninvasively. In the 90's, we developed a method which allows noninvasive quantifying of the AC. The aim of this project was to determine whether the magnitude of the AC can help to individualise setting of pacemakers and to choose between a single-chamber or dual-chamber pulse generator. In 2001, a total of 22 patients with preserved atrial activity were enrolled. All patients gave their informed consents. Quantification of the AC was performed successfully in 20 patients. All patients were randomly assigned into two groups which were followed and underwent the exercise testing on bicycle ergometry. Each patient underwent the exercise testing in both the VVIR and DDDR pacing mode according to the cross-over design of the study. All patients repeatedly filled in the quality of life questionnaire (QOL) after two fourteen-day periods in their assigned pacing mode (VVIR or DDDR). On the follow-up, seven serious adverse events (SAE) were noted in the group set to the VVIR mode (1 stroke, 1 permanent atrial fibrillation, 2 cases of congestive heart failure and 3 cases of pacemaker syndrome) while only 1 SAE was noted in the group set to the DDDR mode (stroke). Significant differences were found in 13 out of the 19 different questions of QOL (P<0.04). All the worse scores were noted in the group of patients set to the VVIR mode. Of special interest were the negative relationships found between the AC and the maximal rate-pressure product (P<0.01) achieved during exercise testing in both the DDDR mode (P<0.01) and in the VVIR mode (P<0.05).

Thus, in patients with preserved atrial activity the DDDR mode seems to be clearly superior to the VVIR mode, possibly also because of a negative influence of the AC in the VVIR mode.

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Influence of memantine on activity of hepatic microsomal CYP1A2, CYP2D6 and CYP3A4

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First, the study was undertaken to estimate the influence of memantine (a new compound important for treatment of dementia) on cytochrome CYP1A2, CYP2D6 and CYP3A4 metabolic activities. In vitro experiments using rats and human liver microsomes show significant inhibition of CYP1A2-associated ethoxyresorufin O-deethylation. K_i (63.75 μM) is much higher than the maximum plasma concentration of memantine recommended for long-term therapy (0.5 μM). Neither CYP2D6 (dextromethorphan O-demethylation) nor CYP3A4 (dextromethorphan N-demethylation) were inhibited by memantine. In conclusion, these data suggest that the potential of memantine for metabolic drug interactions is very low. Second, concerning hepatic clearance of drugs, a model of isolated perfused rat liver (IPRL) was optimized to enable an evaluation of drug interaction at the level of hepatobiliary excretion (biliary clearance). The interaction between methotrexate (MTX – 40 μM) and cyclosporine A (CsA – 1 mg/L) was analyzed using single-pass perfusion (at steady state conditions). Preliminary results seem to demonstrate that co-administration of CsA reduces biliary excretion of MTX by 38 %. Mechanisms of this interaction and its clinical importance are to be analyzed

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Innovation of laboratory classes in histology and embryology: Equipment for digital microscopy

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The project was utilized for equipping the Department of Histology and Embryology classroom with apparatuses for taking microphotographs (consisting of the light microscope Olympus BX-51 and digital camera Camedia C4040) and their projection to students (using two videomonitors). To ensure intelligibility and audibility of the teacher's comments, the classroom was equipped with microphones,

speakers, and power mixer and amplifier. Integration of all elements was carried out with the use of a graphic and multimedial station allowing multitask processing. This station was used for recording and archiving of microphotographs, didactic diagrams and cartoons, preparation of computerized teaching presentations and supplemented with necessary software. All the components were installed in the classroom and they will be utilized in teaching from the summer semester 2002. Furthermore, the project allowed us to supply the departmental library with modern textbooks as well as instruction manuals describing management of the installed software. The installation of this equipment guarantees objective teaching of practical histology while increasing the effectiveness of the teaching process.

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Conversion of neural stem cells into endodermal and blood cells

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It has been postulated that organ-specific stem cells (SCs) give rise to progeny that differentiates exclusively into cells specific for a given organ. However, recent data confirm that the differentiation potential of SCs is broader and under suitable stimulation, SCs may generate cells of any kind. The aim of this project was to characterize plasticity of neural SCs. We used neural SCs isolated from the forebrain of E14 Balb/c mouse fetuses using the neurosphere assay. Spontaneous differentiation of multipotent NSCs (induced by neural grafting or by replacement of EGF and FGF-2 from culture medium with serum) resulted in production of neuronal and glial cells. To find out whether NSCs can be stimulated to produce non-neural cell types, we exposed NSCs to different conditions. In in vitro experiments, NSCs labelled with exogenous beta-galactosidase were mixed with embryonic stem (ES) cells in hanging drops to form chimeric embryoid bodies. Cystic embryoid bodies were harvested after 9-25 days, stained with X-Gal (to identify labelled NSCs) and processed for histology. Sections revealed that in this assay, neural SCs generate mainly endodermal cells; only sporadic blood cells located in generated blood islands contained X-Gal positivity. For in vivo tests, NSCs were injected into sublethally irradiated recipient mice. Counting CFU-GM colonies yielded from the spleen and bone marrow of irradiated animals confirmed that mice injected with NSCs revealed increased haematopoiesis when compared with untreated irradiated animals. Our results indicate that NSCs exhibit features of remarkable plasticity and they can produce non-neural cell types. Chimeric embryoid bodies represent a novel in vitro model for testing stem cell plasticity.

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Newly formed endothelial cells express intermediate filament nestin

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The intermediate filament nestin is temporarily expressed in sporadic cell types during certain phases of their development. Terminal cell differentiation is associated with the loss of immunoreactivity. We observed the same phenomenon in developing endothelial cells. Our previous findings have shown that abundant nestin expression occurs during angiogenesis in vascular endothelial cells in a variety of developing tissues incl. extraembryonic structures (chorion, umbilical cord, placenta) and intraembryonic connective tissue and developing organs (e.g. brain, liver, lung, heart, spleen, gut, limb buds). To answer the question whether endothelial nestin is expressed during vasculogenesis, we examined the immunoreactivity of blood vessels in cystic embryoid bodies. Surprisingly, endothelium lining blood islands or primitive vessels in embryoid bodies did not express nestin indicating that molecular regulation of angiogenesis differs from the process of vasculogenesis. As tissues and their blood vessels matured, they lost nestin from the cytoskeleton of endothelial cells (nestin being gradually replaced with vimentin). Occasional identification of nestin in sporadic endothelial cells of adult tissues is associated with physiological turnover of these cells. When we detected nestin in sections of adult rat brains transplanted with neural grafts or glioma cells, i.e. the tissue whose growth induced neovascularization, we observed widespread immunoreactivity for nestin in all capillaries that vascularized the growing implanted tissue. No reactivity was seen in vessels of distant and intact areas of the host brain. We conclude that IF nestin represents one of the structural molecules that are crucial for formation of new blood vessels in the process of angiogenesis.

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Reactive oxygen species in pathogenesis of acute pancreatitis and antioxidative treatment

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Objectives: 1. To prove enhanced activity of reactive oxygen species (ROS) in the course of acute pancreatitis (AP) by evaluation of markers of activity of ROS and antioxidants in blood. 2. To compare the dynamics of markers of

activity of ROS and the dynamics of antioxidants in patients suffering from AP with the dynamics of markers of activity of ROS and the dynamics of antioxidants in patients with other illness located in the abdominal cavity who were admitted to hospital for elective laparotomy. Results: Blood samples from a group of 17 patients with AP and from a group of 12 patients with other illnesses in the abdominal cavity were analysed on admission, on the 4th and the 8th day of hospitalisation. Blood samples from a group of 12 healthy persons were analysed. We investigated these parameters: Concentration of selenium in plasma, red cell and big toe nail, concentration of vitamin C, vitamin E, alpha-tocopherol, beta-carotene, vitamin A, conjugated dienes (thiobarbituric acid reactive substances) in plasma, activity of superoxide dismutase and glutathione peroxidase in red cells. Our preliminary results suggest: 1. Depletion of selenium in patients with AP compared to the group of patients with other illnesses in the abdominal cavity and the group of healthy persons. 2. Increased consumption of antioxidants in patients in the course of AP and in patients with other illnesses in the abdominal cavity during postoperative period due to increased activity of ROS.

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Incorporation of immunohistochemical methods into the routine education of normal histology

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Thanks to new technologies (especially immunohistochemistry), histopathology has become a central part of the practice of clinical medicine. More and more, in vivo diagnosis of disease rests on microscopic examination of small samples of tissue (classical and fine - needle biopsy). It seems evident that the pedagogy lags behind the practical use of immunohistochemistry; that must be expressly incorporated into the curriculum of normal histology. The purpose of this project has been: 1) For practical classes, to supply the students and the staff with slides showing various immunohistochemical detections, accompanied by slides of some special histological techniques, as well as corresponding microphotos and legends. Histochemical detections cover epithelial markers (Cytokeratins, Epithelial membrane antigen), antigens of mesenchymal tissue (Vimentin, S 100 protein, histocytic and endothelial markers), markers of muscle tissue (Desmin), antigens of neural tissue (Synaptophysin, Neurofilaments, Glial-fibrillar acidic protein), endocrine markers (Chromogranin, hormones of all adenohypophyseal cells, Langerhans islet

cells, some cells of the diffuse neuro-endocrine system (Somatostatin, GASTIN, Serotonin), and cellular proliferation marker (PCNA). 2) To show that some classical histological techniques are nowadays replaced by the more specific immunohistochemical techniques (neurofilaments for neurofibrillar impregnation; glial-fibrillar acidic protein for Cajal's impregnation of astrocytes, etc.). Moreover, immunodetection can better reveal some classical microscopical views (eg. angioarchitecture of the lienal red pulp).

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The effect of L-carnitine pretreatment on acetylcholinesterase inhibition of intracerebroventricularly (i.c.v.) administered 7-methoxytacrine

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Background: Previous results show that L-carnitine (CRT) administered i.p. and 7-methoxytacrine (7-MEOTA) administered i.m. lead to inhibition of brain acetylcholinesterase (ACHE) following CRT pretreatment. It could result either from a change in the blood-brain barrier affected by CRT or from its direct influence on 7-MEOTA inhibition efficacy. The aim of this study was to differentiate these effects using i.c.v. MEOTA administration. Methods: Male Wistar rats were divided into 4 groups: A: i.c.v. administration of saline solution. B: i.c.v. admin. of 0.25 mg 7-MEOTA. C: 3-day admin. of 200 mg/kg i.p. CRT, 30 min. after third CRT inj. i.c.v. 7-MEOTA. D: 3-day admin. of 400mg/kg CRT i.p., 30 min. after third CRT inj. i.c.v. 7-MEOTA. The animals were sacrificed 30 min after the last administration, and frontal cortex (FC) and basal ganglia (BG) were prepared. ACHE activity in the homogenates was expressed as nmol of acetylthiocholine hydrolyzed/min/100 mg of tissue wet weight. Results are expressed as means, S.E.M are not indicated. ACHE activity in FC was: A: 420.7, B: 377.8, C: 402.6, D: 419.6. ACHE activity in BG was: A: 1460.0, B: 1017.6, C: 1038.1, D: 1045.4. Conclusion: CRT pretreatment leads to higher inhibition of brain ACHE by systemic 7-MEOTA administration. When MEOTA was administered i.c.v. after 3 day CRT pretreatment, the inhibition was less expressed in comparison with systemic 7-MEOTA administration.

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Catheter radiofrequency ablation and hemostatic changes

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Catheter radiofrequency ablation (RFA) is a standard non-pharmacological method used in the treatment of cardiac arrhythmias at present. Thromboembolic complications are rare, but serious. Recently published works confirmed activation of the hemostatic system during electrophysiologic study and RFA, but mechanisms of thrombogenicity are still unclear. Also, there is no unification in the use of antithrombotic therapy for prevention of thromboembolic complications in connection with RFA. The authors plan to observe chosen hemostasis activation markers in a group of 100 patients who are treated with RFA for supraventricular tachycardias (AV nodal reentry, AV reentry and atrial flutter). Blood is taken from a catheter in arteria pulmonalis and peripheral vessels before the procedures, after diagnostic electrophysiologic study, and after RFA. Levels of prothrombotic markers are compared with the parameters of the used procedures. We have included the first 20 patients in our study so far. The aim of the research study is to contribute to clarification of pathophysiological mechanisms of prothrombotic state during RFA. Another benefit may also be in identification of risk factors of genesis of thromboembolic complications during RFA and in forming of suggestions for preventive antithrombotic arrangements.

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Reconstruction of the joint surface

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Treatment of defects of the joint cartilage is very much of an orthopaedic problem. It is quite frequently seen, and when in younger patients, it is all the more important because of arthritic changes occurring at a very young age with very complicated medical treatment programmes plus social and financial implications due to arthritis. The project proposes a solution to the treatment of joint cartilage defects by healing through a full-value hyaline cartilage.

Recently the new approach to treatment of articular cartilage defects based on use of cultured autologous chondrocytes was introduced to clinical practice. The results of the treatment by autologous culture of chondrocytes will be compared with patients who will be treated by the Hangody method using autologous osteochondral grafts. The results of these two different methods will be compared by a clinical examination, magnetic resonance, control arthroscopy and biopsy. The experimental part of the project - use of bioactive glass-ceramic material as a carrier of autologous cultured chondrocytes - will form the basis for future research aimed at its use in clinical practice. We carried out cultivation of autologous chondrocytes two times. Transplantation of autologous chondrocytes suspension was used in one patient suffering from a femur condyle defect. Transplantation of osteochondral grafts was carried out in three patients. After healing, we did a control arthroscopic examination in 2 patients. The defect was healed by a compact cartilaginous tissue. Bioptic samples were taken from the transplantate in two patients. The microscopic finding consisted of hyaline cartilage (55%) and fibrocartilage (45%). Five patients were examined with magnetic resonance. The defect was healed with cartilaginous tissue.

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Preparation of digital videosequences for cell biology practical classes

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Digital imaging is an integral part of contemporary biomedicine, including cell biology. The main goal of our project was to prepare digitized videosequences which will serve as reference material for demonstrating chosen cell activities during cell biology practical classes at the Department of Medical Biology and Genetics, Charles University in Prague, Faculty of Medicine in Hradec Králové, Czech Republic. First, we set up a new working place for digitizing videosequences which consisted of: PC - based workstation (Matrox RT200 hardware, Adobe Premiere v. 6.1 software), JVC SVHS videorecorder with time-base correction, TV monitor and UPS. Next, we recorded the behaviour of cells cultivated in vitro using time-lapse phase-contrast videomicroscopy (microscope OLYMPUS IMT2, analogue JVC videocamera and analogue Mitsubishi time-lapse videorecorder). These analogue videosequences demonstrating chosen cell activities - cell division, cell movement, cell death (apoptosis) with membrane blebbing, ruffling, and phagocytosis of calcium

phosphate crystals - were digitized, edited and converted to mpeg-1 format. The resulting videostreams were linked to the Department of Medical Biology and Genetics homepage - internet address: <http://biologie.lfhk.cuni.cz>. There they will serve as teaching material for more than 150 1st year General Medicine and Dentistry students of Charles University, Faculty of Medicine in Hradec Králové, Czech Republic.

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The investigation of gastric emptying using 13C-octanoic acid breath test in the neonates and CF patients

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The non-invasive 13C-octanoic acid breath test (13C-OABT) is a method for investigating the gastric emptying (GE) rate in adults, children and newborns. The only time-limited step of OA metabolism is GE. Labelling of OA with carbon 13C stable isotope enables us to follow the GE rate, which is reflected by 13CO₂/12CO₂ ratio change in the expired breath. We hypothesized the GE rate changes according to the amount of enteral nutrition in different groups of prematures. Gastrointestinal motility is widely believed to be disturbed in cystic fibrosis (CF) patients. We monitored gastric motility in CF patients using 13C-OABT. The original methods were designed: special nasal mask for newborns and infants for the collection of exhaled breath and original software for mathematical analysis of the results was created. Patients: I. 17 stable infants: 31-37 week of gestation, 1400-2680 g birth weight, 5 small for gestational age, 12 average for gestational age. The postnatal age of infants was 1-10 days. There were two pairs of twins in the study. II. 15 children suffered from CF, average age 12.5 yrs. Results: I. The personal GE manner of one neonate is stable under circumstances of the same food (pasteurised breast milk) and increasing doses of it. The GE half-time computed using AUC was generally statistically insignificant intrapersonally. The small for gestational age prematures, with increasing milk doses, tend to have higher intraindividual GE half-time variation compared to appropriate one for gestational age infants. There was no difference in the GE half-time between prematures below 32. w. g. and older ones. The overall half-emptying time of the GE was 46.3 min. II. The GE half-time corresponded to normal value in CF patients.

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Applied anatomy of the head and neck - a new subject in the dental curriculum

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The object of our assignment was to design a curriculum of a new subject - Topographic Anatomy of head and neck - which will be taught in the 3rd year of dentistry studies. First - after consultations with clinicians and using modern teaching methods - the syllabus was established. The new subject was designated APPLIED ANATOMY OF the HEAD and NECK. According to the project plan, we have obtained appropriate textbooks and literature, teaching models and instruments. With fellow clinicians (dentists, anesthesiologist, dental surgeons, neurosurgeon, ENT specialist and radiologist) 3 teaching video films were produced: Regional anesthesiology in the head and neck, Examination of cranial nerves, and Endoscopy of paranasal sinuses. The topic was divided into the six 5 hour teaching modules. Each comprises lectures of a clinician or anatomist, case presentations, demonstrations, training of manual skills and practical classes. The approved list of required manual skills will be a prerequisite for the final examination, together with an essay on a given topic, written by each student individually. Also a spot test for self-assessment was prepared (www.lfhk.cuni.cz/anatomie) together with an educational CD-ROM, comprised of a set of instructive topographic pictures and lectures, especially on pain in the head and neck. A set of final examination questions is a part of the curriculum.

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Modernization and digitalization of the specimens collection for cellular biology instruction

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Cellular biology instruction depends to a large degree on the use of standard microscopes and first-rate collections of specimens. Over the last years, always with generous support of the Grant agency of the Ministry of Education, we have managed to equip our practical classroom with new student Nikon SE microscopes along with a special teacher's workplace including a digital camera connected to the Nikon Eclipse E-400 microscope, computer, and beamer. The specimens collection we have used previously, on the other hand, had not been upgraded for more than twenty years, and it is therefore not capable of meeting the demands of modern trends in biomedical education. To change this situation, we prepared 21 new specimens, each in 50 copies, and digitized them simultaneously using a di-

gital camera Nikon COOLPIX 950. The specimens comprise mostly different cell lines cultivated in vitro (fixed and stained), but there are also tissues as well as subcellular structures such as chromosomes. The prepared digital microphotographs are being placed onto the departmental homepage: www.biology-lfhk.cz/ where they will serve both for instruction and as reference material for students and teachers of other subjects where the morphological methods are accentuated as well (for example histology and pathologic anatomy). We hope that with this approach we will contribute to further coordination and integration of the individual subjects taught at medical faculties.

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Improving the counseling services for medical students at the Faculty of Medicine, Charles University in Hradec Králové

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Being aware of the importance of student development during study at a medical faculty, we have applied for a grant in order to improve the counseling services at our Faculty of Medicine. Due to the grant provided, we were allowed to purchase recent literature on the subject of counseling as well as psychotherapy. This will obviously improve our understanding of the application of different theories in student counseling. Not only have we improved the actual counseling, but we have also started running a voluntary psychotherapy group for students in need. We have also commenced the counseling service for foreign students in the Faculty. Last but not least, we were enabled to equip the counseling room with a new PC as well as with new and comfortable furniture. Thus we have laid the basis for a modern student counseling service at the Faculty of Medicine, Charles University in Hradec Králové.

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Multimedial programs in practical classes of medical microbiology at the Faculty of Medicine of Charles University in Hradec Králové

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Practical classes of medical microbiology are an important part of the study program at the Faculty of Medicine. The aim of the research project was to use multimedial techniques in some study programs with practical classes: clinical specimen taking, streptococcal infections, staphylococcal in-

fections, anaerobic infections, diagnosis of viral infections. Prepared study programs including pictorial documentation (photographs of clinical signs of diseases, microbial cultures, microphotographs, graphs, schemas) were placed on our new web pages, and in such a way that they are easily available to students (<http://www.lfhk.cuni.cz/klinmikrob>). Also multiple-choice tests were added to be used by students for revision of the topics. Results of public inquiry among students show that they prefer electronic forms of lectures and practical classes supplemented by laboratory work. The created web pages concerning the teaching of microbiology were evaluated very positively by students. They consider these presented forms of instruction to be both quantitative and qualitative contributions to their knowledge.

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Influence of the ratio of arterial and portal liver perfusion on insulin resistance and liver function in liver cirrhosis

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Purpose: Liver cirrhosis causes grand changes of liver microcirculation and of its portal and arterial perfusion. This influences the liver function. A portosystemic shunt further alters these conditions. We measured these liver circulation changes and analyzed their relation to liver function and also to insulin resistance which is known to be altered in liver cirrhosis. **Methods:** Using a published method of perfusion liver scintigraphy, we applied ⁹⁹Tc-DTPA before, on the 1st day and on the 7th day after the portosystemic shunt (TIPS) creation. At the same time, we measured insulin resistance using the euglycemic clamp and standard biochemical and clinical data. We obtained records of activity course above the liver which we analyzed by several described methods. **Results:** We succeeded in completing consecutive measurements in 23 subjects undergoing elective TIPS. We found individually variable alteration of liver perfusion in liver cirrhosis and manifold follow-up clinical data. After the portosystemic shunt creation, further dramatic change of liver perfusion was noted and developed during the following week. We processed the parameters of obtained curves using several different methods described in literature and though there seemed to be some tendencies in correlation with the data of clinical outcome, we did not find any statistical significance. Concerning glucose metabolism, we also did not find a significant change of glucose resistance after the abrupt change of liver perfusion following the shunt creation. **Conclusion:** The changes of glucose metabolism in patients with the liver cirrhosis do not seem to correlate with microcirculation changes. Though liver perfusion changes seemed promising con-

cerning the prognosis of liver function development, using the method of scintigraphic perfusion we did not find an exact parameter to predict it.

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Introduction of multimedial electronics in the instruction of medical physiology

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This project of modernizing the theoretical and practical instruction of medical physiology aimed to allow all the teaching staff of our department to use the multimedial electronics to prepare their professional presentations and lectures. The equipment of our faculty allows the use of dataprojection for both lectures and seminars and also for practical classes. We missed only the facilities to process the multimedial data and prepare electronic presentations accessible to all our teachers. For this purpose, we bought a computer of adequate performance together with appropriate input and output devices. The complete system includes: computer with video processing master card and DVD-ROM and CD-RW units, graphic tablet, scanner, videorecorder, digital camera and both black-white laser and color ink-jet printers. This now allows us to use: digitization of analogue video and graphic pictures, capturing and recording video in real time, cutting and mixing video and audio recordings of different independent resources, selecting and processing videosequences and even sole snapshots in different formats, creation, processing and archiving graphic and photodocumentation, creation of graphic figures and charts (even 3D), and animations. All these can be implemented into projected presentations or printed publications or shared through the electronic network. We have learned to use these new techniques, and we hope to have instituted the groundwork for continual growth of intelligibility and effectiveness of instruction of medical physiology utilizing up-to-date information technology.

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Risk perception and level of health self-assessment of employees in different professions

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The aim of our study is to administer an anonymous questionnaire concerning the self-perception of health risk

of workers in various professions. Their knowledge, attitudes and behaviour in essential health questions were also assessed. During the 3 years of the study, we want to contact at least 750 respondents from various professions ("blue-collar" workers, medical workers, teachers of all school levels, representatives of town authorities) chosen in such a manner so that we would be able to evaluate relationship between socioeconomic status, educational level, age, gender, and health risk perception and knowledge. During the first year of the grant we have obtained answers from 411 respondents (242 from teachers, 124 from medical workers and 45 from factory workers). Preliminary results from the answers of teachers and medical workers show marked risk underestimation in spite of a high level of education. In our set 33 % of the teachers, 28 % of the nurses and 6 % of the doctors smoke. A significant difference was found in participants' preventive check-ups. The worst situation was found in the group of medical doctors, where 70 % of them don't participate in preventive check-ups by general practitioners (GP), 30 % of the doctors don't participate in stomatological prevention and 33 % of female doctors don't participate in preventive gynaecology screening. The situation among medical nurses is slightly better - in preventive check-ups (in the order - GP, stomatology, gynaecology) they don't participate in 30 %, 17 % and 19 %. As for the teachers, only 50 % of them participate in GP's preventive investigations, 12 % in stomatological, and 14% in gynaecological prevention. In the year 2002 we will continue in data-gathering and statistical analysis.

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The difference between the influence of glutamine and glutamate in parenteral nutrition

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The principal goal of this project is to answer the question of whether the administration of an amino-acid solution that contains glutamic acid in the quantity equivalent to the amount found in the standard diet is equally as effective as a solution containing glutamine in the form of dipeptide. To date eight stable patients whose clinical situation required total parenteral nutrition for more than 14 days (supposed duration of study) were suggested for the study. After information about the purpose of the study, they were randomised (method of closed envelopes) to start either with glutamate (regimen 1) or with glutamine (regimen 2), respectively. After 12 hours of saline administration (equivalent to a period of fasting) the first blood sample was drawn. Routine laboratory analysis was performed together

with the measurement of albumin, prealbumin, transferrin and cholinesterase levels in serum. Plasma samples were also deproteinized and frozen to -70 degrees C until amino acid analysis. Then the patients were infused with parenteral nutrition based either on glutamic acid (18 g per day) or glutamate (14 g per day). During infusion and after 12 hours of saline administration, blood samples were again drawn for biochemical analysis. Then the patient's regimens were reversed and blood samples were collected in the same way as during the first regimen. The rest of the patients (8 subjects), amino-acid analysis and statistical evaluation will be done in 2002.

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Coronary heart disease and lipid risk factors in nonagenarians

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The aim of the present study was the bioanalysis of lipid metabolism in aged patients with coronary heart disease and to study the relationship between these biochemical markers and longevity. Twelve nonagenarians, 10 women and 2 men, aged 94 ± 3 years (90-101 years) and eleven control patients, 7 women and 4 men, aged 84 ± 5 years (77-89 years), followed for coronary artery disease at the Department of Metabolic Care and Gerontology, Charles University, Teaching Hospital entered the study. All subjects were self-sufficient, without any acute major illnesses and living without assistance. At the start of the project the free fatty acids (FFA), thiobarbituric reactive substances (TBARS), retinol, alpha tocopherol, ascorbic acid, cholesterol, triacylglycerols, phospholipids in serum, in lipoprotein fractions and fatty acids (FA) and phospholipids in erythrocyte membrane were determined. We found significantly higher LDL polyunsaturated fatty acids (PUFA) 18:3n-6 ($p = 0.022$) and 22:6n-3 ($p = 0.012$) and a significant increase of HDL alpha tocopherol/ cholesterol ratio ($p = 0.012$) in nonagenarians. There were not any significant differences in erythrocyte membrane fatty acids and phospholipids. In serum we found significantly higher levels of TBARS (3.23 ± 1.20 vs. 2.12 ± 0.83 $\mu\text{mol/l}$, $p = 0.025$) and lauric acid ($p = 0.023$) in nonagenarians; other parameters were not changed significantly. We also found significant correlation between dodecahexaenoic acid in LDL and level of TBARS in serum ($p = 0.024$) and HDL alpha tocopherol / cholesterol ratio ($p = 0.018$). The higher concentrations of PUFAs in LDL and alpha tocopherol in HDL might be parameters related to longevity in nonagenarians with coronary heart disease.

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Human brain project

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The Human Brain Project is a US federally-funded research project joined by many laboratories. It supports advanced technologies and novel ways to acquire, store, retrieve, analyze, visualize, synthesize, disseminate and share data about the brain. Via its Internet website the Laboratory of Synapse Structure and Function of The Department of Biology, Boston University, developed software and prepared three-dimensional data available to other brain research laboratories or university centers. The author supplied the website with The Atlas of Ultrastructural Neurocytology containing more than 400 high quality electron micrographs and three-dimensional reconstructions. The Atlas as well as other components of the website is widely used as an up-to-date educational resource for students of neurobiology (e.g., in University of Boston, New York, Liverpool, Basel). The total number of logged accesses (website hits) as revealed by the Microsoft site server express analysis (server statistics) made by more than 1.5 million since November 2000, reaching up to more than 2,500 visits per week during the last months. The "Synapse Website" was mentioned in SCIENCE journal, named "Page of the Month" in January 2000, and it received an award for outstanding contribution to psychology on the internet. Reference: <http://synapses.bu.edu/>

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BAS-0 bioactive glass-ceramic material as a bone graft substitute (group of bone cysts)

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Introduction: The aim of the study is to assess the long-term results of BAS-0 bioactive glass-ceramic material (LASAK, Prague, Czech Republic) used as a bone tissue substitute in the treatment of bone cysts. **Materials and methods:** 29 patients were treated by curettage of the bone lesion and filling of the defect with bioactive glass-ceramic material BAS-0 at the Dept. of Orthopaedic Surgery in Hradec Králové between October 1990 and January 2001. Patients with a recurrence of bone cysts were excluded and only these following sites of involvement were included in our study: femur in 7 cases, humerus and tibia in 3 cases each. There were 9 men and 3 women and the average age

was 14 years (range 8 to 23 years). The primary diagnoses were unicameral bone cysts in 9 patients and aneurysmal bone cysts in 3 patients. The mean follow-up after surgery was 7 years (range 2 to 9 years). As a follow-up clinical examination, plain radiographs and bone scintigraphy were performed. Results: No inflammatory changes of soft tissues with normal carrying capacity of the extremity were found in any of the cases. On plain radiographs no signs of glass-ceramic material loosening and no periosteal reaction were observed. The monitored level of osteoblastic activity of bone tissue in the area of implanted material was normal in 5 cases, increased in 4 cases and high in 3 cases (all 3 patients were seen after treatment of bone cysts located in the diaphysis and suffered pain). Conclusions: By the use of the granular form of BAS-0 glass-ceramic material after bone ingrowth, the elastic properties of this filling get near to normal bone tissue. The level of osteoblastic activity in the area of treated bone cyst will be compared to a group of patients after filling of the bone defect with bone grafts.

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The possibility of using and the safety of radioactive stents, with activity brought about by the cyclotron, in the prevention of restenosis

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Background: The aims of this study were: 1) Preparation of the methodology to activate coronary stents in the cyclotron. 2) Finding out the safety of implantation of radioactive stents for patients. 3) Finding out if radioactive stents have a positive effect on the incidence of restenosis after the implantation of coronary stents. 4) Finding out the effect of radioactive stents on vessel remodeling and plaque formation. **Methods and Results:** We successfully implanted 14 radioactive stents into 14 patients during the period from September 2000 to January 2001. We used 18 mm long BX Velocity stents. The radioactivity of the stents has been brought about by the cyclotron in the Department of Cyclotron Academy of Science, in Řež, the Czech Republic. Beta emission (⁵⁵Co) is the dominant source of radiation of the stents. We implanted the stents with a mean activity of 41.14 ± 1.23 °C at the time of the implantation. The reference diameter of the treated artery was 3.049 ± 0.57 mm. The minimal lumen diameter increased from 1.02 ± 0.47 mm pre-procedure to 3.19 ± 0.62

mm post-procedure. The minimal luminal diameter at follow-up was 1.78 mm on average, and later loss was 1.26 mm. Angiographic restenosis (> 50 %) was observed in 7 patients (50 %). Target lesion revascularization was performed in 5 patients (35.7 %). We didn't have any subacute or late thrombosis of the stents during the follow-up period. We didn't observe any death or myocardial infarction during the procedure or during the follow-up. Conclusion: The present type of radioactive stents isn't acceptable for clinical application due to the high incidence of restenosis.

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Introduction of methods based on UV-spectrophotometry into biochemistry practical courses

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The aim of this project was to buy an UV-spectrophotometer and introduce a set of enzymic methods based on decrease of absorbance at 340 nm into the biochemistry practical courses. The system lactate dehydrogenase (enzyme) – pyruvate (substrate) – NADH (cosubstrate) was chosen. In this system, pyruvate is reduced to lactate in the presence of NADH and lactate dehydrogenase, which results in a decrease of absorbance at 340 nm. We modified the method to investigate: – the effect of enzyme concentration on the reaction rate, – the effect of substrate concentration, – the effect of cosubstrate concentration, – the effect of temperature, – the effect of pH. The determination of aspartate aminotransferase and creatine kinase was also introduced to complete the set of enzymes important in the heart infarction diagnostics.

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Innovation in medical biophysics practical instruction – Basic Audiometry

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The main goal of the project was the introduction of a new measurement task "Audiometry" into the practical training of medical biophysics instruction. To fulfill this goal, we established an audiometric workplace equipped with a clinical audiometer DA 65. For practical courses we have prepared complete manuals and documentation concerning the task. The content of the task is the measurement of threshold audiogram for air and bone conduction and the evaluation of hearing loss by the Fowler method.

Students were assigned the topic "Audiometry" for the first time in academic year 2001/2002. The success of Audiometry was measured by a questionnaire, which students filled out after finishing the practical training from biophysics. The result of enquiry shows that students appreciate work with technology used in clinical practice; they consider the new practical task suitable and useful.

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Development of the databases and software for biosignals analysis and improvement of the quality of education

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In correspondence with the proposal (see <http://camelot.lfhk.cuni.cz/fyzika/reference> Granty) the third year activity was concentrated on: 1) the development of the application of programs for analysis of mechanical properties of tissues and materials for bioimplants, 2) the analysis of motion-onset visual evoked potentials connected with sensory information processing and mechanism of non-specific adaptation, 3) the analysis of cataract surgery with a capsular tension ring, and traumatic cataracts in children and refractive lensectomy, 4) the development of the hypertext program for undergraduate students in fields of biostatistics, pharmacotherapy training, and case studies in hygiene, 5) 3D database for the reconstruction of apoptotic nuclei, 6) the writing of software for transfer and storing of data from stabilographic plane and statokinesiometric examinations. For backup and support of the above-mentioned goals, new hardware was purchased: 4 notebooks, 2 servers, 4 personal computers, ISES measuring system and 2 digital cameras. Software which was paid from the allocated funds included renewal of STATISTICA v.6, MATHLAB licenses and new LabView program.

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Photopic electroretinography in diabetics

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There is no doubt about the importance of the scotopic electroretinography (ERG) for diagnosis and prognosis of the diabetic retinopathy. The aim of this study is to detect abnormalities of the photopic ERG in diabetics. The ERG examinations of three groups of diabetics were continued:

1. diabetics without diabetic retinopathy (n=38), 2. diabetics with simple diabetic retinopathy (n=24), 3. diabetics with preproliferative and proliferative diabetic retinopathy before and after panretinal photocoagulation (n=20). Besides the rod and the maximal ERG responses, the photopic ERG responses were evaluated. The abnormalities of the photopic responses were confirmed; the manuscript is in preparation. In the clinical electroretinography, the subnormal oscillatory potentials were found in patients with the optic nerve pits complicated by maculopathy. It will be the first description of such a finding in the literature. The ERG potentials were examined in 40 patients with stenosis of the carotid artery. The possible dependence of the ERG potentials on the laterality of the carotid artery stenosis was proved, and the extent of the ERG changes in bilateral carotid artery stenosis was tested. The interesting ERG findings in 5 patients with congenital stationary night blindness are supposed to enable more precise evaluation of the cone's contribution to the size of the oscillatory potentials and the efficient power. The spectrum of the examination methods was enlarged. Examination with the 15 Hz flicker stimulus was introduced. Eleven gelatine neutral gray filters which enable changing the intensity of the illumination in smaller steps (Nd1-Nd2) were provided.

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Use of videorecordings in preventive medicine education

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To obtain a good quality of medical care in preventive medicine it is necessary to teach medical students basic information concerning all possible aspects of the occupational environment. Till recent times we have been making use of the field visits into various enterprises. Such an educational approach was laden with specific risks. The visits were time-consuming, and there existed the danger of possible injuries and poor oral communication in a noisy environment. That's why we looked for financial support from the grant system. We succeeded in getting a TV set with a large screen, a videorecorder, and a movie camera. This equipment at our disposal can be used for videorecording the chosen activities in various professions. In the year 2001 we recorded 17 video shots of our own production, and moreover obtained 7 professional videorecordings. These video tapes have already been used during our pedagogic activity in this school year. These visual aids make lectures/seminars more interesting and increase the active participation of students.

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The standardization of clinical tests on a force platform

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The upright posture in humans represents maintaining an equilibrium between various disturbances and contractions of musculature resulting in continuous body-sway. Body-sway measurement is usually accomplished by evaluation of the trajectory of the center of pressure (CP) of the vertical foot-ground reaction forces by using a force platform (FP) equipped with strain gauge sensors. The trajectory of the CP is related to the asymmetry of the contact area of the feet with ground, i.e. between the length and the width of both feet. It also corresponds to the form projection of the center of gravity. The sway of 50 healthy persons (20 males, 30 females), aged 18-27 years, all students of two Universities in Hradec Králové, were examined. Their overall body status (fitness) was evaluated before FP investigation (e. g. body symmetry, muscle development, medical history etc.). Swaying in 20 sec periods described time intervals: i/ 2D trajectory of the CP and ii/ X- and Y- components of the CP deflection in time, resembling a time series. Pilot results represented by the grand average component range in both groups show no significance. In the second year statistics were extended also the recording data from different tests. It was confirmed by statistics that 50 young individuals can be used as standard for comparisons. We continued to evaluate aging people. We investigated 7 individuals 30-49 year old and 10 individuals 50 and older to compare statistics from elderly and younger people to have standards for the aging population. References: Benda BJ, Riely PO, Krebs DE. Biomechanical relationship between center of pressure during standing. IEEE. Trans Rehab Eng 1994;2:13-25.

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Therapeutic impact on the cognitive functions in schizophrenia

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Schizophrenia is, in variable degrees, accompanied by a broad spectrum of cognitive impairments. Numerous reports document lower premorbid IQ scores, impairments of psychomotor development, and premorbid speech abnormalities in children who later developed schizophrenia. These findings suggest that early developmental anomalies might be responsible for the cognitive impairment observed in schizophrenic patients. Considerable evidence now

points towards cognitive deficits as an enduring trait of schizophrenia. Poor cognitive function is a major factor interfering with patients' social and occupational functioning. The key domains of cognitive dysfunction are memory, attention and executive function impairments. Novel antipsychotics such as risperidone may help optimise treatment and long-term functional outcome. Considerable evidence exists suggesting an important role for cholinergic neurotransmission in various aspect of learning and memory. The aim of this project is to evaluate the effectiveness of the combination of risperidone and the cholinergic drug donepezil on cognitive functions in a double blind placebo controlled study in schizophrenic patients. The first four patients have entered the study between October and December 2001.

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Impression taking by reversible hydrocolloids (agar-agar)

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The device allowing the impression taking of prepared teeth in fixed prosthodontics by the reversible hydrocolloid impression materials was bought. This apparatus is installed at the prosthetic department of the dental clinic, and it is used in both the preclinical and clinical dental prosthetic instruction. We are now able to demonstrate the impression material and technique, which were discussed only theoretically before. Reversible hydrocolloid agar-agar is a natural hydrophilic impression material which sets, due to the physical process during cooling, without practically any dimensional changes. The hydrophilic properties are very important in fixed prosthodontics assuring the reproduction of the preparation details placed in the wet gingival crevice. The materials at our disposal so far are non-hydrophilic. Hydrocolloid materials are supplied by manufacturers in the set gelatinous state in collapsible plastic tubes or small glass cylinders. They are liquified during a 10 min. immersion in the boiling water bath in the first compartment of the purchased apparatus called a hydroprocessor. The material in the sol state can be stored for 5 days in the second compartment of the hydroprocesor - in the storing water bath. Immediately before the impression, the material placed in the specialized impression tray is tempered to the temperature of 45 Celsius degrees at which the material remains still in the sol state suitable for impression taking. Introduction of this impression material and technique contributes both to the enlargement of treatment methods in prosthetic dentistry, and to the enrichment of the dental instruction.

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Changes of lipid metabolism and its mediator-related effects in patients with colorectal carcinoma - prospective impact on nutritional support.

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Lipids are the most important substrates for energy production, components of cellular membranes and metabolic mediators (polyunsaturated fatty acids, precursors of cholesterol biosynthesis etc.). In addition, short chain fatty acids are important metabolites as energy fuel for colonocytes. The research project is composed of three parts: 1) Experimentally, the effect of selective inhibitors on enzymatic cascade in production of arachidonic acid metabolites was studied, and in the second step the interaction of these products with TNF-alfa. This experiments demonstrated increased sensitivity of tumor cells (colon cancer cells HT-29) to apoptosis after polyunsaturated fatty acid treatment. 2) The clinical section is focused on the development of methods applicable for monitoring of SCFA (short chain fatty acid) production. Method based on the methane analysis in the breath enables the determination of value of dietetic fibres in nutritional manipulations. The new method based on breath analysis has been successfully accomplished, practical clinical application proved, and patent recently prepared. 3) Precursors of cholesterol biosynthesis represent a group of compounds important in the composition and function of cellular membranes. Isoprenoids, mevalonic acid and squalene are precursors of ubiquinones, and anchoring molecules for Ras proteins. Cholesterol biosynthesis precursors were determined by GCMS method in colon cancer patients and in control group and the results are prepared for publication. Some methodologies or results of pilot studies were accepted for publication or already published (example): European Journal of Cancer 2000;36:1844-52: TNF modulates differentiation induced by butyrate in the HT-29 human colon adenocarcinoma cell line.

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Analysis of expired gases using GC/MS for observation of metabolic disorders

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The aim of this project was the development and elaboration of sampling and analytical methods for the determi-

nation of cholesterol precursors and intestinal anaerobic fermentation product with an emphasis on expired gas analysis. The sampling and analytical methods for the determination of isoprene (Solid Phase Microextraction-GC-MS) and methane (GC-FID) in expired breath were developed. As a reference parameter of cholesterol synthesis, the analytical method for determination of cholesterol precursors (squalene, lanosterole, lathosterole) and phytosterols (sitosterole, campesterole, stigmasterole) in blood plasma. The method for determination of hydrogen sulphide concentration in whole blood (extractive alkylation and GC-MS). Expired breath is not suitable sampling material due to the interference of dental plaque bacteria. Successful project realisation was facilitated by close cooperation between regional universities. Developed methods are used in medical research of metabolic disorders and processes accompanying critical care. The analytical methods and their clinical application were published in impacted journals and presented at international conferences. References: Hyšpler R, Crhová Š, Gasparič J, Zadák Z, Čížková M, Balasová V, J Chromatography B. 2000;739:183-90, ISSN 0378-4347. Hyšpler R, Tichá A, Indrová M et al. J Chromatography B, accepted for publication, in press. Zadák Z, Hyšpler R, Bakalář B, Crhová Š. Vnitř Lék 2000;46(11):779-81, ISSN 0042-773X. Zadák Z, Hyšpler R, Bakalář B et al. Clinical Nutrition 2001;20(Supplement 3), ISSN 0261-5614.

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The relation between selected biochemical parameters in cadaveric kidney donors and post-transplantation graft function

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The aim of the study was to assess the relationship between plasma selenium concentration in cadaveric kidney donors and graft function in recipients in the early stage after transplantation. Patients and methods: The study protocol was approved by the local ethical committee. Plasma selenium concentrations (Se, umol/l) were measured in cadaveric kidney donors at the time of brain death diagnosis established by brain angiography. The markers of graft functioning in recipients were observed during a 5-day period after transplantation. Results: Grafts with a worse function were from these donors: women (Se - 0.74 v.s. men 0.59), time period between insult and death longer than 48 h (Se - 0.67 v.s. shorter 0.59), hemoglobine concentrations higher than 120 g/l (Se - 0.72 v.s. lower 0.52), no treatment with desmopressini acetate (Se - 0.68 v.s. desmopressini acetate treatment 0.57). Higher plasma selenium concentrations were the common sign of all these donors. Grafts with signs of good function: early decrease of

serum urea (mmol/l - 10.3 v.s. worse function 34.0), creatinine (umol/l - 175 v.s. worse function 562) and rapid increase of creatinine clearance (ml/s - 0.84 v.s. worse function 0.20) were from donors with lower plasma selenium concentrations. Conclusion: We assume that higher plasma selenium concentration is the result of the reduced ability of cells to utilize this trace element for antioxidant defence reactions. There is a higher risk of reactive oxygen species formation, which impaired the kidney and made a poor graft for future transplantation. Our results suggest that desmopressini acetate treatment of donors was linked with lower plasma selenium concentration in donors and with better early post-transplantation graft function in recipients.

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THE INFLUENCE OF ANTICHOLINERGIC DRUG AND OXIME SELECTION ON THE EFFECTIVENESS OF ANTIDOTAL TREATMENT AGAINST TABUN-INDUCED POISONING IN MICE

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Summary: 1. The influence of oximes (pralidoxime, obidoxime, HI-6) and anticholinergic drugs (atropine, benactyzine, biperiden, scopolamine) on the effectiveness of antidotal treatment to eliminate tabun-induced lethal effects was studied in mice. 2. Obidoxime seems to be the most efficacious oxime for the elimination of tabun-induced lethal effects in mice, although the difference in the efficacy of obidoxime and HI-6 is not significant when they are combined with atropine. 3. Obidoxime and HI-6 when combined with centrally acting anticholinergic drugs (benactyzine, biperiden and scopolamine) seem to be more efficacious in the elimination of toxic effects of the lethal dose of tabun than their combination with atropine. 4. The findings support the hypothesis that the choice of acetylcholinesterase reactivators as well as the anticholinergic drug selection are important for the effectiveness of antidotal mixture in the case of antidotal treatment of tabun-induced acute poisoning.

Key words: *Tabun; Oximes; Anticholinergic drugs; Acute toxicity; Mice*

Introduction

Despite the entry into force in April 1997 of the Chemical Weapons Convention forbidding the development, production, stockpiling and use of chemical warfare agents, the world has seen a rapid proliferation of such agents (21). The chemical warfare agents include nerve agents, very dangerous highly toxic organophosphorus compounds (OPs) that exert their toxic effects by phosphorylation and subsequent inactivation of acetylcholinesterase (AChE, EC 3.1.1.7). The inactivation of this enzyme allows the accumulation of acetylcholine (ACh) in the synaptic terminals of the central and peripheral nervous systems with subsequent widespread overstimulation of cholinergic receptors (18, 25). Effective management of nerve agent-induced cholinergic overstimulation is critical for immediate casualty treatment and for a rapid and full recovery from the effects of nerve agent exposure. Unfortunately, certain OPs were found to be resistant to the standard antidotal treatment which consists of anticholinergic drugs to counteract the accumulation of ACh and oximes to reactivate nerve agent-inhibited AChE (7,18).

Tabun (O-ethyl-N,N-dimethyl phosphoramidocyanidate) is probably one of the most dangerous compounds among the warfare nerve agents, since its deleterious effects are extraordinarily difficult to counteract because of the existence of a lone electron pair located on an amidic group

that makes the nucleophilic attack of oximes almost impossible (4,7,9,11,16).

As the ability of currently used monopyridinium (e.g. pralidoxime) and bispyridinium oximes (e.g. obidoxime) to eliminate toxic effects of nerve agents is generally rather low, the H oximes have been developed to improve the effectiveness of oximes to reactivate nerve agent-inhibited AChE and eliminate nerve agent-induced lethal effects (14,17). Among the series of H oximes, the HI-6 has been the most studied because it seems to be the most efficacious oxime, yet found, against toxic effects of soman (1,12,23). Unfortunately, the reactivating efficacy of HI-6 for tabun-inhibited AChE is not so high as in the case of soman poisoning (5,6,16,22,26,28).

Not only the choice of oximes but also the anticholinergic drug selection could influence the effectiveness of antidotes against OPs. The OP-induced poisoning is usually treated by an oxime in combination with the anticholinergic drug atropine. Nevertheless, there are other anticholinergic drugs that seem to be suitable adjuncts to oxime treatment of nerve agent-induced poisoning, especially in the case of poisonings with centrally acting nerve agents such as soman and tabun (26). It has been described that some centrally acting anticholinergic drugs such as benactyzine are able to increase the ability of HI-6 to eliminate nerve agent-induced lethal toxic effects in comparison with atropine (13).

The present study compares the effects of currently used oximes (pralidoxime, obidoxime) and H oximes (HI-6) in combination with various anticholinergic drugs (atropine, benactyzine, biperiden, scopolamine) against tabun-induced acute poisoning in mice.

Methods

Male mice weighing between 22 and 25 g were obtained from Konárovice. The animals were maintained in an air-conditioned room with light from 07.00 to 19.00 h and were allowed free access to standard food and tap water. The principles of laboratory animal care were followed and the handling of animals was made under the supervision of the Ethics Committee of Medical Faculty of Charles University and Purkyně Military Medical Academy in Hradec Králové.

Tabun of 95% purity was purchased from Military Technical Institute Brno. Its purity was assayed by acidimetric titration. The oxime HI-6 of 98.5% purity was synthesized at the Department of Toxicology of Purkyně Military Medical Academy in Hradec Králové. Its purity was analyzed using HPLC. All other chemicals and drugs of analytical grade were obtained commercially and used without further purification.

In the first part of the experiments, tabun-poisoned mice were treated intramuscularly (i.m.) with one of tested oximes (pralidoxime, obidoxime and the oxime HI-6) at equieffective doses (5% LD₅₀) in combination with atropine (21 mg/kg) one minute after the challenge of tabun. In the second part of the experiments, tabun-poisoned mice were treated i.m. with obidoxime or the oxime HI-6 in combination with one of tested anticholinergic drugs (atropine, benactyzine, biperiden and scopolamine). The oxime as well as anticholinergic drugs were used at equieffective doses (5% LD₅₀).

The effectiveness of tested antidotal mixtures was evaluated by the assessment of the LD₅₀ values and their 95% confidence limits using probit-logarithmical analysis of death occurring within 24 h after i.m. administration of tabun at five different doses with six mice per dose (24). The efficacy of tested antidotal mixtures was expressed as protective ratio (LD₅₀ value of tabun in protected mice/ LD₅₀ value of tabun in unprotected mice).

Statistical significance was determined by the use of Student's t-test and differences were considered significant when $p < 0.05$.

Results

The therapeutic efficacy of antidotal mixtures consisting of various oximes in combination with atropine is presented in Table 1. These results show that obidoxime seems to be the most efficacious reactivator of tabun-inhibited AChE in the elimination tabun-induced lethal effects in

mice, although the difference in the efficacy between obidoxime and the oxime HI-6 is not significant. On the other hand, obidoxime as well as the oxime HI-6 are significantly more efficacious ($p < 0.05$) to protect the mice from the lethal effects of tabun than pralidoxime.

The efficacy of obidoxime or the oxime HI-6 in combination with various anticholinergic drugs is shown in Tables 2 and 3. The data clearly demonstrate the higher effectiveness of centrally acting anticholinergic drugs (benactyzine, biperiden, scopolamine) to eliminate tabun-induced lethal effects in comparison with atropine in the case of the combination of anticholinergic drugs with obidoxime ($p < 0.05$) (Tab. 2). On the other hand, the therapeutic efficacy of the combination of the oxime HI-6 with centrally acting anticholinergic drugs is only slightly higher than the combination of HI-6 with atropine (Tab. 3). In addition, obidoxime appears to be significantly more efficacious than the oxime HI-6 when combined with anticholinergic drugs with pronounced central effects such as benactyzine, biperiden and scopolamine ($p < 0.05$).

Tab. 1: Therapeutic effect of oximes, administered at a dose of their 5% LD₅₀ value in combination with atropine (21 mg/kg) 1 min after poisoning, on the LD₅₀ value of tabun. * significantly different from the untreated group at the level of $p < 0.05$, ^x significantly different from the group treated with pralidoxime at the level of $p < 0.05$.

Treatment	LD ₅₀ (µg/kg) ± 95% IS	Protective ratio
—	275.4 (269.3–281.6)	—
HI-6 + atropine	430.2 (414.0–447.1) ^{*x}	1.56
Obidoxime + atropine	454.9 (421.8–490.6) ^{*x}	1.65
Pralidoxime + atropine	377.7 (355.8–400.9) [*]	1.37

Tab. 2: Therapeutic effect of obidoxime in combination with various anticholinergic drugs, administered at a dose of their 5% LD₅₀ values 1 min after poisoning, on the LD₅₀ value of tabun.

* significantly different from the untreated group at the level of $p < 0.05$, ^x significantly different from the group treated with obidoxime and atropine at the level of $p < 0.05$.

Treatment	LD ₅₀ (µg/kg) ± 95% IS	Protective ratio
—	275.4 (269.3–281.6)	—
Obidoxime + atropine	454.9 (421.8–490.6) [*]	1.65
Obidoxime + benactyzine	773.2 (636.7–939.1) ^{*x}	2.81
Obidoxime + biperiden	716.9 (685.6–749.6) ^{*x}	2.60
Obidoxime + scopolamine	716.9 (657.3–780.0) ^{*x}	2.60

Tab. 3: Therapeutic effect of HI-6 in combination with various anticholinergic drugs, administered at a dose of their 5% LD₅₀ values 1 min after poisoning, on the LD₅₀ value of tabun.

* significantly different from the untreated group at the level of $p < 0.05$, ^x significantly different from the group treated with HI-6 and atropine at the level of $p < 0.05$.

Treatment	LD ₅₀ (µg/kg) ± 95% IS	Protective ratio
—	275.4 (269.3–281.6)	—
HI-6 + atropine	430.2 (414.0–447.1)*	1.56
HI-6 + benactyzine	462.1 (432.6–493.5)*	1.68
HI-6 + biperiden	524.5 (503.3–546.6)* ^x	1.91
HI-6 + scopolamine	460.2 (444.4–476.5)*	1.68

Discussion

Nerve agents are still considered to be the most important chemical warfare agents. With the existing threat of the use of chemical weapons not only in military conflicts but also in terrorist attacks, the search for effective protection is the central concern of different laboratories both civilian and military (7,20).

The effectiveness of antidotal treatment of acute poisoning with tabun is not sufficient regardless of the choice of the oxime because tabun-inhibited AChE is very difficult to reactivate (6,11,22,26,28). The reason for the weak reactivation potency of the oximes is not the rate of aging of phosphorylated AChE that is relatively low (10) but the presence of lone electron pair located on an amidic nitrogen. This lone electron pair makes the nucleophilic attack very difficult (9). Therefore, the oxime HI-6, that is rather effective against soman (12,14), is not too effective against tabun (6,22). According to our results, obidoxime seems to be more effective to eliminate tabun-induced lethal effects in mice than the oxime HI-6. Till now, it is not known what is the reason for higher effectiveness of obidoxime in comparison with the oxime HI-6. Generally, the difference between the stereochemic arrangement of obidoxime and the oxime HI-6 can play a role in the difference in therapeutic efficacy of both oximes.

The effectiveness of oximes against the toxic effects of nerve agents including tabun is usually tested in combination with atropine (6,22,27). Nevertheless, some other anticholinergic drugs (e.g. benactyzine, biperiden) should be more advantageous than atropine for the elimination of toxic effects of nerve agents because of their central antimuscarinic effects (2,13,26). Our results confirm the influence of anticholinergic drug selection on the efficacy of oximes in antagonizing the toxic effects of tabun at the supra-lethal doses. Both tested oximes, obidoxime and HI-6, were more

efficacious in eliminating tabun-induced toxicity when they were combined with centrally acting anticholinergic drugs in comparison with their combination with atropine although the increase in the therapeutic efficacy of the oxime HI-6 was not significant.

Our results can be explained by the difference in the central antimuscarinic effects of anticholinergic drugs studied that are very important for the prevention of tabun-induced central respiratory depression and, thus, the survival of tabun-poisoned mice. Benactyzine, biperiden as well as scopolamine differ from atropine in that they are more potent in the central nervous system as antimuscarinics due to their relatively high affinity to the central muscarinic receptors (15) and their lipophilicity making them possible to readily cross the blood-brain barrier (3,19,29). Atropine is also able to cross the blood-brain barrier but a relatively large dose of atropine is necessary to achieve the central antimuscarinic effects because of its lower lipophilicity and affinity to the central muscarinic receptors when compared to other anticholinergic drugs studied (3,8).

In conclusion, our data indicate that the correct choice of AChE reactivators as well as anticholinergic drugs is important for the survival of tabun-poisoned experimental animals. The most efficacious antidotal mixture against lethal effects of tabun in mice seems to be obidoxime in combination with some of centrally acting anticholinergic drugs.

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SERUM LIPID AND LIPOPROTEINS IN GALLSTONE PATIENTS

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Summary: Serum lipid and lipoprotein concentrations were determined in a group of thirty two gallstone patients and compared to the values obtained in thirty two healthy control subjects. The serum levels of high density lipoprotein cholesterol and lipoprotein(a) in gallstone patients were found to be lower than that of the control group. Total cholesterol, triglyceride, low density lipoprotein cholesterol, apoprotein-A and apoprotein-B concentrations were observed to be higher in gallstone patients than the controls. The differences in triglyceride and low density lipoprotein cholesterol concentration were statistically significant. However, there was no significant difference in high density lipoprotein cholesterol, lipoprotein(a), apoprotein-A, apoprotein-B, and total cholesterol concentrations between groups. It is concluded that gallstone formation may be related to a disturbance in lipid and lipoprotein metabolism.

Key words: Apoprotein; Gallstone disease; Lipoprotein; Lipid

Introduction

The prevalence of gallstone disease is high in many European countries as well as in the USA (15). Serum lipids and lipoproteins were suggested to be related with the pathogenesis of gallstones (18).

Although there have been numerous studies evaluating the serum lipid status of gallstone patients, these studies yielded inconsistent results. For example, decreased (15) and increased (17) total cholesterol (TC) levels have been reported in gallstone patients. The highest gallstone formation has been reported to be observed at low HDL-C levels and high triglyceride levels (18). Conversely, an unchanged high density lipoprotein cholesterol (HDL-C) and triglyceride (TG), and reduced serum low density lipoprotein cholesterol (LDL-C) were reported in gallstone patients (17).

The aim of this study was to investigate the possible relationship between serum lipids, lipoproteins and gallstone disease, and compare it with previous studies.

Materials and Methods

In this study 32 hospitalised gallstone patients undergoing elective cholecystectomy operation (27 women, 5 men; 28–65 years; mean: 47.6 years) and 32 healthy donors (control group, 27 women and 5 men; 26–60 years; mean: 43.4 years) were allocated to the study. Those having diseases

other than cholelithiasis were not included to the study in gallstone patients group. In gallstone group patients, there were no cholestasis, and the number of stones were two or more. There were no differences in demographic characteristics of patients between groups, including body mass index, and fasting blood sugar, as morbid obese patients, hypertensive patients and patients with fasting blood sugar of more than 120 g/dL were not included to the study.

Blood was obtained in the fasting state and serum samples were analysed for TC, HDL-C, TG, lipoprotein(a) (Lp(a)), apoprotein A (Apo-A), and apoprotein B (Apo-B) concentrations. The serum TC and TG concentrations were determined using an enzymatic method (UV test, Boehringer Mannheim) and by using auto-analyser (Hitachi 705). Lp(a), Apo-A, and Apo-B values were determined by nephelometry. LDL-C values were calculated by the Friedewald formula using TC, HDL-C and TG values obtained previously (4).

All the results are presented as mean value \pm standard error (SE). The statistical analyses were performed using the analysis of variance (Anova).

Results

There was no difference in patient characteristics between groups. Mean (\pm SE) values for fasting serum lipid fractions (TC, HDL-C, LDL-C, TG) and the concentra-

tions of the Lp(a), Apo-A, and Apo-B in gallstone patients and control group are presented as mg/dL in Tables 1 and 2, respectively.

The values for LDL-C, TG, TC, Apo-A, and Apo-B in the gallstone patients were found to be higher than the values obtained in the control group, and these differences were statistically significant ($p < 0.05$). On the other hand, HDL-C, Lp(a) levels were found to be lower in gallstone patients, but this difference was not statistically significant.

Tab 1: Serum lipid concentrations in control and gallstone groups.

Group	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	TG (mg/dL)
Control n=32				
Mean±SE	185.60±19.3	48.40±7.8	95.35±13.7	136.40±23.2
Gallstone n=32				
Mean±SE	209.23±11.6	43.35±3.6	132.70±8.0	186.91±9.4
Significance	NS	NS	P<0.05	P<0.05

TC indicates total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglyceride. NS, Not significant.

Tab 2: Serum lipoprotein concentrations in control and gallstone groups.

Group	Lp(a) (mg/dL)	Apo-A (mg/dL)	Apo-B (mg/dL)
Control n=32			
Mean±SE	46.72±13.6	151.46±8.3	91.12±8.3
Gallstone n=32			
Mean±SE	42.63±7.2	158.94±9.4	98.60±6.2
Significance	NS	NS	NS

Lp(a) indicates lipoprotein(a); Apo-A, apoprotein A; Apo-B, apoprotein B. Ns, Not significant.

Discussion

It is a widely accepted idea that the gallstone formation is closely related to a disorder in the lipid and lipoprotein metabolism (1). It was reported in a study that the average levels of serum lipids in patients with gallstones were higher than subjects without gallstones (11). However many metabolic factors may affect the gallstone formation (13). In order to standardise the groups in this study hypertensive, atherosclerotic and morbid obese patients were not included to the study. Since other patient characteristics were similar between groups, determinant factors of gallstone formation in these patients seem to be related to the lipid metabolism.

Triglycerides are converted by fatty tissue lipase into free fatty acids and transported in the blood stream to the various organs where, in the presence of glucose, they are resynthesized to triglycerides. In the liver, a large part of the free fatty acids is utilized for the synthesis of the lipids, primarily phospholipids. When the supply of free fatty acids exhausts the metabolic capacity of the liver, as, for example, in fat-rich diets, triglycerides accumulate in the liver cells and may possibly be excreted in the bile. Free fatty acids also stimulate mucin hypersecretion in the gallbladder (12). This mucosal hypersecretion has been assigned a significant role in the formation of gallbladder stones (8).

It is suggested that HDL-C might be a protective factor against gallstone formation in gallbladder (5). HDL-C has been reported to be negatively associated with gallbladder disease or the presence of gallstones (16). Also, Carel found the highest gallstone risk at low HDL-C levels and high TG levels (18). This study confirms results of earlier studies of serum TG (7,15,18) and HDL-C (5,18) levels in gallstone patients.

Contradictory to some studies (17,15) and in accordance with others (5,7), we observed higher concentrations of TC and LDL-C in gallstone patients than did control subjects, although the difference for TC was not statistically significant.

It was reported that serum apolipoproteins might be more sensitive parameters than serum lipids in distinguishing patients with stones from those without stones (5, 19). Similar to these findings we have found increased concentrations of serum Apo-A, Apo-B, LDL-C, TC, and TG in gallstone patients.

Data on the association between cholelithiasis and diabetes often are controversial and are mostly based on autopsies or on hospital series. However De Santis et al (2) reported that an altered glucose metabolism may increase the risk of developing cholelithiasis in certain subjects. Gebhard et al (6) reported that obese persons were at risk for cholesterol gallstones because their bile is saturated with cholesterol. Nevertheless, neither diabetic nor obese patients were included in our study to get the accurate results.

In conclusion, the results of this study may indicate that a disturbance in the lipid and lipoprotein metabolism may have a role in gallstone formation.

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IMPROVEMENT OF SKIN SYMPTOMS AND MINERAL IMBALANCE BY DRINKING DEEP SEA WATER IN PATIENTS WITH ATOPIC ECZEMA/DERMATITIS SYNDROME (AEDS)

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Summary: Deep sea water intake improves skin symptoms and mineral imbalance and decreases serum IgE levels and IgE-inducing cytokines, IL-4, IL-13 and IL-18 in patients with atopic eczema/dermatitis syndrome (AEDS), while distilled water intake fails to do so.

Key words: Atopics eczema/dermatitis syndrome; Deep sea water; Hair analysis; IgE; Magnesium

Introduction

Magnesium (Mg) is involved in the regulation of immune responses. It has been reported that in Mg deficient rats, serum levels of substance P, histamine, interleukin-1 (IL-1), IL-6 and tumor necrosis factor-alpha were elevated (5). Mg deficiency also caused atopic eczema/dermatitis syndrome (AEDS)-like symptoms in hairless rats (3).

We have previously reported that drinking of refined deep sea water that contains Mg as its main cation significantly reduces whole blood flow time and blood pressure in healthy volunteers (4). We have now studied the effect of drinking deep sea water on patients with AEDS.

Materials and method

Deep sea water, Amami no Mizu Hardness 1000 (Ako Kasei Co. Ltd.), obtained at 344 m depth and refined, contained Mg as well as other minerals. 500 ml of it contained Mg (100.0 mg), Na (37.0 mg), Ca (35.5 mg), and K (34.5 mg) and other trace minerals (Zn: 2.0 µg, Cu: 2.2 µg, I: 4.5 µg, P: 4.5 µg, Se: 0.2 µg). After obtaining the informed consent, 8 patients with AEDS (range: 21–52 years, mean age: 36 years), drank 500 ml/day of deep sea water (Amami no Mizu Hardness 1000). As control group, 10 patients with AEDS (range: 20–53 years, mean age: 36 years) drank 500 ml/day of distilled water, which did not contain minerals. At baseline and after 1 year, skin symptoms were assessed by skin scoring system as previously reported (1). Briefly, assessment was done on a scale of 0 to 2 in ascending order of severity with respect to inflammation. These signs

were assessed on 15 areas of the body (face, scalp, neck, right arm, left arm, right hand, left hand, trunk, back, pelvis, buttocks, right leg, left leg, tight foot and left foot). The maximum possible score was 30. Hair minerals were analyzed by inductively coupled plasma mass spectrometry. Simultaneously, serum levels of anti-mite IgE, anti-Japanese cedar pollen IgE, IL-4, IL-13 and IL-18 were measured by ELISA (2). Patients were asked to maintain their skin cares with washing by povidone iodine followed with applying azulene ointment. Since Mg, K and Ca interact mutually, the results of them were shown as Mg/K and Ca/Mg ratios, while the levels of Al, Mg, Pb were shown as absolute values (PPM).

Results

As shown in Table 1, after 1 year, skin symptoms by skin score were significantly improved in deep sea water group, while they were unchanged in control group.

Hair analysis revealed elevated levels of Mg/K and Ca/Mg ratios, Al, Hg and Pb levels at baseline in both groups of patients. However, in deep sea group they returned to normal levels, while they were unchanged in control group. In both groups, serum levels of Mg/K and Ca/Mg ratios were within normal range at baseline, and they were unchanged after 1 year (data not shown). Moreover, serum levels of anti-*Dermatophagoides pteronyssinus* and anti-Japanese cedar pollen IgE, and IgE-inducing cytokines IL-4, IL-13 and IL-18 were decreased significantly in deep water sea group, while they were not decreased in control group.

Tab. 1: Effect of drinking deep sea water in patients with AEDS.

	Deep sea water (n 8)			Distilled water (n 10)		
	Baseline	1 year	P	Baseline	1 year	P
Skin score	15.0±0.5	6.1±0.2	<0.01	14.6±0.6	14.8±0.7	NS
Hair						
Mg/K ratio	11.5±1.6	2.3±0.4	<0.01	11.1±1.9	10.9±1.8	NS
Ca/Mg ratio	19.4±0.8	13.7±0.7	<0.01	18.7±0.7	19.3±0.6	NS
Al (PPM)	10.7±1.1	6.9±0.7	<0.01	10.2±1.2	10.4±1.0	NS
Hg (PPM)	2.8±0.2	1.2±0.1	<0.01	2.6±0.2	2.5±0.2	NS
Pb (PPM)	3.5±0.2	1.1±0.1	<0.01	3.4±0.2	3.5±0.2	NS
Serum						
Anti-mite IgE*	71.8±5.6	42.8±5.3	<0.01	70.7±5.8	72.9±6.5	NS
Anti-JCP IgE**	76.3±4.2	32.5±3.9	<0.01	73.5±5.1	75.5±6.2	NS
IL-4 (pg/ml)	19.3±0.1	7.7±0.1	<0.01	18.2±0.1	18.5±0.1	NS
IL-13 (pg/ml)	9.3±0.1	5.1±0.1	<0.01	10.5±0.1	10.9±0.1	NS
IL-18 (pg/ml)	8.2±0.1	5.2±0.1	<0.01	8.5±0.1	9.0±0.1	NS

Values are the mean + SEM.

*Anti-*Dermatophagoides pteronyssinus* specific IgE (UA/ml)

**Anti-Japanese cedar pollen specific IgE (UA/ml)

Discussion

Although number is small, these results suggest that deep sea water intake may improve skin symptoms and mineral imbalance and decrease IgE production and IgE-inducing cytokines, IL-4, IL-13 and IL-18. Large scale of study which has been currently in progress is needed.

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