

### CSF LEVELS OF TAU PROTEIN AND BETA AMYLOID IN DIAGNOSIS OF ALZHEIMER'S DISEASE

C. Andrys<sup>1</sup>, R. Taláb<sup>2</sup>, M. Vališ<sup>2</sup>

<sup>1</sup>Department of Clinical Immunology and Allergology Charles University, Hradec Králové, <sup>2</sup> Department of Neurology, Charles University, Hradec Králové, e-mail: andrys@lfhk.cuni.cz

About two thirds of cases of senile dementia are due to Alzheimer's disease (AD). Definite diagnosis of AD is based on the neuropathological changes in the brain characterized by the presence of senile amyloid plaques, neurofibrillary tangles and neuronal cell loss. Sensitive assays showed the presence of tau and beta amyloid in CSF and change in levels of these molecules have been suggested to be a possible markers of AD.

Cerebrospinal fluid samples from total of 102 subjects consisting of 24 patients with AD and 78 control patients with other neurological diseases were examined by ELISA of tau protein and beta amyloid (Innogenetics, Belgium).

As compared to the control group, the concentrations of tau protein were significantly higher in AD patients ( $p < 0.0001$ ) in contrast to levels of beta amyloid, where are significantly decreased ( $p < 0.0001$ ). ROC (Receiver Operating Characteristic) analysis was performed to define cut-off values for maximized sensitivity and specificity (tau protein 219 pg/ml, sensitivity 70.8 %, specificity 74.4 % and beta amyloid 900 pg/ml, sensitivity 75 %, specificity 83 %). Cerebrospinal fluid analysis showing low levels of beta amyloid and high levels of tau protein come closest to fulfilling criteria for a useful biomarker for Alzheimer's disease.

#### Key words:

Alzheimer's disease, cerebrospinal fluid, tau protein, beta amyloid, ROC.

### THE EFFECT OF WHOLE-BODY AND LOCAL HIGH-FREQUENCY ELECTROMAGNETIC FIELD EXPOSURE ON THE BRAIN ELECTROGENY

J. Barcal, J. Cendelín, F. Vožeh, V. Žalud

Department of Pathophysiology, Charles University, Medical Faculty in Pilsen, Czech Republic, e-mail: jan.barcal@lfp.cuni.cz

A direct registration of brain cortical and hippocampal activity during an exposure to high-frequency electromagnetic field (HF EMF) and a depiction of possible changes in the hippocampal rhythmicity were performed. All experimental procedures were done in anesthetised adult Lurcher mutant mice, wild-type (healthy animals) were used as controls. This mutation represents a natural model of genetically determined olivocerebellar degeneration. Experimental animals were exposed to HF EMF with frequency of 870 MHz. Our method is based on use of gel electrodes (silicon and glass tubes filled with agar) and a connection with platinum electrodes is located out of HF EMF space. Spontaneous electrocorticogram (ECoG) was measured as 2 min segments from continuously recorded activity either without HF EMF or with it. Final calculation with Fourier analysis and averaging were performed off-line on DISYS-system (Software for data acquisition and analysis). The hippocampal activity was recorded by glass capillary filled with agar (used as a registration electrode), grounding electrode was silicon tube filled with agar. Stereotactic coordinates - ref. point bregma: 2.0; 1.5; 1.3 (CA1) - 2.0 (hilus DG). DISYS-system for a final averaging according to frequency spectra with emphasis on a theta-oscillation has been used. ECoG evaluation showed a distinct shift to lower frequency components but significant effect only in wild - type (healthy littermates) has been observed whereas in Lurcher mutant mice only gentle differences between frequency spectra were found. On the other hand, changes of the hippocampal activity to higher frequencies in both types of animals (healthy and mutant) were observed as well as changes of theta rhythmicity. Our findings suggest that HF EMF (corresponding to cellular phone frequency) is not without effect on the electrical brain activity. There were no significant differences between local and whole-body HF EMF exposure.

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### NEUROVEGETATIVE RESPONSES AND RESUSCITATION EFFECTS OF TRIGEMINAL AND GLOSSOPHARYNGEAL REFLEXES

R. Beňačka<sup>1</sup>, Z. Tomori<sup>2</sup>

<sup>1</sup>Department of Pathophysiology, <sup>2</sup>Department of Physiology, Medical Faculty, P. J. Šafarik University, Košice, Slovakia, e-mail: roman@central.medic.upjs.sk

Electrocortical power spectra recordings (EcoG) as well as brainstem auditory- and sensory-evoked potentials (BAEPs and SEPs) from cortical areas were used to study the effects of reflex stimulations in the epipharynx (EPS), and infraorbital-nasolabial trigeminal areas (NPS) in recovery from apnoea in 15 pentobarbitone anaesthetized nonparalyzed cats under severe hypoxia. Changes in heart rate (HR), systemic blood pressure (BP), tracheal air-flows and ABS were recorded in comparison. EPS applied within 90 sec of apnoea (pH 6.9 -7.3; P<sub>a</sub>O<sub>2</sub> 9-13 mmHg, P<sub>a</sub>CO<sub>2</sub> 54 - 82 mmHg) lead to short-latency (3-5 s) increase in both BP (2-3x) and HR (4x) with concurrent renewal of missing pontine BAEPs (P2-P4) and near field SEPs (III-V) components. Their amplitudes and latencies returned to control level 60-120 s together with far field thalamo-cortical SEPs. Suppression in ECoG was reversed gradually from 20-30 s after EPS as indicated by 3-4 fold increase of mean spectral power and shift of median frequency from 2-3 Hz to 5-8 Hz). Rhythmic breathing reappeared spontaneously 40-60 s after EPS. NPS could reactivate cardiovascular, respiratory and brain activity (BAEPs, persistent P1, N1; near-field SEPs, wave I and II) even 120 -150 s after the onset of apnoea (87 % of cases). Increases in HR and BP (both-2-4-times) showed independent onsets and dynamics. Respiration renewed by series of 4-10 gasp-like bursts (216±12 ms) resuming the normal rhythm. When BP >30-40 mmHg repetitive reflexes could preserve automatic breathing even after cortical death.

We conclude that reflex cardio-respiratory recovery during apnoea is achieved by widespread brain arousal and profound sympathetic overexcitation and reperfusion effect within functionally corresponding brainstem structures.

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### DIVING REFLEX – SENSITIVE REGIONS AND EXERCISE - DEPENDENT EFFECTS

R. Beňačka, P. Pobeha, V. Ratvaj

Department of Pathophysiology, P. J. Šafarik University Medical Faculty, Košice, Slovakia, e-mail: roman@central.medic.upjs.sk

Sudden cold-water facial immersion with concomitant breath-holding evokes a set of reflexes called trigeminal diving reflex (DR) comprising laryngeal closure, bradycardia, peripheral vasoconstriction and adaptive metabolic changes lowering tissue expenditure of oxygen. The study was designed to find the most effective responsive sites of DR and to test whether DR can be effective in reversal of exertional tachycardia.

Changes in heart rate (HR) and arterial blood pressures (BP) evoked by 20-60 s long cold-wet stimuli (CW, water or gel-filled plastic applicator; < 10°C) to orbital (OA) and frontal (FA) trigeminal areas, respectively, were evaluated in 16 volunteers (17- 42y.) during the rest conditions with (n=4; CW+A) and without (n=12; CW-A) breath-holding (A, apnoea). Effects of upper facial CW stimuli (OA+FA) were examined during sustained tachycardic-hypertensive response modelled by continuous exercise on bicycle ergometer (HR ≥150 b.min<sup>-1</sup>, mean BP >140 mmHg). CW-A stimulation produced immediate fall in HR both in OA (n=9/11, Δ10,5 %; in 4 cases significant - Δ 19,4 %; Δ15,33±3,28 c.min<sup>-1</sup>) and FA (n=6/11; Δ13,6 %). Bradycardia was more regular and stronger in OA than in FA, which, however was lasting longer (up to 60 s). BP changed only inconsistently. In CW+A regiment stimuli evoked slightly stronger bradycardic response in FA compared to OA (Δ15±4 % vs. Δ13±5 %, M±SD) and both effects were significantly higher than those from lower face (9-11 %). CW stimuli to OA+FA resulted in immediate (< 20 s) reversal of exertional tachycardia (Δ14,05±3,4 c.min<sup>-1</sup>, Δ 9,3 %). With little changes in BP, fall in HR was further strengthened over 60-80 s of exercise (Δ 19,2±9,95 c.min<sup>-1</sup>, Δ12,2 %). In conclusion, the strongest bradycardic effects of DR is achieved from FA and OA. This may be further reinforced or modified by apnoea. DR can efficiently reverse sustained tachycardia and might be simple and effective method for treatment of paroxysmal tachycardic dysrhythmias.

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### **PATHOPHYSIOLOGY IN CURRICULUM OF SCHOOL OF MEDICINE, COMENIUS UNIVERSITY, BRATISLAVA. SOME RESULTS OF SURVEY BETWEEN GRADUATES 2000-2003**

M. Bernadič<sup>1</sup>, M. Benedeková<sup>2</sup>, E. Kukurová<sup>3</sup>, P. Traubner<sup>4</sup>, I. Hulín<sup>1</sup>  
<sup>1</sup>Department of Pathophysiology, <sup>2</sup>The 1st Department of Paediatrics,  
<sup>3</sup>Department of Biophysics, <sup>4</sup>The 1st Department of Neurology, School  
of Medicine, Comenius University, Bratislava, Slovakia, e-mail:  
marian.bernadac@fmed.uniba.sk

In order to obtain information how were the tasks in education and teaching processes realized a questionnaire investigation is performed from 2000 to 2003 every year. The questionnaire oriented to study and possibilities to find employment after graduation was sent to all graduates. Statistical analysis of data led to conclusions: The admission examinations to School of Medicine were objective (more than 90 % students). Most of admitted students have already chosen the discipline where they would like to work (58 %), after gaining experience during teaching process (lectures, seminars, practical lessons etc.) 16 % of students had reserved working place during the study, 41 % at the graduation term. 67 % working places were in accord with those chosen by students. Interesting are the results of questions regarding the content and level of education a teaching process and the objectivity of knowledge evaluation. 30 % of graduates estimated the level of teaching process as high, 56 % as medium and 16 % as low. Students studied a part of study at foreign universities evaluated the level of education process highest. The method of knowledge evaluation was estimated as correct by 35 % of graduates and the objectivity of examinations was considered low in 39 %. An example of a true medicine doctor was found by 73 % of students during the study. The most useful disciplines for practical medicine are according the responders: internal medicine (86 %), surgery (43 %), pathophysiology (34 %) and pharmacology (31 %). The less useful disciplines are social medicine (57 %), biophysics (52 %), preventive medicine (37 %), chemistry and biochemistry (12 %). Questionnaire investigation as a project will continue in future. We are preparing the project of anonymous investigation of teaching by means of questionnaire with students during the study of medicine. The aim is the actualisation and modernisation of practical and theoretical approach to gathering of ration knowledge and preparation of teaching program transformation in accord with ECTS.

### **SELECTED CYTOGENETIC AND IMMUNOLOGICAL CHANGES AFTER DERMAL EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS (PAH)**

L. Borska<sup>1</sup>, Z. Fiala<sup>2</sup>, K. Hamakova<sup>3</sup>, C. Andrys<sup>4</sup>, J. Krejsek<sup>4</sup>, D. Vokurkova<sup>4</sup>, J. Smejkalova<sup>2</sup>, J. Kremlacek<sup>1</sup>  
<sup>1</sup>Institute of Pathological Physiology, Charles University, Medical Faculty, <sup>2</sup>Institute of Hygiene and Preventive Medicine, Charles University, Medical Faculty, <sup>3</sup>Clinic of Dermal and Venereal Diseases, University Hospital, <sup>4</sup>Institute of Clinical Immunology and Allergology, Charles University, Medical Faculty, Hradec Králové, Czech Republic, e-mail: borka@lfhk.cuni.cz

Selected cytogenetic and immunological changes after dermal exposure to PAH were observed.

Patients with psoriasis cured by therapeutic coal tar ointments created observed group (coal tar ointments contain PAH). Blood samples were collected from patients before the therapy and after last application. The levels of chromosomal aberrations in peripheral lymphocytes (CA) and immunological parameters (IgM, IgA, IgE, IgG, C3 complement, Neopterin, Beta-2-microglobulin, Alpha-2-macroglobulin, Orosomucoid, Prealbumin, Haptoglobin, Transferrin, CD3, CD4, CD8, CD4+/CD8+, HLA-DR+, CD3+/HLA-DR+, CD3+/HLA-DR+ of CD3+, CD8+/HLA-DR+, CD8+/HLA-DR+ of CD8+, CD4+/CD45RA+, CD4+/CD45RA+ of CD4+, CD4+/CD45RO+, CD4+/CD45RO+ of CD4+) were determined by standard methods. PASI scores (Psoriasis Area and Severity Index) evaluated the objective therapy effect.

The statistic analysis showed significant elevation of CA after the therapy ( $p < 0,001$ ). The levels of IgM ( $p < 0,05$ ), beta-2-microglobulin ( $p < 0,05$ ), alpha-2-macroglobulin ( $p < 0,01$ ) and transferrin ( $p < 0,01$ ) decreased significantly after the therapy. The level of PASI scores decreased as much as 89 %.

Some results indicate that the psoriatic patients, treated by therapeutic coal tar ointment could be endangered by increased health risk. Nevertheless, the problem needs further research.

### **REPEATED NASAL ALLERGEN CHALLENGES IN SENSITIZED GUINEA PIGS AND COUGH RESPONSE**

M. Brozmanová<sup>1</sup>, V. Čalkovský<sup>2</sup>, J. Plevková<sup>1</sup>, M. Tatár<sup>1</sup>  
<sup>1</sup>Department of Pathophysiology and <sup>2</sup>Clinic of Otorhinolaryngology,  
Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia,  
e-mail: brozmanova@jfm.uniba.sk

Asthma and allergic rhinitis are the most frequent causes of the chronic cough. The sensitization of the afferent nerve-endings due to microaspiration of nasal secretion through an action of inflammatory mediators and the facilitation of the central cough generator from nasal reflex input are still discussed mechanisms. The aim of this study was to evaluate the cough sensitivity during a period of an early and late allergic response in sensitized and challenged guinea pigs. 45 Trik strain guinea pigs weighing 250-350g were used and divided into two separate experiments. All animals were passively sensitized with OA (1). Four weeks later to develop a model of allergic rhinitis 0.5 % OA (0.015ml) was instilled into both nostrils of animals. Sensitized animals were repeatedly intranasally challenged by OA (experimental group) or saline (control group) in 7-day intervals for nine weeks. Nine experimental animals were inhaling aerosol of beclomethasone dipropionate (350 µg for 3 min) seven days between the eight and the ninth OA challenges. Cough was induced by the inhalation of citric acid aerosols of gradually increased concentration (from 0.05 to 1.6 M) for 30 sec. Cough was evaluated in the first experimental (n=15) and control (n=11) group 1 hr after the 6<sup>th</sup> nasal challenge (NCH), 17 hrs after the 9<sup>th</sup> NCH and 1 hr after the 10<sup>th</sup> NCH in animals treated with corticosteroids; in the second group of experimental (n=9) and control (n=10) animals 3 hrs after the 6<sup>th</sup> NCH and 24 hrs after the 9<sup>th</sup> NCH. The intensity of cough was significantly increased 1 hr [18(14-23) vs 8(3-10);  $p = 0.0002$ ] and 3 hrs [11(10-18) vs 5(3-8);  $p = 0.008$ ] after repeated NCH comparing to controls. Cough responses evoked 17 and 24 hrs after repeated NCH were not significantly different. *Conclusion:* Enhanced cough sensitivity only corresponds with early allergic response after repeated nasal challenges. The inhaled corticosteroids therapy blocked the stimulating effect of experimental allergic rhinitis on the chemically - induced cough in awake guinea pigs.

1. Underwood et al.: Eur Respir J 1995, 8: 2104-2113

### **CIRCATRIGINTAN CYCLE OF SALIVARY TESTOSTERONE IN HUMAN MALE**

P. Celec<sup>1,2</sup>, D. Ostatníková<sup>3</sup>, Z. Putz<sup>4</sup>, J. Hodosy<sup>1,5</sup>, P. Burský<sup>6</sup>, L. Stárka<sup>7</sup>, R. Hamp<sup>7</sup>, M. Kúdela<sup>8</sup>  
<sup>1</sup>Institute of Pathophysiology, Comenius University, Bratislava, Slovakia, <sup>2</sup>Department of Molecular Biology, Comenius University, Bratislava, Slovakia, <sup>3</sup>Institute of Physiology, Comenius University, Bratislava, Slovakia, <sup>4</sup>Institute of Endocrinology, Lubochňa, Slovakia, <sup>5</sup>Department of Animal Physiology and Ethology, Comenius University, Bratislava, Slovakia, <sup>6</sup>Faculty of Technical Engineering and Informatics, Slovak Technical University, Bratislava, Slovakia, <sup>7</sup>Institute of Endocrinology, Praha, Czech Republic, <sup>8</sup>Department of Zoology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia

Circadian and circannual cycles of testosterone (T) in men are known. The results of our previous study have shown indices for the existence of a male circalunar cycle of T similar to the female one.

The purpose of this study was to investigate possible infradian rhythms of salivary T in young healthy men.

Saliva samples from 31 healthy men (18–25 years of age) were collected every second day (for 1 month) and every third day (during the following 1.5 months). T levels were determined by radioimmunoassay. Data were statistically analyzed for infradian rhythmic changes using two different methods: moving averages – zones of extremes, and analysis of rhythmic variance (ANORVA).

The data showed rhythmic variations with 2 different infradian periods. The differences between extreme values of the circatrigrantan (period of 30 days) and circavigintan (period of 20 days) cycles were found to be highly significant ( $p < 1E-9$ ;  $p < 1E-8$ ).

We believe that this is the first study demonstrating the existence of circatrigrantan and circavigintan rhythms of testosterone in human males. Our findings might have implications in human physiology and pathophysiology.

Keywords: Salivary testosterone, circalunar cycle, biorhythms, chronobiology, steroid, hormones, infradian variations.

### MODERNIZATION OF PRACTICAL EXPERIMENTAL EDUCATION IN PATHOPHYSIOLOGY

J. Cendelin, F. Vožeh, J. Barcal, I. Korelusová V. Markvartová, P. Sobotka, V. Štenglová, F. Vožeh, J. Záhava, V. Žalud  
*Department of Pathophysiology, Faculty of Medicine in Pilsen, Charles University, Pilsen, e-mail: jan.cendelin@lfp.cuni.cz*

Education in pathophysiology requires experimental demonstration of pathophysiological mechanisms. For such experiments a suitable laboratory equipment is necessary. In our workplace we modernized laboratories which are used for practical education as well as for research – microscopic laboratory, laboratory for behavioural experiments in animals and laboratory for recording of various physiological parameters in both humans and animals. Most experiments are performed using Lurcher mutant mice, which represent a natural model of genetically determined olivocerebellar degeneration known also in human pathology. Microscopic laboratory enables to demonstrate various neurohistological methods and to visualize functional-morphological changes in the brain. Students can interpret these changes in the neurodefective mutants and after pharmacological influencing or after behavioral experiments (learning). Experimental system EthoVision automatically observes behavior of laboratory animals. The device records trajectory, speed of movements and position of animals during the experiment and stores the data for off-line analysis. The method is absolutely objective, what is necessary for statistical evaluation. EthoVision is used for demonstration of behavioral consequences of the neurodegeneration and pharmacological influencing it, e.g. in the open field or Morris water maze. The PowerLab system is an education set, with high variability of measurement of biological functions by means of direct electrophysiological methods (ECG, EEG, EMG) and also other important nonbioelectrical parameters (blood pressure, temperature, spirometry, pulse). It can demonstrate their changes during physiological and pathological states and conditions. All these methods use personal computers with sophisticated software and can be presented with data projection for large group of students.

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### THE ROLE OF MOTOR AND COGNITIVE ABILITIES IN SPATIAL LEARNING TASK IN LURCHER MUTANT MICE

J. Cendelin, F. Vožeh  
*Department of Pathophysiology, Faculty of Medicine in Pilsen, Charles University, Plzeň, e-mail: jan.cendelin@lfp.cuni.cz*

Lurcher mutant mice represent a natural model of genetically determined olivocerebellar degeneration. They suffer from cerebellar ataxia and deterioration of cognitive functions. They show worse performance in the Morris water maze as compared with their healthy wild type littermates. The aim of our work was to assess the role of motor coordination disorder and of cognitive deficit in spatial learning task in Lurcher mutant mice derived from two strains. Young adult wild type and Lurcher mutant mice of the C3H and C57Bl/7 strains were tested in the Morris water maze. For first 5 days the platform was visible and its position changed from trial to trial. For next 5 days the platform was hidden under the water and the localization was stable. Platform reaching latencies were measured. Latencies of Lurchers were longer than in wild type mice at the beginning of the experiment. In mice of the C57Bl/7 strain the latencies shortened during the training with visible platform and by the 5th day Lurchers reached almost the same results as wild type. After hiding the platform the latencies increased slightly in wild type, significantly in Lurcher mutant mice. Wild type mice needed short training with hidden platform to reach the latencies as in the phase with the visible platform. Lurchers learned too, but they never reached results of wild type. Latencies of both Lurcher mutant and wild type C3H mice increased more after hiding of the platform and were longer than in C57Bl/7 mice for next course of the experiment. The experiments showed that after training Lurcher mutant mice are able to reach visible platform as well as wild type. Their worse results in the following task with the invisible platform are caused by a cognitive deficit and can not be attributed only to the motor disorder. Worse performance of both Lurcher mutant and wild type C3H mice indicates lower spatial learning ability.

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### EXPRESSION OF HEAT SHOCK PROTEINS IN HEPG2 CELLS UNDER THE INFLUENCE OF DIFFERENT STRESS FACTORS

K. Červenková, M. Rypka, L. Uherková, H. Poczatková, R. Pucek, D. Riegrová  
*Department of Pathological Physiology, Medical Faculty, Palacký University, Olomouc, e-mail: katerinacervenkova@seznam.cz*

It is well known that heat shock, UV radiation, heavy metals, hypoxia, hypoglycaemia, and other stress factors increase expression of heat shock proteins (Hsp) in cells. Moreover, these proteins have been implicated in pathogenesis of major civilization disorders (1).

We have focused on expression of individual Hsp protein family members such as Hsp47, Hsp60, Hsp70, Grp78, Hsp90, and Grp94 and on their changes induced by different factors. Using an HepG2 cell model, we tested the following inducing factors: a) heat shock; b) lack of glutamine; c) abundance of free fatty acids in an incubation medium. Experimental data have been obtained based on electrophoresis, immunoblotting, and RT(Real-Time)-PCR methods.

The experiments performed thus far have revealed changes in Hsp60, Hsp70, Hsp90, and Grp94 at protein and/or mRNA levels. The work features in a broader study dealing with mechanisms of polyfactorial diseases.

1. Csermely P.: Trends Genet. 17:701-704, 2001.

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### GLYCATED HAEMOGLOBIN – COMPARISON OF ACTUAL RESULTS WITH THOSE CALCULATED ACCORDING TO THE MATHEMATICAL MODEL OF GLYCATION

P. Dombrovský, O. Rác  
*Institute of Pathological Physiology, Medical School, Šafárik University, Košice, Slovakia, e-mail: patfyz@central.medic.upjs.sk*

The mathematical model of haemoglobin glycation makes calculation of glycated haemoglobin (GHb) concentration from blood glucose values possible. In this study we analysed the agreement between calculated and measured values of 373 results from 282 patients with diabetes mellitus.

The values were sorted into 3 classes. In the first class there was agreement between calculated and measured results (16,9 % of all results). The actual value of GHb was lower than the calculated one in almost two thirds (62,2 %) of results. Higher measured GHb than calculated was in 20,9 % of results.

The course of regression line calculated from linear regression analysis between GHb and blood glucose values was in agreement with the model only in the first class of results. In both classes where there was no agreement between calculated and measured GHb values the course of line was considerably flatter than expected. Agreement was not achieved by changing the values of constants applied in the equation either.

According to the analysis the value of GHb is at relatively low values of blood glucose higher than expected from the model. At very high values of blood glucose the opposite is true. This „seesaw“ effect can be explained by the fact that blood glucose values do not reflect exactly the glycaemic compensation. The results confirm the view that assessment of compensation is possible only through complex view on both short and long term parameters of glycaemic control.

### EXCRETION OF SELECTED URINARY METABOLITES AFTER DERMAL EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS (PAH)

Z. Fiala<sup>1</sup>, L. Borska<sup>1</sup>, K. Hamakova<sup>2</sup>, V. Krajak<sup>3</sup>

<sup>1</sup>Charles University Medical Faculty Hradec Kralove, <sup>2</sup>University Hospital Hradec Kralove, <sup>3</sup>Health Institute Pardubice, Czech Republic, e-mail: fiala@lfhk.cuni.cz

Excretion of selected urinary metabolites after dermal exposure to PAH. Patients with psoriasis cured by therapeutic coal tar ointments created observed group. Concentrations of 15 selected PAH were determined in therapeutic coal tar ointments by gas chromatography/mass spectrometry (GC/MS) after extraction by n-hexane. Urine samples were collected before the therapy, after first application of ointment, in the middle, before last application and after last application. At selected patients full amount of urine was collected over the time of first application. Urinary levels of 1-hydroxypyrene, 1-,2-,3-, and 4-hydroxyphenanthrene were analyzed by GC/MS after extraction (solid phase extraction - SPE) and derivatization. In parallel, urinary levels of creatinine were analyzed by spectrophotometric method.

The ointments contain 5 % of coal tar and the concentrations of total PAH in coal tar samples varied from 29 to 36 %. The range of ointment applications varied from 30 to 60 % of patient's body surface with application time of 12 - 25 hours. The results of urinary metabolite measurements (ranges of values) are: 1-hydroxypyrene (0.18 - 32.71 µg/g creatinine), 1-hydroxyphenanthrene (0.18 - 19.45 µg/g creatinine), 2-hydroxyphenanthrene (0.09 - 13.88 µg/g creatinine), 3-hydroxyphenanthrene (0.18 - 24.75 µg/g creatinine), 4-hydroxyphenanthrene (0.09 - 1.50 µg/g creatinine).

The excretion characteristics of selected metabolites varied markedly in dependence with the area and time of application. These findings indicate that the determination of described metabolites could be useful method for exposure assessment (exposure control) of given therapy. Nevertheless, the problem needs further research.

### THE ONSET OF PHYSIOLOGICAL GASTROINTESTINAL ACTIVITY IN THE POSTOPERATIVE PERIOD-COMPARATIVE STUDY WITH PROKINETIC DRUG GANATON®

R. Fraško (1), P. Maruna (2), R. Gürlich (1)

<sup>1</sup>Ist. Surgical department of 1st Faculty of Medicine, Prague, <sup>2</sup> Dept of Pathophysiology, 1st. Faculty of Medicine, Prague, e-mail: rfras@lf1.cuni.cz

Postoperative gastroparesis lowers patient's comfort and stalls beginning of realimentation. In presented study authors tried to evaluate effect of prokinetic drug Ganaton® (itoprid hydrochlorid) used in perioperative time by patients undergoing laparoscopic intervention. This prospective, non-randomized study involved 20 patients before projected laparoscopic cholecystectomy and next 20 patients before laparoscopic non-adjustable gastric banding. 24 patients with the same surgical procedure using no prokinetic drug created control groups. Gastrointestinal activity has been observed by evaluation of patient's sensations, using auscultation and method of percutaneous electrogastrography one day before operation, then three hours after the surgical intervention and next two days more.

Most common finding during postoperative period on the electrogastrography pattern was bradygastria, sometimes associated with nausea and vomiting. No real postoperative gastroparesis has been observed.

Return to physiologic electrogastrography patterns was quicker in both groups of patients using prokinetic drug. We conclude that Itoprid hydrochlorid used during perioperative period accelerate return of physiologic gastrointestinal activity.

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### ANGIOTENSINOGEN GENE POLYMORPHISMS IN CHRONIC HEART FAILURE AND TRIPLE-VESSEL DISEASE

M. Goldbergová<sup>1</sup>, L. Špinarová<sup>2</sup>, J. Špinar<sup>3</sup>, J. Vitovec<sup>2</sup>, A. Vašků<sup>1</sup>  
<sup>1</sup>Institute of Pathological Physiology, Faculty of Medicine, Masaryk University Brno, Czech Republic, <sup>2</sup>I. Department of Internal Medicine, St. Ann's Faculty Hospital, Masaryk University Brno, Czech Republic, <sup>3</sup>Department of Internal Cardiology, Faculty Hospital Brno, Masaryk University Brno, Czech Republic, e-mail: goldberg@med.muni.cz

The RAS has been proved to be an important regulator of cardiovascular and renal structure and function. The association of genetic variants of angiotensinogen gene promoter polymorphism (-6G/A) and polymorphism M235T in coding sequence with chronic heart failure and triple-vessel disease (TVD) in the Czech population was investigated. A total of 240 patients with chronic heart failure, 196 patients with TVD and control group of 204 subjects of similar age and sex distribution without any personal history of cardiovascular diseases, were included in the study.

We detected no differences in genotype distributions and/or allele frequencies of AGT M235T polymorphism within chronic heart failure and triple-vessel disease in case-control design, and between chronic heart failure and TVD patients. Also no association of AGT (-6G/A) polymorphism to chronic heart failure and TVD in case-control design was found. Significant association observed was between chronic heart failure and TVD patients (Pg=0.001), with higher prevalence of AG genotype within the latter group (OR=1.97; P=0.0004). A significant difference was also observed between TVD patients and subgroup of chronic heart failure patients with main diagnosis of ischemic heart disease (P=0.007), with the same trend of higher AG genotype prevalence (OR=2.08; P=0.001).

The results obtained in this study provide the evidence of an association of AGT gene promoter polymorphism with chronic heart failure and triple-vessel disease, with distribution differences of genetic variants between both diagnoses.

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### PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS GENE POLYMORPHISMS IN CHRONIC HEART FAILURE AND TRIPLE-VESSEL DISEASE

M. Goldbergová<sup>1</sup>, L. Špinarová<sup>2</sup>, J. Špinar<sup>3</sup>, J. Vitovec<sup>2</sup>, A. Vašků<sup>1</sup>  
<sup>1</sup>Institute of Pathological Physiology, Faculty of Medicine, Masaryk University Brno, Czech Republic, <sup>2</sup>I. Department of Internal Medicine, St. Ann's Faculty Hospital, Masaryk University Brno, Czech Republic, <sup>3</sup>Department of Internal Cardiology, Faculty Hospital Brno, Masaryk University Brno, Czech Republic, e-mail: goldberg@med.muni.cz

Peroxisome proliferator-activated receptors (PPARs) belong to a superfamily of nuclear receptors. PPAR $\alpha$  and  $\gamma$  are both characterized by their ability to influence lipid metabolism, glucose homeostasis, cell proliferation, differentiation and apoptosis, as well as the inflammatory response, by transcriptional activation of target genes. The association of genetic variants of PPAR $\alpha$  gene polymorphism L162V and PPAR $\gamma$  gene polymorphism C161T, both in coding sequence, with chronic heart failure and triple-vessel disease (TVD) in the Czech population was investigated. A total of 240 patients with chronic heart failure, 196 patients with TVD and control group of 204 subjects of similar age and sex distribution without any personal history of cardiovascular diseases, were included in the study. No differences in genotype distributions and/or allele frequencies of PPAR $\alpha$  L162V polymorphism as well as of PPAR $\gamma$  C161T polymorphism within chronic heart failure and TVD in case-control design were observed. But, significant difference in PPAR $\alpha$  L162V polymorphism was found between TVD females and control females (Pg=0.04, Pa=0.008). Higher G allele frequency was observed within control females in comparison to control males (Pg=0.02, Pa=0.007). Significant differences in PPAR $\gamma$  C161T allelic frequencies were found between TVD males and chronic heart failure males (Pa=0.04), and within chronic heart failure females in comparison to chronic heart failure males (Pg=0.02, Pa=0.008).

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### CARDIOTOXICITY AND BAROREFLEX SENSITIVITY IN CHILDREN TREATED WITH ANTHRACYCLINE FOR LEUKAEMIA

H. Hrstková<sup>1</sup>, L. Elbl<sup>2</sup>, Z. Nováková<sup>3</sup>, E. Závodná<sup>3</sup>, B. Fišer<sup>3</sup>, N. Honzík<sup>3</sup>

<sup>1</sup>1<sup>st</sup> Clinic of Paediatric Internal Medicine and <sup>2</sup>Department of Internal Cardiology Medicine, Faculty Hospital in Brno, <sup>3</sup>Department of Physiology, Faculty of Medicine, Masaryk University in Brno, e-mail: znovak@med.muni.cz

Cardiotoxicity of anthracycline used for leukaemia treatment is well known. Recently we observed decrease of systolic (SBP) and diastolic pressure (DBP) after the anthracycline treatment, which is seen still after several years. The link between cardiotoxicity and the blood pressure regulation was examined in the present study.

Methods: Forty patients (9-25 years) after anthracycline treatment were examined. Ejection fraction (EF) was measured by echocardiography. Baroreflex sensitivity in ms/mmHg (BRS) and in Hz/mmHg (BRSf) was determined with spectral method (five minutes non-invasive continuous blood pressure recording by Finapres at metronome controlled breathing). Patients were divided in two groups of 20 subjects according to the magnitude of EF: group A with higher EF (EF between 70 % and 65 %) and group B with lower EF (EF between 65 % and 54 %). The values of SBP, DBP, BRS and BRSf were normalized according to age by linear regression and compared by Wilcoxon test.

Results: Following values normalized for age 15 years (mean±SD) were found: SBP (A:104.3±13.8; B:95.1±14.4 mmHg; p<0.05); DBP(A:60.2±7.7; B:55.2±9.4 mmHg; p=0.07); BRS (A:7.1±4.5; B:7.3±3.7 ms/mmHg; ns) and BRSf (A:0.0126±0.006; B: 0.0119±0.005 Hz/mmHg; ns).

Conclusion: Significant decrease of SBP in patients with low EF indicates a link between blood pressure decrease and anthracycline cardiotoxicity. Baroreflex sensitivity is not influenced.

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### GENDER-SPECIFIC ASSOCIATION OF NITRIC OXIDE SYNTHASE-3 (NOS3) GENE POLYMORPHISMS WITH ATOPIC DISEASES IN THE CZECH POPULATION

L. Izakovičová Hollá, A. Stejskalová, D. Bučková, M. Schüuller, V. Znojil, J. Vácha, A. Vašků

Department of Pathological Physiology, Medical Faculty, Masaryk University Brno, Czech Republic, e-mail: holla@med.muni.cz

Several studies suggest that abnormalities in nitric oxide (NO) synthesis may contribute to the development of pulmonary diseases. We have, therefore, performed a case-control study to investigate a plausible association between two promoter polymorphism in the NOS3 gene and respiratory allergic diseases.

This study included 901 subjects – 604 patients with clinically manifested allergy (asthma and/or rhinitis) and 297 unrelated controls. Screening for the -786C/T and -691 C/T polymorphisms in the promoter of the NOS3 gene was carried out by PCR-RFLP analyses.

Although no association was found regarding the NOS3 -691C/T polymorphism, marginally significant difference was observed in frequencies of the NOS3 -786C/T polymorphism between patients and controls (P=0.04). However, further analysis of this variant showed considerable effect of gender. The NOS3 -786 T allele (and TT genotype) were significantly underrepresented in male patients with asthma and/or rhinitis in comparison with control male subjects (35.9 versus 45.9 %, p=0.02, p<sub>corr</sub> < 0.05; odds ratio OR=0.73, 95 % CI: 0.55-0.98), while no differences were shown between female patients and controls.

We conclude that the presence of the T allele of the NOS3 -786 polymorphism could be a marker of decreased risk of developing respiratory allergy in male subjects.

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### ASSOCIATION OF FUNCTIONAL SINGLE NUCLEOTIDE POLYMORPHISMS IN MATRIX METALLOPROTEINASES (MMPS) GENES WITH COLORECTAL AND BREAST CANCER PROGRESSION

M. Jurajda, J. Vácha,

Department of Pathological Physiology, Faculty of Medicine, Masaryk University Brno, e-mail: mjuraj@med.muni.cz

The degradation of the microenvironment of cancer cells, composed of extracellular matrix (ECM), plays an important role in tumor progression and development of metastases. The most of this degradation is mediated by matrix metalloproteinases. The increased levels of MMPs activity in tumor tissue were reported. Functional promoter single nucleotide polymorphisms (SNP) in MMP-1 and MMP-3 genes increase their transcription levels and consequently their enzymatic activity. An insertion of G at -1607 bp in MMP-1 promoter creates an Ets transcription factor family binding site and thus increases MMP-1 transcription. A deletion of A at -1171 bp increases MMP-3 transcription. These polymorphisms probably play an important role in tumor progression and metastasis. Recently, several studies reported associations of these polymorphisms with breast, colorectal, lung and renal carcinomas. Thus we have genotyped 150 patients with colorectal carcinoma and 164 patients with breast cancer for above mentioned SNPs and analyzed allelic frequencies in subgroups with and without metastases. Our results show that there is a weak association between 5A allele and metastases development in colorectal cancer patients (p=0.04375). Since both MMP genes are located in chromosome 11 we have examined their linkage disequilibrium by chi-square test. 1G allele of MMP-1 promoter is closely linked with 5A allele of MMP-3 promoter (p<0,01). The results suggest the role of studied polymorphisms in metastases development is unequivocal.

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### VASCULAR FUNCTION - RISK FACTORS AND SUPPLEMENTATION

E. Kohlíková<sup>1</sup>, M. Petr<sup>1</sup>

<sup>1</sup>Department of Physiology, Charles University, Faculty of Physical Education and Sport, Prague, Czech Republic

Cardiovascular disease has a multifactorial aetiology that is influenced by both genetics and environmental factors (2,3,4,5). Independent risk factor for the development of cardiovascular disease is hyperhomocysteinemia (2,3), 30-50 % mild hyperhomocysteinemia has genetic aetiology (5,7). Exposure of endothelial cells to elevated levels of homocysteine [Hcy] results in decreased availability of nitric oxide [NO] and impaired vascular function, both of which are early events in atherogenesis. In patients with the genetic load and clinical manifestation of cardiovascular diseases [heart attack, ictus, reconstruction of the artery] increased the mean total homocysteine [tHcy] plasma concentration (7), in women load test tHcy was positively associated with body mass index, but not related to plasma HDL, LDL and cholesterol (5,7). In conclusion, the total homocysteine blood level is considered a product of genetic and lifestyle interactions [f.e. hypertension, diabetes, raised cholesterol and cholesterol levels, smoking, obesity, hormone changes and exercise]. Supplementation of folic acid, pyridoxine and recently creatine may represent a practical strategy for decreasing plasma homocysteine level (3,4,5,6). Adequate supplemental intakes of creatine can suppress creatine suppress creatine synthesis by inhibiting expression of the enzyme arginine-glycine transamidase(1).

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### LYMPHOKINE ACTIVATED KILLER CELLS DURING LONG TERM CULTIVATION - PHENOTYPICAL ANALYSIS

O. Kopecký, M. Zichová, J. Krejssek

*Institute of Clinical Immunology and Allergology, University Hospital, Hradec Králové, Czech Republic, e-mail: kopecky@lfhk.cuni.cz*

Lymphokine induced killer cells (LAK) are heterogenous population of cytotoxic cells. These cells demonstrate the highest activity against tumor cells. We tested feasibility of generating LAK cells exhibiting potent cytotoxic activity against target K-562 cell line. The peripheral mononuclear cells from 21 healthy blood donors were cultivated for 14 days in RPMI medium + 4 % human albumine with rGM-CSF (final concentration 100ng per 1ml cultivation medium) and rIL-2 (final concentration 600IU per 1ml cultivation medium). The phenotypic analysis of activated cells were performed on the 1<sup>st</sup>, 3<sup>rd</sup>, 7<sup>th</sup>, 11<sup>th</sup> and 14<sup>th</sup> day of cultivation. We determined expression of CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>/CD57<sup>+</sup>, CD19<sup>+</sup>, CD3<sup>+</sup>CD16<sup>+</sup>/CD56<sup>+</sup> cells, together with determination of activation and costimulatory markers, CD25<sup>+</sup>, CD45RA<sup>+</sup>, CD45RO<sup>+</sup>, HLA DR<sup>+</sup>, CD95<sup>+</sup>, CD28<sup>+</sup>, CD80<sup>+</sup>, CD86<sup>+</sup> and CD152<sup>+</sup> by flow cytometry. Cytotoxicity of cultivated cells was determined at the 14<sup>th</sup> day of cultivation.

We found the increase of proliferation activity of cytotoxic cells during the cultivation period. This proliferation was accompanied by increased expression of activation and costimulatory molecules. The cytotoxic cells display significantly higher cytotoxic activity in the end of 14 days of cultivation. These result suggest that expansion and activation of peripheral mononuclear cells is useful strategy to improve their antitumor potential in nonspecific immunotherapy. There is the possibility to combine this approach to therapy with other immunotherapeutical strategies.

### HUMAN BRAIN PATHOLOGY SIMULATION

J. Kremláček, M. Kuba, F. Vit,

*Dept. of Pathophysiology, Charles University – Faculty of Medicine in Hradec Králové, Czech Republic, e-mail: jan.kremlacek@lfhk.cuni.cz*

Spatially defined brain lesions serve as a gold standard for examining the brain function (1). The recent technological advantages allow creating of transient virtual lesions in the human brain non-invasively via transcranial magnetic stimulation (TMS) that is based on the Faraday's principle of electromagnetic induction. The strong magnetic pulse (from 1 up to 4 T) penetrates through the skull and induces ion currents in superficial part (1-2 cm) of the cortex. Direct measurements showed that current density peaks at 12  $\mu\text{A}/\text{cm}^2$  for the magnetic field of 0.14 T (2). Currently there is increasing use of the TMS in basic research, but the TMS applications also include several diagnostic and therapeutic approaches.

TMS of the cortex can have several types of manifestation. The direct excitation can result in motoric muscle reactions or a perception interruption or even in illusory perceptions. The low repetition TMS can modulate excitatory or inhibitory brain mechanisms and couple of these experiments demonstrate brain plasticity. (3)

The idea that TMS can serve as a tool for demonstration of brain pathology in medical education is not used so far probably because of safety issues. However, the recent review arguing that a single pulse TMS is safe, non-invasive and nearly painless (4) is supporting a possible use of TMS for educational purposes - as a tool for reversible brain functional disruption.

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### EEG BIOFEEDBACK – PRINCIPLES AND REALISTIC EVALUATION OF POSSIBLE EFFECTS

M. Kuba, F. Vit, J. Kremláček, L. Borská, Z. Kubová, J. Chlubnová, *Dept. of Pathophysiology, Charles Univ. – Faculty of Medicine in Hradec Králové, Czech Republic, e-mail: kuba@lfhk.cuni.cz*

EEG biofeedback (synonyms - neurofeedback, neurotherapy) has been advertised for more than 20 years as a therapeutic tool for a large spectrum of neuro-psychiatric disorders. The used hypothetical explanations of its possible effects interfere, however, with the known background of these diseases and moreover, the method is not covered by health insurance in majority of countries and so it represents mainly a commercial activity. Therefore, we try to provide a summary of the recent knowledge in this field to medical students to prevent its non-critical acceptance and misuse in patients.

Formerly this method was recommended only to those who could not relax spontaneously - their increased "psychic tension" (stress) led then to manifestation of various psychosomatic disorders. This application was based on providing of feedback information about the level of resting alpha activity (ca 8 – 13 Hz frequencies in the human EEG) with the use of simple frequency filters or analysers. This variant is in agreement with the physiological understanding of EEG formation and its frequency changes, and the effect was recently well proved. Later on, however, more applications appeared (e.g. <http://www.eegspectrum.com> or <http://www.eegbiofeedback.cz>) and the reasoning for their use is either missing or very vague, without being supported by respectable research studies sufficiently. It is almost impossible to verify that the found effects of EEG biofeedback are specific just for a particular application when the prospective improvement in any of the indicated problems is explainable via many simultaneous psychological (psychotherapeutical) factors - e.g. simply by increase of attention paid to the patients (mainly in children with learning disabilities).

In our understanding, according to the recent state of knowledge, it is not possible to recognise significant beneficial changes in the EEG frequency spectrum (specific for EEG biofeedback), when EEG inter-individual variability is so large and intra-individual changes can be attributed to many influencing factors.

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### MATURATION AND AGEING OF CENTRAL NERVOUS SYSTEM ACCORDING TO VISUAL EVOKED POTENTIALS PARAMETERS

M. Kuba, J. Chlubnová, J. Kremláček, Z. Kubová,

*Dept. of Pathophysiology, Charles University – Faculty of Medicine in Hradec Králové, Czech Republic, e-mail: kuba@lfhk.cuni.cz*

We verified suspected differences in dynamics of maturation and ageing processes on various levels of cortical visual information processing. Three types of visual evoked potentials (VEPs) representing activation of different parts of the visual pathway and visual cortical areas were used (details at <http://www.lfhk.cuni.cz/elf>):

- High contrast transient pattern-reversal VEPs (using 40', 20' and 10' element sizes) for testing of the parvocellular system and of the primary visual cortex (recorded from Oz derivation)
- Low contrast motion-onset VEPs to linear motion of isolated checks and radial motion of randomly expanding/contracting concentric rings (with decreasing spatial frequency and increasing motion velocity toward periphery of the stimulus field) for magnocellular system and associate secondary visual areas examination (recorded from lateral temporo-occipital derivations)
- Cognitive VEPs using the "oddball" paradigm (recognition of "X" letter - "non-target" stimulus and randomly presented digits 1 - 9 counted by subjects - "target stimuli") were applied for evaluation of the highest cognitive cortical functions (recorded from Pz, Cz and Fz derivations).

In the group of 70 healthy subjects in the age span of 6 - 60 years, it was proved that there are no significant changes of latencies but systematic decrease of amplitudes in pattern-reversal VEPs. Most significant criterion for maturation completion seems to be the latency of the motion-onset VEPs that is prolonged up to about 15 years of age compared to adult subjects (over 20 years) and documents rather slow maturation of the magnocellular pathway. Toward elderly, the highest correlation with age displayed the latencies of the cognitive VEPs that were significantly prolonged from the age of 50 years indicating function deterioration on the level of the highest cortical processes.

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### TEACHING OF PATHOPHYSIOLOGY - HOW TO ORGANISE IT?

Z. Kubová, M. Kuba,

*Dept. of Pathophysiology, Charles University – Faculty of Medicine in Hradec Králové, Czech Republic, e-mail: kubova@lfhk.cuni.cz*

Pathophysiology represents very important part of the medical curriculum of all Medical Schools. However, there are large differences how the Pathophysiology courses are organised. Although it is a tradition in the Czech Republic and in some other European countries to teach Pathophysiology as a separate subject, independently on Physiology and before the Internal Medicine starts, it seems to be an actual topic for discussion so far, what is the optimal arrangement of the Pathophysiological course. Time relationships mainly with respect to the preceding Physiology but also to subsequent clinical subjects differ among the schools as well as the structure of courses. Advantages and disadvantages of the "problem based" teaching compared to standard scheme according to the body systems were not definitely evaluated yet. There is also a problem of sufficient teaching staff qualification for enough synthetic approach to pathological processes explanation simultaneously with providing of up-to date information about their aetiology (that is frequently based on the very specialised level of molecular biology). Lecturers should be specialised to some parts (body systems) of Physiology and Pathophysiology only, since they cannot cover the very large field of Physiology and Pathophysiology completely. This, however, is not matched with the existing system of postgraduate specialisation and separate academic degrees in Physiology and Pathophysiology. Moreover, the hypothesised specialisation would not enable to the teachers to be fully qualified for topics requiring very complex knowledge of both subjects. Thus, it seems that some clear optimal arrangement does not exist and each Pathophysiology department has to compromise the specified situation. Nevertheless, some perspectives concerning the future strategy of Pathophysiology teaching should be discussed and proposed.

### A REVIEW OF NEGATIVE EFFECTS OF CARDIOPULMONARY BYPASS (CPB)

V. Lonský, J. Mandák, J. Dominik, J. Kubiček

*University Department of Cardiac Surgery, Charles' University Hospital Hradec Králové, Czech Republic, e-mail: vlonsky@seznam.cz*

**Introduction:** It was 50 years since the first successful application of extracorporeal circulation with an artificial heart-lung machine last year (John Gibbon 1953). Since this time the method has been progressively improved by development and simplification of the equipment and by better understanding of the body response. Despite of it injury to some degree of another still occurs in all patients who undergo CPB.

**Aim:** To give the brief summary of the main negative effects of CPB.

**Review:** The "normal physiology" of CPB, which considerably differs from normal physiology of the body, is connected with nonpulsatile and lower ( $2,2 \text{ l/m}^2/\text{min}^{-1}$  -  $2,4 \text{ l/m}^2/\text{min}^{-1}$ ) blood flow, blood contact with nonendothelial surface of the system, hemodilution and temperature changes (cooling and rewarming of the blood). Subsequent changes in neurological (central emboli, decrease cerebral flow, temporary deficits), renal (decrease glomerular filtration rate, emboli to kidneys, renal failure), lung (atelectasis, increased interstitial water, leukoembolization), hematological (coagulopathy, reduced platelet count, decreased hematocrit), complement system, endocrine and hepatic functions are secondary to these substitutions. It is necessary to say that in approx. 98 % of pts there are only subclinical or laboratory signs of this so called post - perfusion syndrome which is almost in 100 % of patients reversible.

**Conclusion:** Despite of above mentioned negative effects CPB became a very safe and routine method which enables to perform more than 750 000 cardiac procedures per year over the world.

### THE EFFECT OF SUBCHRONIC CADMIUM INTOXICATION ON ANTIOXIDANT STATUS IN RATS

E. Lovásová<sup>1</sup>, A. Šipulová<sup>2</sup>, F. Ništiar<sup>1</sup>, O. Rácz<sup>1</sup>,

*<sup>1</sup>Institute of Pathophysiology and <sup>2</sup>Institute of Experimental Medicine, Medical School, Šafárik University, Košice, Slovakia. E-mail: evalov@central.med.cupjs.sk*

Transition metals both in elementary and compound form are known to cause serious health damage due to their accumulation and toxicity in living organisms. Cadmium (Cd) is one of the most widespread environmental pollutants. In cells, Cd binds to the sulphhydryl groups of proteins. It can affect various metabolic processes, especially energy metabolism, membrane transport and protein synthesis. In this study the effect of subchronic cadmium intoxication on red cell antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and plasma total antioxidant status (TAS) was investigated in rats. Cadmium (as CdCl<sub>2</sub>) was administered to 45 adult albino rats in 5 different doses from 1,83 to 29,27 mg.kg<sup>-1</sup>.day<sup>-1</sup> (experimental groups Cd1 – Cd5) in tap water for 30 days. The control group (CdC, n = 8) drank clear tap water. Antioxidant enzymes and TAS were determined in blood collected from the heart at the end of experiment. SOD, GPX and TAS were measured by spectrophotometric methods (RANDOX, UK) on Cobas Mira automatic analyser (Roche), CAT by UV spectrophotometric method on Specord M40. Subchronic administration of Cd led to elevation of SOD in all experimental groups - significantly in the group with the highest dose of Cd (Cd5, p < 0,05). GPX activity increased in all experimental groups but the increase was not statistically significant. The CAT activity decreased in three groups with the lowest doses of Cd (Cd1–Cd3) and increased in the groups receiving the two highest doses (Cd4 – Cd5). These changes were nonsignificant. TAS decreased in all experimental groups, significantly in Cd2, Cd3 and Cd4 groups (p < 0,05 – 0,005). The results show that subchronic cadmium intoxication increases the activity of red cell antioxidant enzymes, especially in the highest doses, which may enhance the antioxidant potential of cells to reduce oxidative stress.

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### INTRAAORTIC BALLOON COUNTERPULSATION IN THE TREATMENT OF HEART FAILURE

J. Mandák, V. Lonský, J. Kubiček, P. Valentová, *Department of Cardiac Surgery, Charles University Hospital, Hradec Králové, Czech Republic, e-mail: jiri.mandak@centrum.cz*

**Introduction:** Intra-aortic balloon counterpulsation (IABP) belongs into the group of contemporary standard methods for support of failing circulation. It is applied both in cardiosurgery and in the intensive care and resuscitation units, coronary units and catheterization laboratories as well.

The principle of counterpulsation is the synchronous inflating and deflating the balloon by inert gas during the heart action, the balloon being placed in the descending aorta. The inflating during the diastole improves the filling of the coronary bloodstream. The deflation of the balloon at the beginning of the systole decreases the intraaortic pressure and the left ventricle will be depleted under a lower resistance.

**Methods:** The indication for initiation of IABP during operation and in the post-operation period, at the Department of Cardiac Surgery, University Hospital, Hradec Kralove, Czech Republic, is the syndrome of low cardiac output (the cardiac output above  $2.0 \text{ l/min.m}^2$ , the systolic pressure above 80 mm Hg, the diuresis lower than 20 ml/hour) and severe acute ischaemic changes of myocardium (do not react to conservative treatment). Prior to the surgical revascularization of the myocardium the IABP is introduced in the patients having at least 2 of the following risk factors at the same time: EF < 20 %, unstable AP, reoperation, stenosis of the main left coronary artery higher than 70 % or haemodynamic support of the circulation by drugs before the procedure.

**Results:** From September 1994 to May 2004, 7503 cardiosurgery operations were performed. The IABP was applied in 263 cases, i.e. in 3.5 %. From the group of 263 counterpulsated patients 145 patients were successfully treated (55.1 %). They were disconnected from IABP and discharged to home care. 118 counterpulsated patients (44.9 %) died due to the surgical procedure (30-days mortality rate). In 15 cases (5.7 %), from group of 263, the IABP was introduced already prior to operations.

**Conclusion:** IABP has an irreplaceable role in interventional treatment of acute heart failure and is a successful method for providing support in circulatory failure.

### **PATHOLOGICAL PHYSIOLOGY IN INTEGRATED TEACHING SYSTEM AT THE THIRD FACULTY OF MEDICINE**

J. Mareš<sup>1</sup>, R. Rokyta<sup>1</sup>,

*Department of Normal, Pathological and Clinical Physiology, 3<sup>rd</sup> Faculty of Medicine, Charles University, Prague*

Since 1997 with the implementation of the new curriculum at the 3<sup>rd</sup> Faculty of Medicine the teaching of physiological disciplines has changed. Physiology has been included in the integrated system in the first two years of curriculum into the Module I.A (the 1<sup>st</sup> circle). It is a combination of morphological disciplines anatomy, histology and embryology and Module I.A dynamic disciplines biochemistry and physiology. Students are to pass exams of Module I.A by the end of the second year.

Pathological Physiology is being taught within the subject of pathology together with the pathological anatomy in the third and fourth year. Pathological Physiology is included in two modules. Module II.A is called Theoretical foundations of clinical medicine (a total of 325 hours and 18 credits) and consists of 6 courses. Tuition in the summer semester continues with the module Basic clinical problems (a total of 300 hours and 17 credits), which also consists of 6 courses. In all these courses there are always one or two seminars taught directly by the Department of Pathological Physiology. Pathological Physiology is also being lectured in normal courses in a total of 45 hours. Module Basic clinical problems continues through the whole fourth year where it is within 5 courses out of 13. We cover 36 out of a total of 810 hours. Connection with the Pathological anatomy enables to leave out useless repeating of some areas in both subjects. From the view of the integration a common examination, which is at the end of the fourth year, is proving to a certain extent well. A regular evaluation of teaching by students at the faculty is taking place and an evaluation by teachers is being prepared.

### **BODY SURFACE MAPPING IN PATIENTS WITH ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY**

Š. Navarčíková<sup>1</sup>, R. Hatala<sup>2</sup>, L. Urban<sup>2</sup>, L. Zlatoš<sup>1</sup>, I. Hulín<sup>1</sup>,

*<sup>1</sup>Institute of Pathological Physiology, Faculty of Medicine, Comenius University, Bratislava, <sup>2</sup>Department of Rhythm Disorders, Slovak Institute of Cardiovascular Disorders, Bratislava, stefania.navarcikova@fmed.uniba.sk*

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a progressive disorder of predominantly right ventricle characterized with arrhythmic events possibly leading to sudden cardiac death. The fibrofatty infiltration is viewed as a healing phenomenon in the setting of a programmed cell death – apoptosis. Body surface mapping provide a noninvasive measure to detect substrate for ventricular arrhythmias as QRST isointegral maps reflect local disparities of ventricular repolarization. The aim of this study was to determine a presumptive nondipolarity in QRST isointegral maps in patients with ARVC. A group of 8 patients with ARVC and a control group of 8 patients with a concealed accessory pathway were studied. All ARVC patients fulfilled the criteria for diagnosis (1). Body surface mapping was performed during sinus rhythm using a 63-lead Savard's system. In all control subjects smooth dipolar QRST maps were observed. In ARVC subjects, QRST isointegral maps were nondipolar with multiple extrema in 3 patients and dipolar with abnormal characteristics in 5 patients. Up till now, nondipolarity, a characteristic feature of body surface integral maps in patients susceptible to arrhythmias, was not observed in ARVC (2,3). We suggest that nondipolarity most likely reflects only gross regional inequalities of repolarization (3). Could regional inequalities of repolarization be a predictor for high-risk patients with ARVC?

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### **TREATMENT OF DIABETES MELLITUS IN BB RATS WITH COMBINATION OF VANADIUM AND INSULIN**

F. Ništiar<sup>1</sup>, O. Ráč<sup>1</sup>, M. Riemerová<sup>1</sup>, A. Lukačinová<sup>2</sup>, A. Ništiarová<sup>3</sup>,

*<sup>1</sup>Institute of Pathological Physiology, <sup>2</sup>Institute of Physiology, and <sup>3</sup>Central Animal Laboratory, Faculty of Medicine, Šafárik University Košice, Slovak Republic, nijfran@central.medic.upjs.sk.*

In this study the effect of vanadium and insulin on metabolism of diabetic BB rats was evaluated in a chronic (3 month) experiment. 120 rats were divided into 5 groups (24 per group). Group I: without treatment; Group II: ammonium vanadate 0,1 mmol/l; Groups III and IV: 2 or 1 U insulin daily (Ultralente, Novo-Nordisk); group V: combination of ammonium vanadate (0,1 mmol/l) in drinking water and 1 U insulin daily

The results summarized in Table indicated that chronic low dose administration of vanadate with combination of insulin was the most effective treatment in this experimental setting. Further studies are necessary to recommend vanadium supplementation in treatment of human diabetes.

**Table**

Group	FI	WI	U	GU	TUG	BG	GI	AQ
I. Diabetic NT	21.6	80.6	68.9	266.6	3.21	18.8	1.78	0.67
II. Vanadate	18.3	42.3	36.8	118.2	0.77	9.7	1.06	0.92
II. 2 U insulin	26.7	35.4	24.6	66.4	0.31	8.1	0.61	0.98
IV. 1 U insulin	22.8	58.8	41.5	132.1	0.84	12.2	1.07	0.93
V. Vanadate + 1 U insulin	20.8	40.3	28.9	35.7	0.18	7.1	0.68	0.98

FI = food intake g/d; WI = water intake ml/d; U = volume of urine ml/d; GU = glucose in urine mmol/l; TUG = glucose in urine g/d; BG = glycaemia mmol/l; GI = glucose index (glycaemia/glucose content in food); AQ = assimilation coefficient (assimilated glucose/glucose content in intaked food/).

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### **FUNCTIONS OF THE NOSE, INTERACTIONS BETWEEN THE UPPER AND THE LOWER AIRWAYS**

J. Plevkova, M. Brozmanova, M. Tatar

*Department of Pathophysiology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovak Republic, e-mail: plevkova@jffmed.uniba.sk*

Nasal cavity is a compartment where the inspired air enters into the respiratory system and it is an integral anatomical and functional component of the respiratory tract. Nasal cavity is responsible for conditioning of the inspired air (warming and humidification), filtration of particles larger than 10 µm via adhesion on the superficial mucus lining and their transportation into the pharynx by mucociliary system. Irritation of the afferent nerve endings in the nose could trigger respiratory defense reflexes (apnoea, sneezing and sniffing). Mucosa of the nose and sinuses provides an immunological monitoring of inspired air, these spaces are important for voice modulation, and last but not least – afferent nerve endings of the olfactory nerve in the roof of the nasal cavity are responsible for smelling.

The relationship between the nose and the lower airways is of great interest both from clinical and pathophysiological stand points. It is well known that disorders of the nose and sinuses are strongly associated with diseases of the lower airways (bronchial asthma, chronic cough in the adults, idiopathic recurrent cough in children).

There are several mechanisms, which may be responsible for this reality. The most commonly known is a post nasal drip syndrome – spreading of the pathological process from the nose by dripping of inflammatory components. Next one is microaspiration of the inflammatory aerosol from the nasal cavity to the larynx and more peripheral airways. Inappropriate conditioning of the air due to lack of nasal functions and nasobronchial reflex are another important mechanisms responsible for nose – lung interaction. A particularly new is a conception of “one airway – one disease” point of view and guessing of spreading of the inflammatory mediators not only via airways, but also via systemic circulation.

Although many unanswered questions still exist regarding the relationship between the nose and lower airway diseases, it is important to know that “nasal disorders” could affect even the lower airways and that treating of the rhinosinopathies could improve the functions of the lower airways.

### EXPERIMENTS ON ANIMALS AND THE ALTERNATIVES IN THE CURRICULUM OF PATHOLOGICAL PHYSIOLOGY – HISTORY, CURRENT STATUS AND PERSPECTIVES

O. Rácz, M. Riemerová, F. Ništiar

*Institute of Pathological Physiology, Medical School, Šafárik University, Košice, Slovakia, e-mail: raczo@central.medic.upjs.sk*

The history of medicine is probably longer than the history of sciences but the association between them was very weak or nonexistent until the XVIII century. The situation dramatically changed from the second half of the XIX century when carefully designed experiments on animals laid down the fundamentals of modern scientific medicine. Our teacher and the founder of modern diabetology in our country, professor Rudolf Korec (1921 – 2003) during his long professional career carried out of thousands of experiments on rats and mice but he treated them as his „patients“ – carefully, not inducing any unnecessary suffering to them. During the years when Prof. Korec was the head of our department almost all practical workshops in pathological physiology were based on simple animal experiments carried out by students of 3<sup>rd</sup> year. In the last year most of these experiments were eliminated from the education, mainly from ethical reasons. Our own activity in this direction was based not only on transformed legislation and demands from students but also on our conviction that in changing world of medical research also the education should be transformed.

video demonstrations of experiments, video presentations dealing with the pathogenesis of different diseases, interactive computer models are gradually introduced into medical education at our department. The number of experiments on animals was reduced to minimum but on the other side these experiments and activities (carried out mostly in general anaesthesia) are crucial according to our view because during these workshops the students learn to apply injections and acquire the basics rules of surgical techniques. These skills are not replaceable with virtual models of experimental animals.

According to our view teaching the history of experimental medicine and information on ethical rules of experimental work and legislation is a very important part of medical education.

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### GENOMICS AND THE ETHICAL ASPECTS OF GENOMIC MEDICINE IN OUR CURRICULUM

O. Rácz<sup>1</sup>, F. Longauer<sup>2</sup>, J. Palaščák<sup>3</sup>, F. Ništiar<sup>1</sup>, R. Beňačka<sup>1</sup>, E. Lovásová<sup>1</sup>

*<sup>1</sup>Institute of Pathological Physiology and <sup>2</sup>Institute of Forensic Medicine Medical School and <sup>3</sup>Christian Academy, Šafárik University, Košice, Slovakia, e-mail: raczo@central.medic.upjs.sk*

In the near future active knowledge of molecular medicine and genomics will be of vital importance for everyday clinical medicine and even for general practitioners. On the other side some years ago we recognized that medical students in the 3<sup>rd</sup> year of medicine are unable to apply the knowledge from molecular biology and genetics in understanding the pathogenesis of hereditary and common diseases. To solve these problems in 1966 we introduced a new subject – Introduction to Molecular Medicine (1). In the frame of this subject (one term – 56 hours) is not possible to teach molecular and genomic medicine in full range but is possible to explain the basic principles of the complicated way from genotype to phenotype, the principles of interactions between nature and nurture and that genes are involved in the pathogenesis of every disease and not only in those which in classic medicine were treated as „hereditary“ diseases. After more than 8 years of experience we can conclude that this approach is successful. In our main subject, Pathological Physiology we can now build on the skills acquired in Molecular Medicine. The permeation of molecular and genomic medicine into everyday medical practice arises also very important ethical questions. We discuss these problems in the subject „Medical Ethics“ in the 4<sup>th</sup> year of medical curriculum and also during different postgradual workshops for general practitioners (2,3).

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### STUDENTS' AND TEACHERS' ATTITUDE TOWARDS THE PROCESS OF EDUCATION IN THE DEPARTMENT OF PATHOPHYSIOLOGY AT THE MEDICAL FACULTY, PALACKÝ UNIVERSITY, OLOMOUC

D. Riegrová, K. Dostálová

*Department of Pathological Physiology, Medical Faculty, Palacký University, Olomouc, e-mail: rie@tunw.upol.cz*

Evaluation of the teaching process by students has been started at the Medical Faculty, UP Olomouc, since the academic year 1999/2000 as a means to increase quality of education and a valuable source of feedback.

In the last three years, this department has used its own version of inquiry forms monitoring the process of teaching instead of a standard form offered by the dean office. Additionally, our department has also used a second special version of inquiry to assess the students' attitude towards experiments using laboratory animals and alternative forms of education.

In 2004, the first form of inquiry has been introduced to students of the 3<sup>rd</sup> year of general medicine and stomatology programmes (117 and 36 students, respectively) while the second form of inquiry has been introduced to 40 students. As for evaluation of non-medical (bachelors and magister) programmes of studies, these are organized by the Department of theory and practice of nursery care and the results are presented to all faculty departments involved in education of those programmes.

The first form turned back answered in nearly 80 %, the second one in about 90 %. The most interesting conclusions were as follows. About half of the students positively appreciated the skills and techniques learned during the tutorials utilizing animals; another part of them expressed negative position concerning animal experiments, however, and some suggested to shift these experiments among the type C, it is optional, subjects. Further, study texts and textbooks were criticized by the students. Also the students recommended evaluating each semester separately and publishing their evaluation results on the web.

### RELATION BETWEEN CAROTID WALL THICKNESS, AGE AND BAROREFLEX SENSITIVITY IN NORMOTENSIVE AND HYPERTENSIVE SUBJECTS

B. Semrád<sup>1</sup>, R. Lábrová<sup>1</sup>, E. Dufková<sup>1</sup>, P. Vysočanová<sup>1</sup>, B. Fišer<sup>2</sup>, N. Honzíkova<sup>2</sup>, Z. Nováková<sup>2</sup>, E. Závadná<sup>2</sup>,

*<sup>1</sup>1<sup>st</sup> Clinic of Internal Medicine, Faculty Hospital in Brno, <sup>2</sup>Department of Physiology, Faculty of Medicine, Masaryk University in Brno, E-mail: znovak@med.muni.cz*

Heart-rate baroreflex sensitivity expressed in ms/mmHg (BRS) or in Hz/mmHg (BRSf) decreases with age. The hypothesis that the age-dependent decrease of BRS and BRSf are caused by age-dependent increase of carotid wall thickness was investigated in the study presented.

Nineteen treated hypertensives (mean age±SD:51±11years) and 7 normotensives (mean age:50±5years) were tested. Intima-media thickness (IMT) of carotid wall was measured by ultrasonography, BRS and BRSf were determined by spectral method (five minutes non-invasive beat-to-beat recording of blood pressure by Finapres Ohmeda at metronome controlled breathing). The results were evaluated by Wilcoxon test and Spearman's correlation coefficient.

The difference in intima-media thickness between hypertensives (0.613±0.098 mm) and normotensives (0.526±0.078 mm, p<0.05) has been found. The difference in BRS (hypertensives vs. normotensives; BRS: 3.281±2.459 vs. 4.871±2.484, p=0.10) was not significant; the difference in BRSf was significant (0.004±0.002 vs. 0.008±0.004, p=0.04). Taking all subjects together we have found the positive correlation between age and left carotid IMT (r=0.4319, p=0.03). The positive correlation coefficient on the right side was insignificant. The correlation between age and BRSf was significant (r=-0.6009, p=0.001), the correlation between age and BRS was not found.

The positive correlation between age and ITM and negative correlation between age and BRSf are in agreement with the hypothesis that the age-dependent decrease of baroreflex sensitivity expressed in Hz/mmHg corresponds to the age-related structural changes of carotid wall.

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### THE EFFECT OF EMBRYONIC CEREBELLUM TRANSPLANTATION ON SIGNS OF ATAXIA IN LURCHER MUTANT MICE

B. Schmidtmayerová, J. Cendelin, F. Vožeh

Department of Pathophysiology, Faculty of Medicine in Pilsen, Charles University, Plzeň, e-mail: jan.cendelin@lfp.cuni.cz

Neurodegenerative diseases are a serious medical problem and a possibility of therapy by transplantation of stem or embryonic neural cells is intensively investigated. Laboratory animals affected with neurodegeneration are a suitable experimental model. One of them are Lurcher mutant mice which suffer from olivocerebellar degeneration and consecutive cerebellar ataxia, which affects their motor abilities. The aim of our work was to assess the effect of the embryonic cerebellar graft transplantation on motor abilities of Lurcher mutant mice. Embryonic cerebellar tissue was obtained from 12-14 days old Green Fluorescent Protein-expressing mice embryos. A solid piece of donor tissue was applied into the cerebellum of adult Lurcher mutant mice through trepanation in occipital bone. To control animals only vehiculum was administered by the same procedure. Motor abilities were examined using a set of three tests (horizontal bar, ladder, rotating cylinder). The tests were performed before and in week intervals after the operation. Then brains of experimental animals were examined histologically. The graft was identified according to the green fluorescence. For detailed morphology visualization the Nissl staining was used. Transplanted grafts developed and grew in the host body. Individual cerebellar cortex layers differentiated. Migration of individual cells and nerve fibres sprouting from the graft to the host tissue was observed. Motor tests showed better results in mice with cerebellar graft and the increase of latencies during repeated motor training was more marked after the transplantation than in sham-operated controls. Cerebellar graft transplanted to mice affected with olivocerebellar degeneration survived and created connections with the host tissue, what is an essential condition for functional involvement into neural circuits interrupted by the disease. The transplantation had a positive effect on motor coordination in Lurcher mutants.

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### TRIGLYCERIDE SECRETION RATE IN HYPERTRIGLYCERIDEMIC RATS: THE EFFECT OF N-3 POLYUNSATURATED FATTY ACIDS

B. Staňková<sup>1</sup>, A. Žák<sup>1</sup>, E. Tvrzická<sup>1</sup>, L. Kazdová<sup>2</sup>, M. Zeman<sup>1</sup>, A. Vrána<sup>2,1</sup>

<sup>1</sup>IV<sup>th</sup> Department of Medicine, <sup>1st</sup> Faculty of Medicine, Charles University, Prague, Czech Republic, <sup>2</sup>Institute for Clinical and Experimental Medicine, Prague, Czech Republic  
E-mail: barsta@volny.cz

Hypertriglyceridemia (HTG) is nowadays supposed to be an independent risk factor for coronary artery disease. Fructose-induced HTG in rats can be used as an experimental model of insulin resistance syndrome. The aim of our work was to elucidate the relation between plasma triglyceride (TG) levels and some metabolic, nutritional, as well as hormonal factors and the effects of dietary n-3 polyunsaturated fatty acids (PUFA) on this relation. Male Wistar rats with dietary sucrose induced HTG were supplemented either by fish oil (FO) or lard/olive oil mixture (CON). Glucose, insulin, and TG secretion rate (TGSR) were measured following 12 hours of fasting. After sacrifice, further analyses of serum lipids, fatty acid profiles in serum lipid classes were performed.

Increased relative liver size and decreased epididymal fat mass were observed in FO group. Concomitantly decreased plasma concentrations of total cholesterol, fasting as well as postprandial TG, glucose, uricaemia, and free fatty acids (FFA) were found. The TGSR rate was decreased as well. Concentrations of fasting plasma TG in CON group correlated with TGSR, glucose and FFA concentration. In the FO group, levels of TG were dependent on TGSR and insulin concentration. Both groups turned to exhibit close correlation of 20:5n-3, 18:0, and 16:0 fatty acid with TGSR, which was the strongest predictor of fasting TG. In conclusion, the supplementation of diet with FO rich in n-3 PUFA normalized plasma lipid levels, glucose, FFA, insulin and uric acid in HTG rats. The fatty acid composition of plasma lipids correlated with TGSR, which showed to be an important predictor of fasting TG concentration.

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### OCCUPATIONAL STRESS ASSESSEMENT OF EMPLOYEES OF DIFFERENT PROFESSIONS

J. Šmejkalová<sup>1</sup>, M. Bendová<sup>2</sup>, H. Skalská<sup>3</sup>, L. Borská<sup>4</sup>, L. Hodačová<sup>5</sup>, D. Fialová<sup>6</sup>

<sup>1</sup>Dept of Hygiene and Prev. Med., Charles Univ. Prague, Fac. Med. Hr. Králové, <sup>2</sup>ERGO, Hr. Králové, <sup>3</sup>Dept. of Informatics Quantitative Methods, Fac. Informatics and Management, Univ. Hradec Králové, <sup>4</sup>Dept. of Pathological Physiology, Charles Univ. Prague, Fac. Med. Hr. Králové, <sup>5</sup>Dept. of Social Medicine, Charles Univ. Prague, Fac. Med. Hr. Králové, <sup>6</sup>Dept. of Physical Education and Sports, Pedagogical Faculty Univ. Hradec Králové

The aim of our study was to conduct an anonymous questionnaire inquiry concerning the health risk self-perception of workers in various professions. Altogether we analysed 825 questionnaires from 242 teachers of all school levels, 172 factory workers of mostly risk professions, 267 health professionals (doctors and nurses) and 144 clerks. For occupational mental stress estimation we used Meister Questionnaire by which chronic effects of working stress exposition – overload or monotony – could be analysed. Questionnaire consists of 10 statements and respondents are asked to assess on five-point scale. Mental stress of all our respondents was high, but the particular risk factors differed according to the type of profession. The best situation was found in the group of clerks. The psychic load of their work was classified to be in category 1, that means without influence on subjective status and working output. In spite of that they complained of *overload factor* – namely the feeling of time pressure and interpersonal conflicts. The level of mental stress of medical workers was classified to be in category 1 with tendency to category 2 (we were can find transient influence on subjective status and working output). Medical workers suffered mostly from the *overload factor* – working under time pressure and high responsibility. The level of mental stress of teachers was assessed in category 2. Teachers complained of frequent interpersonal conflicts, great responsibility and time pressure. In this professional group there was expressed namely the *factor of stress reaction of organism* – nervousness, psychic fatigue, and the feeling of not being able to accomplish this profession for a long time. For factory workers there was typical the *factor of monotony* in combination with the overload factor. Workers are not satisfied with their job, which they take for being uninteresting. Their mental stress was characterised to be in category 2.

### ANTIOXIDANT ENZYMES IN CORD AND NEONATAL BLOOD

A. Šipulová<sup>1</sup>, O. Rácz<sup>2</sup>, D. de Furia<sup>2</sup>

<sup>1</sup>Institute of Experimental Medicine and <sup>2</sup>Institute of Pathophysiology, Medical School, Šafárik University, Košice, Slovakia, e-mail: asipul@central.medic.upjs.sk

In vitro, bilirubin is an antioxidant, but in vivo its antioxidant capacity is questionable. The aim of study was to investigate the activity of erythrocyte antioxidant enzymes in cord and infant blood in relation to concentration of bilirubin (Bi). We determined activity of superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase and concentration of bilirubin in 65 paired umbilical cord samples (day 0) and neonatal samples collected on the fourth postpartum day (day 4). The newborn infants were divided into three groups according to their Bi concentration: I. - Bi < 85 µmol/l, II. - Bi = 85 – 205 µmol/l, III. - Bi > 205 µmol/l (n = 17, 26 and 22 respectively).

SOD and GPX were measured by spectrophotometric methods (RANSOD and RANSEL from Randox, UK) on Cobas Mira automatic analyzer (Roche, Switzerland). The activity of catalase was determined by UV spectrophotometric method according to Luck. The results are summarised in Table as mean ± SD

Group		Catalase [mkatal/gHb]	GPX [U/gHb]	SOD [U/gHb]
I. Bi < 85 µmol/l	day 0	4.04 ± 0.95	41.3 ± 7.6	1062 ± 133
	day 4	4.16 ± 1.00	41.6 ± 5.5	1106 ± 169
II. Bi = 85-205 µmol/l	day 0	3.56 ± 0.93 <sup>S</sup>	36.7 ± 11.1	1009 ± 186
	day 4	3.36 ± 0.75 <sup>#</sup>	42.8 ± 9.3 <sup>*</sup>	967 ± 176 <sup>#</sup>
III. Bi > 205 mmol/l	day 0	3.96 ± 0.70	35.8 ± 6.4 <sup>®</sup>	1090 ± 100
	day 4	3.76 ± 0.74 <sup>@1</sup>	38.7 ± 6.7	1027 ± 159

\$ p < 0.05 (I/II, day 0); & p < 0.05 (I/III, day 0); # p < 0.05 (I/II, day 4), @ p < 0.05 (I/III, day 4); ! p < 0.05 (II/III, day 4); p < 0.05 (II, day 0/4). The activity of CAT and GPX was significantly decreased in umbilical cord blood and neonatal blood in infants with hyperbilirubinemia. This result does not contradict with the view that bilirubin is an antioxidant in vitro but hyperbilirubinemia in vivo can be associated with decreased antioxidant system capacity.

### **PATHOPHYSIOLOGICAL AND CLINICAL SIGNIFICANCE OF FATTY ACIDS IN HUMAN ORGANISM**

E. Tvrzická, A. Žák, M. Vecka, B. Staňková  
*IV<sup>th</sup> Department of Medicine, 1<sup>st</sup> Faculty of Medicine, Charles University, Prague, Czech Republic, e-mail: tvrzicka@volny.cz*

Fatty acids are substantial component of lipids, which represent one of the three major components of biological matter (along with proteins and carbohydrates). Chemically are lipids esters of fatty acids and organic alcohols - cholesterol, glycerol, sphingosine. Pathophysiological role of fatty acids is derived from that of individual lipids.

Fatty acids are synthesized ad hoc in cytoplasm from two-carbon precursors, with the aid of acyl carrier protein, NADPH and acetyl-CoA-carboxylase. Their degradation by  $\beta$ -oxidation in mitochondria is accompanied by energy-release.

Fatty acids in mammalian organism reach chain-length 12-24 carbon atoms, with 1-6 double bonds. Their composition is species as well as tissue specific. Endogenous acids can be desaturated up to  $\Delta 9$  position, desaturation to farther position is possible only from exogenous (essential) acids (linoleic,  $\alpha$ -linolenic).

Circulating lipids (in form of lipoproteins) consist of cholesteryl esters and triglycerides in nonpolar core and phosphatidylcholine and sphingomyelin in polar envelope. Nonesterified fatty acids (product of lipolysis and source for lipid synthesis) are bound to plasma albumin.

Membrane lipids, which ensure its fluidity and other functions, consist of phosphatidylcholine, phosphatidylethanolamine, sphingomyelin and further minor phospholipids. Unsaturated fatty acids with 18-20 carbon atoms are precursors of prostaglandins, leucotrienes and thromboxanes, which have a broad scale of autocrine as well as paracrine effects. Fatty acids are ligands of several nuclear receptors, which take part in a number of metabolic pathways. Covalent modification of proteins by acylation enables their incorporation into membranes.

Number of pathological stages is accompanied with changes in fatty acid composition, often expressed as decreased content of unsaturated and increased content of saturated fatty acids (dyslipidemia, malnutrition, inflammation, inherited diseases).

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### **A HAPLOTYPE CONSTITUTED OF FOUR MMP-2 PROMOTER POLYMORPHISMS (-1575G/A, -13056C/T, -790T/G AND -735C/T), TOTAL CHOLESTEROL AND CHRONIC HEART FAILURE**

A. Vašková<sup>1</sup>, M. Goldbergová<sup>1</sup>, L. Špinarová<sup>2</sup>  
<sup>1</sup>*Institute of Pathological Physiology, Faculty of Medicine, Masaryk University Brno, Czech Republic,* <sup>2</sup>*Department of Internal Medicine, St. Ann's Faculty Hospital, Masaryk University Brno, Czech Republic, e-mail: avasku@med.muni.cz*

Remodelling of extracellular matrix by activated matrix metalloproteinases is considered to contribute to progression of ventricle remodelling during chronic heart failure (CHF). The changes in lipid metabolisms are supposed to participate in pathogenesis of cardiovascular diseases as risk factors. The aim of the study was to test association among variability in MMP-2 promoter (four polymorphisms) and biochemical levels of lipid metabolism compounds (total cholesterol, LDL, HDL and triglycerides) in chronic heart failure. A total of 239 patients with CHF (aged 56 years, range 21-91), 185 men and 54 women, was included in the study. Hyperlipoproteinemia was diagnosed in 108 patients (46.4 %). The DNA genotyping in four SNPs was performed using simple PCR methods with restriction analyses.

A significantly lower odds ratio (OR) was proved for haplotype ATGT in CHF patients with the total cholesterol above 5 mmol/l compared to those with the lower level of cholesterol (OR=0.07, P=0.0008, P<sub>corr</sub>=0.006). The power of the test was 85 % at the level 5 %. No differences in MMP-2 haplotype frequencies were found for either higher and lower levels of LDL, HDL or triglycerides or for diagnosed hyperlipoproteinemia.

The ATGT promoter haplotype of MMP-2 gene is associated with lower risk for chronic heart failure patients with the total cholesterol level above 5 mmol/l.

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### **PATHOPHYSIOLOGY AND CLINICAL SIGNIFICANCE OF NON-CHOLESTEROL STEROLS**

M. Vecka, A. Žák, E. Tvrzická  
*IV<sup>th</sup> Department of Medicine, 1<sup>st</sup> Faculty of Medicine, Charles University, Prague, Czech Republic, e-mail: marvec@volny.cz*

Sterols are steroids carrying a hydroxyl group at carbon three of the base skeleton, which in turn are part of the big family of isoprenoids. Apart from cholesterol, in human plasma can be found many sterols derived either from endogenous biosynthesis or from dietary sources, which are mainly of plant origin and therefore called phytosterols. The most important precursors of cholesterol include lathosterol, desmosterol, 7-dehydrocholesterol and lanosterol, commonly used for evaluation of cholesterol biosynthesis. The pattern of phytosterols is far much broader, but only some of them are efficiently absorbed in the intestine and appear in plasma, as it is true for  $\beta$ -sitosterol and campesterol.

Together with phytosterols, cholesterol derived from diet is absorbed by a complex process with several important steps including micelle formation, absorption and re-excretion on the apical side of enterocyte, esterification by acylCoA: cholesterol acyltransferase, formation of chylomicrons and finally releasing the particle on the basolateral side of enterocyte. Phytosterols were found to accompany the cholesterol molecule throughout all these steps, thus allowing estimation of fractional absorption of dietary cholesterol on one hand and lowering of cholesterol intake by supplementation of phytosterols on the other. Nevertheless, these compounds disturb electrochemical and fluid properties of animal membranes that is why enterocytes discriminate phytosterols from entering the circulation in the step of steryl ester formation. Nonesterified pool of sterols is then subjected to re-excretion from enterocyte through ABCG5/G8 transporter dimer that is inflicted with mutations of either encoding gene in sitosterolemia.

Since excess cholesterol as well as phytosterol absorption can be detrimental by contributing to atherosclerosis, understanding the mechanisms of sterol absorption helped to synthesize new classes of cholesterol lowering drugs as ezetimibe, avasimibe and inhibitors of microsomal triglyceride transfer protein.

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### **INTEGRATED PRESSURE-VOLUME REGULATION OF CARDIOVASCULAR FUNCTION**

J. Veselý, K. Dostálová, D. Riegrová, J. Mačáková, M. Rypka  
*Department of Pathological Physiology, Medical Faculty, Palacký University, Olomouc, e-mail: vesely@tunw.upol.cz*

About a century ago a classical concept has been implicated in cardiovascular physiology that cardiac output (CO) is regulated primarily by two major peripheral factors. The first of these is the ratio of blood volume to capacity of the circulation and the second is the local resistance autoregulation accomplished by peripheral tissues themselves. Only under failing conditions, such as when the pumping capacity of the heart becomes too small or when inflow into heart becomes excessively increased, the heart becomes the limiting factor in CO regulation.

Nevertheless, many physiologists, pharmacologists, and clinicians recently believe that CO is primarily controlled by heart itself and by nervous and hormonal reflexes to the heart and/or resistance vessels. Also, in hypertension, increased CO accompanied by "normal" resistance, or increased total peripheral resistance (TPR), the two major clinically observable abnormalities, are considered as the primary hypertensive factors. Recalling the fact that only about one fifth (or even less) of the hypertensives are controlled to current goals, it can be deduced that this erroneous belief holds up necessary progress in research, diagnosis and therapy of hypertension.

In analysis of circulation based on Ohm's law, despite of its boom, CO and TPR falsely became nominated independent variables. Hence the confusion our texts and teaching in this field create when reducing complexity of the system to the simple, linear, and mechanistic explanation. It is hardly any wonder that our medical students, clinicians, and practitioners experience so serious difficulties in grasping the complete system and, as a result, take refuge in learning a few relationships by rote.

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## HYPOVOLEMIA AND ITS CORRECTION – CONTEMPORARY KNOWLEDGE AND CONTROVERSIES

L. Vokrouhlický<sup>1</sup>, L. Červinka<sup>2</sup>, R. Souček<sup>1</sup>,  
<sup>1</sup>Dept. of Clinical Pharmacology, RE&D VÚFB, Prague, <sup>2</sup>Dept. of Development and Clinical Evaluation, Infusia, L.t.d., Division Prague.

Serious pathological states are currently associated with fluid balance disturbances and adequate correction of these disturbances represents an important therapeutic action. The main aims of fluid resuscitation in critically ill patients are: restoration of normovolemia, renovation of a sufficient perfusion pressure of all organs, restoration of a normal equilibrium between different compartments of body fluids, achievement of an adequate colloid-osmotic plasmatic pressure, amelioration of the microcirculation and oxygen/nutrients delivery to tissue cells, prevention of reperfusion damage and if need be prevention of hypercoagulation and/or activation of inflammatory cascade systems. At present, fundamental controversies concern three questions: the use of crystalloid or colloid solutions, the use of albumin and the question of an most suitable colloid solution. Valuable metaanalyses have prove that an individual approach in accordance with the etiology of the critical state must be applied. When extravascular fluids depletion is prevailing, use of crystalloid solutions is suitable, in case of hypovolemia colloid solutions.

Contemporary opinions agree that routine use of albumin is inappropriate. Its application in critically ill patients provoked a higher mortality rate caused mainly by capillary leak of albumin.

Also the question of the choice of an optimum colloid solution is not yet solved and new discussions run since 1998. Frozen plasma carries the risk of transmitting infection and of unfavourable influence on the immunocompetence; therefore it is not recommended. The use of gelatine preparations is mostly abandoned due to their short intravascular persistence and more marked risk of anaphylactic reactions. The most appropriate and all over the world most used are dextrans and hydroxyethylstarches which exert approximately the same efficacy. Advantages of dextrans consist in their antithrombotic effect and in their expressive effect on leukocyte-endothelial interaction (improvement of microcirculatory blood flow). In distinction from hydroxyethylstarches dextrans do not form any tissue deposits and an effective prevention of anaphylactic reactions by means of monovalence dextran 1 use exists.

## GENE EXPRESSION OF HEPICIDIN IN EXPERIMENTAL IRON KINETICS DISTURBANCES

M. Vokurka, J. Krijt, E. Nečas  
 Institute of Pathological Physiology, 1<sup>st</sup> Medical Faculty, Charles University, Prague, Czech Republic, e-mail: mvoku@lf1.cuni.cz

Hepcidin, a disulfide-bonded peptide synthesized predominantly in the liver and secreted in the plasma, is a central regulator in iron homeostasis. It decreases iron intestinal absorption and leads to iron sequestration in macrophages. Apart from iron, hepcidin gene is up-regulated in mice by lipopolysaccharide and IL-6. In humans, hepcidin is supposed to play a role in the pathogenesis of anemia of chronic diseases, its mutations cause rare forms of juvenile hemochromatosis (type 2B) and its dysregulation was described also in other types (1, 2A and 3) of hereditary hemochromatosis. However, regulation of hepcidin gene expression and mechanisms of its co-operation with other molecules involved in iron kinetics (HFE, hemojuvelin, transferrin receptors, ferroportin 1 etc.) as well as the mechanisms of its action still remain largely unknown.

We studied hepatic gene expression of hepcidin in male mice C57BL/6 under several conditions – phenylhydrazine-induced hemolysis alone or combined with sublethal irradiation, erythropoietin administration and transfusion-induced polycythemia. Amount of mRNA for hepcidin and other genes was determined by real-time PCR and expressed as relative to the amount of mRNA for beta-actin.

Important increase of hepcidin gene expression (up to 35 fold) was observed after redistribution of iron to the liver due to phenylhydrazine-induced hemolysis combined with radiation-suppressed erythropoiesis. Acute or chronic hemolysis did not lead to significant hepcidin changes, irradiation and transfusion-induced polycythemia increased hepcidin expression maximally 5 fold while erythropoietin administration caused its significant decrease.

Based on these observations, we assume that hepcidin gene expression is mainly sensitive to iron flux to or from storage organs.

## SURFACE LEUKOCYTE ACTIVATION MARKERS IN CORD BLOOD OF TERM HEALTHY NEONATES

D. Vokurková<sup>1</sup>, P. Rozsival<sup>2</sup>, B. Buriánková<sup>2</sup>, E. Pařízková<sup>2</sup>, C. Andrýs<sup>1</sup>  
<sup>1</sup>Institute of Clinical Immunology and Allergology, Charles University, Medical Faculty, Hradec Králové, Czech Republic, <sup>2</sup>Department of Pediatrics, Charles University School of Medicine, University Hospital, Hradec Králové, e-mail: vokurkovad@lfhk.cuni.cz

To assess leukocyte activation markers expression in cord blood of healthy term neonates. Diagnosis of perinatal infection in the newborn is difficult and the use of combination of markers seems necessary.

13 healthy newborns without infectious risk were evaluated. Cord blood was analyzed using fluorescein labeled monoclonal antibodies and three-color flow cytometry. The phenotype of leukocytes was characterized for expression of activation markers on T lymphocytes (CD3+/CD69+, CD3+/CD25+) and B lymphocytes (CD19+/CD69+, CD19+/CD23+). Presence of early (CD45RA+) and late (CD45RO+) activation marker on CD4+, CD38+ on CD8+, expression of HLA-DR+ and CD16+ on monocytes, CD64+ and CD11b+ on granulocytes and NK cells was also assessed.

Flow cytometric analysis of cells using monoclonal antibodies showed these relative values of leukocyte subsets (mean±SD): CD3+ cells: 70.4±10.1 %; CD4+ cells: 48.7±8.9 %; CD8+ cells: 27.4±6.4 %; CD19+ cells: 13.8±8.6 %; NK cells: 8.9±3.7 %; CD3+/CD69+: 1.1±0.4 %; CD3+/CD25+: 7.0±2.1 %; CD19+/CD69+: 1.3±1.0 %; CD4+/CD45RA+: 63.0±7.5 %; CD4+/CD45RO+: 2.3±0.8 %; CD8+/CD38+: 92.5±2.0 %; CD14+/DR+: 61.3±18.6 %; CD14+/CD16+: 6.2±2.9 %; CD16+/CD64+: 2.8±5.8 %; CD16+/CD11b+: 99.7±0.9 %.

We characterized a wide panel of leukocyte activation markers in cord blood of healthy term neonates, in contrast to literary works, where the number of investigated markers is usually lower. Thus within several hours, we can see the whole “profile” of neonate’s leukocytes and hopefully diagnose infection early. Investigation of leukocyte activation markers expression changes depending on gestation duration is necessary.

Literature: Ottolini et al.: *Pediatr. Infect. Dis. J.* 22(5): 430-4, 2003. Skrzeczynska et al.: *Scand. J. Immunol.* 55: 629-38, 2002. Marchant et al.: *J. Clin. Invest.* 111(11): 1747-55, 2003. Nupponen et al: *Pediatrics* 108(1), 2001.

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## IMMUNOPHENOTYPIC ANALYSIS OF LYMPHOCYTE APOPTOSIS UPON GAMMA IRRADIATION IN VITRO

D. Vokurková<sup>1</sup>, J. Šinkora<sup>2</sup>, J. Vávrová<sup>3</sup>, M. Řezáčová  
<sup>1</sup>Inst. Clin. Immunol. Allergol., Charles Univ., Medical Faculty, Hradec Králové, <sup>2</sup>DakoCytomation AG, Brno, <sup>3</sup>Dept. Radiobiol., Purkyně Milit. Med. Acad., Hradec Králové, <sup>4</sup>Dept. Med. Biochem., Medical Faculty, Hradec Králové, Charles Univ., Hradec Králové, Czech Republic, E-mail: vokurkovad@lfhk.cuni.cz

The aim of this study was to introduce a simple and reliable flow cytometric method to detect apoptosis in human lymphocyte subsets upon irradiation *in vitro*.

Peripheral blood mononuclear cells (PBMC) isolated from healthy volunteers were irradiated using a <sup>60</sup>Co gamma-ray source with a dose of 3, 5, 7, 10 a 20Gy, respectively, and incubated for 16 hrs in fetal calf serum-supplemented medium in humidified atmosphere at 37°C. After that, cell surfaces were stained with the combination of RPE- and APC-labeled monoclonal antibodies directed against T cell markers and FITC-conjugated Annexin V (AnnV), which specifically binds to phosphatidyl serine exposed on the cell surface during early stages of apoptosis due to the loss of plasma membrane asymmetry. Plasma membrane permeability has been measured by propidium iodide (PI) exclusion. Four-colour analysis has been performed on the FACSCalibur flow cytometer.

The combination of fluorochromes used has allowed for distinguishing major T cell subsets in different stages of apoptosis. AnnV- and PI-negative cells were supposed to represent intact cells while binding of AnnV to PI-negative cells represented early apoptosis. The latter cells could be classified into major T-cell subsets as AnnV-FITC fluorescence did not interfere with R-PE and APC emission. Interestingly, the loss of membrane asymmetry has been accompanied by a decrease of both CD4 and CD8 expression while the surface density of CD3 was not affected at this stage. Low PI fluorescence events corresponded to cells possessing residual amounts of CD markers while PI-bright objects appeared to represent apoptotic bodies with no surface phenotype. CD8<sup>+</sup> T cells appeared to be more sensitive to both *in vitro* cultivation and gamma irradiation than CD4<sup>+</sup> ones.

**SOME NEURO-IMMUNE CONSEQUENCES OF NEURO-DEGENERATION IN LURCHER MICE DERIVED FROM TWO STRAINS**F. Vožeh<sup>1</sup>, P. Šíma<sup>2</sup>, M. Virtová<sup>2</sup>

<sup>1</sup>Department of Pathophysiology, Faculty of Medicine, Charles University, Pilsen, Czech Republic, <sup>2</sup>Division of Immunology and Gnotobiology, Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic,  
E-mail: frantisek.vozeh@lfp.cuni.cz

In our previous papers we described special functional and morphological changes in the brain as well as in some endocrine and immune organs of neurodefective Lurcher mutant mice derived from C3H strain (1,2). In this work we analyzed and compared some functional immune parameters in Lurcher mutants derived from two different strains (C3H, C57Bl/7). In the thymus where we already described signs of so called "accidental involution" in C3H Lurcher mutants, in this work a significant reduction in the proliferation index and lower representation of CD4<sup>+</sup> and CD8<sup>+</sup> cells in 3 month old Lurchers of both strains were found. Differentiation stages of double negative thymocytes exhibited significant differences only in Lurchers C3H when compared with healthy controls, while in C57Bl/7 animals the differences were insignificant. In Lurchers' peripheral immune organs (spleen, axillar lymph nodes) and in peripheral blood we obtained results showing affected immune functions in a sense of weakening. On the other hand there were also evident strain dependent differences which mildly modified the results with relationship to the strain origin. Results obtained confirmed that changes of immune functions found in Lurcher mutant mice are the consequence of disturbance of regulatory role of the neuroendocrine system affected by neurodegeneration despite clear but not substantial strain differences. The work further complements knowledge about multilateral functional relationships between neuroendocrine and immune systems in both normal and pathological conditions.

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