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FREQUENCY ANALYSIS OF INSPIRATORY ELECTRICAL ACTIVITIES DURING QUIET BREATHING AND COUGHING BY FAST FOURIER AND WAVELET TRANSFORMS

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The frequency analysis (Fast Fourier and Wavelet transforms) of the phrenic nerve activity was performed during inspiratory phase of the tracheobronchial cough and quiet inspiration of eupnoeic breathing. Mechanically induced tracheobronchial cough was evoked in cats anaesthetized either by chloralose or pentobarbital. Four frequency bands, which represent percentage intervals of total spectral power were analyzed by Fast Fourier Transform. Five frequency bands determined by morlet as mother wavelet were analyzed by Wavelet Transform. Both methods resulted in comparable results. It was shown that total power of inspiratory phase of the tracheobronchial cough was several times higher, comparing to quiet inspiration ($p<0.001$). The power was concentrated mostly within the range of lower frequencies (below 80 Hz) for tracheobronchial cough, comparing to quiet inspirations. The effect of anesthesia was manifested by different frequency width of power bands. The spectral power of quiet inspirations was cumulated in the space of low frequencies for chloralose compared to pentobarbital anesthesia. It seems that the kind of anesthesia differently affected the activity of individual brainstem neuronal populations leading to modified phrenic motoneuronal output and consequently altered spectral characteristics. The analysis of the electrophysiological signals from respiratory nerves and muscles is useful way how to gather new informations about the neuronal mechanisms of breathing and the airway reflexes. This study was supported by the grant APVV 20 - 047705.

THE VIABILITY OF HUMAN OVARIAN CARCINOMA CELL LINES AFTER ULTRASOUND EXPOSURE AND CISPLATIN TREATMENT

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The use of cytostatic drugs based on heavy metal complexes represents one of the present treatment possibilities in cancer therapy. However, despite their benefits, utilisation is limited by several factors, e.g., by the resistance of the cancer cells to the cytostatic drugs used. This resistance can be caused, among others, by a change of cytoplasmic membrane proteins which reduces transport of the drug into the cell or increases its efflux. The action of ultrasound on cellular structures, particularly the possibility of influencing biological membranes seems a possible way of enhancing the uptake of cytostatic drugs by cells. Considering the nature of the action of ultrasound, i.e. rearrangement and change of porosity of cellular membranes, this treatment seems suitable for overcoming the resistance to chemotherapy. As a model *in vitro* system, we have chosen two human ovarian carcinoma cell lines A2780 and A2780cis (the latter one is cisplatin resistant) treated by a cytostatic platinum derivative *cis*-(diamminedichloro) platinum(II) - cisplatin. The main goal of this work was to study how the viability of the chosen carcinoma cell lines is influenced by ultrasound of given frequencies and intensities applied simultaneously with the cisplatin treatment. During the experiments, cell proliferation was compared in the following experimental groups: non-treated control cells, ultrasound treated cells, ultrasound treated cells in the presence of cispaltin (cisPt+us), ultrasound treated cells followed by treatment with cisplatin (us+cisPt), and cells treated by cisplatin only (cisPt). The A2780 cells were exposed to a continuous horizontal ultrasound beam with intensities of 0.5 W/cm^2, 1 W/cm^2 and 1.5 W/cm^2 for 10 min. at 37 °C. The A2780cis cells were treated in the same way but only at 1 W/cm^2. We used an ultrasound therapeutic generator BeautyLine BTL-7 (1 MHz, probe active surface area 4 cm^2). After the treatment, the cells were pipetted into 96-well microplates and incubated with cisplatin for 72 hours. Their viability was then evaluated by means of the MTT test. The blue product was measured by an EL800 microplate reader at 570 nm. Cancer cell viability was affected by the presence of cisplatin, by ultrasound or by a combination of both. It is evident from the results obtained, that the viability was influenced not only by different intensity of ultrasound but also by experimental design (experimental groups cisPt+us and us+cisPt). In the A2780 cells, a statistically significant difference was found after ultrasound treatment at intensities of 0.5 W/cm^2 and 1 W/cm^2 but not at 1.5 W/cm^2. In the experiments with a combined action of cisplatin and ultrasound, it was found that cell viability in the group cisPt+us (ultrasound treatment in presence of cisplatin) was lower than in the group us+cisPt. In our opinion, a possible explanation of this finding consists in the expected action of ultrasound on cellular membranes with respect to increase in membrane porosity and loosened cell surface. At the highest intensity of ultrasound (1.5 W/cm^2) the ultrasound effect dominates. In the experiment with cisplatin resistant A2780cis cells, the statistically significant difference between both groups (cisPt+us and us+cisPt) was not demonstrated, however, the viability in cisPt+us expressed as median is lower than in the groups us+cisPt and cisPt.

EFFECT OF ACUTE SMOKING ON PERIPHERAL PULSE WAVE CONTOUR

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Pulse wave analysis is one of many methods used to assess arterial stifness. The most popular non-invasive methods are based on pletysmographic principles. Others include computer oscilometry, ultrasonography and applied tonometry. The shape of the pulse wave is determined by a number of factors, as age, sex, body height or physical fittness. Pulse wave analysis is also often used for predicting cardiovascular diseases. Smoking is one of the most important determinants of increased arterial stiffness and this accelerates the sclerotic process. The main goal of this study was to investigate the effect of acute smoking on arterial stiffness at a peripheral site using pulse wave analysis. Forty five smokers (19 males, 26 females) of average age 24,3 ± 2,4 year were included in the study. Each person had to smoke one cigarette of nicotine content of 0,9 mg. Parameters of pulse wave were measured before and after smoking. Four parameters, PWV (pulse wave velocity), RI (reflection index), CT (crest time) and IWD (interwave distance) were evaluated by means of an adapted device based on pletysmographic principles that transform volume changes to voltage changes. PWV was 1,12 m/s higher after smoking which presents 13% increase. RI was also significantly higher after smoking (42,49 ± 6,7% versus 49,55 ± 7,71%, $P<0,001$). IWD increased after smoking from 9,21 ± 0,83% to 11,34 ± 1,1%, ($P<0,001$). We detected a small augmentation in CT (0,01 s) after smoking. Acute tobacco smoking is associated with endothelial dysfunction. We found increased values for all assessed parameters after smoking. Both PWV and RI are considered to be good predictors of augmented arterial stiffness. Although we only investigated the effect of acute smoking it can be assumed that long-term effect of smoking could markedly deteriorate a function of cardiovascular system. This work was supported by the Ministry of Education of the Czech Republic MSM 6198959215.

LONG-TERM FUNCTIONAL EFFECT OF THE AMBLYOGENIC FACTORS´ SCREENING: METHODOLOGICAL ASPECTS

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Amblyopia represents the most frequent causation of the insufficient monocular vision in working age. Inception of therapy in early childhood is considered to be a crucial prerequisite for optimal long-term results of therapy. Hence, the methodology of screening for amblyogenic factors is in the focus of research. Our retrospective study

was targetted to the estimation how the toddlers´ participation at screening programme modify their chances for good binocular vision in early school age. We have analysed retrospective data (years 2000-2007) of two patients´ groups. 419 randomly selected children indicated to paedo-ophthalmological care due to positive result at photoscreening test set up a group SC. Second group, matched by age and intersexual ratio, was constituted from 263 children referred by paediatric practitioners (group PP). Two causal relations were studied: (1) the degree of monocular vision reduction (amblyopia) as dependent variable related to the degree of (inborn) anizometropia and age (independent variables) and (2) the degree of binocular function reduction (strabismus) as dependent variable related to the degree of (inborn) hypermetropia and age (independent variables). Due to data comparativity, we systematically subdivided SC and PP groups according to (1) monocular, best corrected central visual acuity (VE % of amblyopic eye), (2) severity of strabismus related binocular disturbance (four degree scale: „ortophoria“, „heterophoria“, „heterotropia with harmonious anomal retinal correspondence“, „heterotropia with strabismc amblyopia“), (3) anisometropia (difference between spherical equivalents of last cycloplegic refraction), (4) hypermetropia (spherical equivalent of last cycloplegic refraction of better eye) and (5) age (three degree scale: „2-3 years“ (24 – 47 months), „4-5 years“ (48 – 71 months) a „6-7 years“ (72 – 95 months). Statistical analysis was applied on the numbers of patients in each sub-grous. The $\chi 2$-tests of (sub)samples distribution homogenity followed by analysis of addjust residuals were performed on the level of all two-dimensional cross-tables. The results of data analysis have documented the significantly lower age during first paedo-ophthalmological examination of patients from SC group compared to PP group. The difference in the functional consequene (i.e. degree of amblyopia) of comparable anizometropia were recorded only in youngest subgroups. Significantly higher (P=0,034) values of VE % were recorded in SC group. In older pre-school children („4-5 years“) and younger school range of „6–7 years“, no intergroup differences were documented. This results could imply the supposition that the participation in photoscreening in toddler age do not offer any advantage in visual acuity of amblyopic eye in long-term perspective if the inception of therapy is not postponed over tree years of age. In contrary, data describing severity of binocular disturbances related to strabismus were consistently worse at PP group in all age ranges. The proportion of heterophorias and heterotropias related to comparable degree of hypermetropia were significantly higher in PP group in age ranges of „2-3 years“ (P<0,001) and „4-5 years“ (P=0,004). The same trend in age range of „6–7 years“ was not significant probably due to small number of samples. This data could emerge the hypothesis about higher age during first paedo-ophthalmological exam of children referred by paediatric practitioner (group PP) due to early symptoms and signs of manifest strabismus reduce the chances for optimal therapy results. The participation in screening programme (otherwise focused to different pathological entity, i.e. amblyopia) offers chance for efficient strabismus therapy. Applied methodology based on sub-dividing of both experimetal group and consequent statistical analysis of samples distribution homogenity approved pertinency for such a study design.

MATHEMATICAL MODEL OF HUMAN VISUAL SYSTEM FOR IMAGE QUALITY EVALUATION

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Testing of image quality is today in the time of digital multimedia very important task. I deal with evaluation of image quality by various methods and comparison of their results. Generally, there are several ways how to assess image quality. Three main approaches are: subjective testing, objective testing and image quality evaluation using a human visual system model (HVS). The subjective testing is based on human perception. A group of observers evaluate a set of images. It is expensive and time demanding, but the results matching with human perception (only for a set of tested images). The objective testing is based on mathematical algorithms that compare image matrixes (e.g. MSE, MAE, PSNR). Objective evaluation is cheap fast and gives the results in real time, but it very often not corresponds with human perception. These methods had technical origin in the measuring of digital transmission systems. The HVS models simulate process of the human vision with respecting the human physiology of perception properties. These methods are cheap quick and give results that have good correlation with human perception. I design HVS model for image quality evaluation. The main requirements on the model were: to evaluate quality of general image scene (independent on the type of distortion), high correlation with human perception, respecting of observer viewing conditions and speed of evaluation. Inputs of the model are two images: original and distorted. Both images are processed by the five computational blocks of model and results of this processing are compared in differential metrics to obtain number that represents image quality. First processing block of the model represent optical part of the eye. Input parameters are surrounding illumination (that give information of the pupil diameter), size of the image and viewing distance (these values are used for diffraction computing of circular aperture). From these values is computed PSF (Point Spread Function) that is applied using convolution on image. The block of optical attenuation is then applied (it simulates CSF - Contrast Sensitivity Function). Next block is colour transformation from input RGB colour space to CIE Lab colour space via XYZ colour space. This simulates human physiology of colour coding (three channels: lightness, red-green and blue-yellow). Following space filtration (simulates high brain processing) image is separated to six frequency octave bands. Than is in each band except base-band applied orientation filtering (in six orientation 0°, 30°, 60°, 90°, 120° a 150°). So computation is made in 31 channels. Results of these filtering are compared for original and distorted image using Minkowski summation. These results are squared, summed, square root and divided by number of points. This gives a number that represent image quality. Model was tested with various scenes including big homogenous regions, text, textures, image edges and face colour and various compression methods (JPEG, JPEG200, LRW, DCT, fractals) in compression ratios form 20 to 260. Results were compared with the Subjective testing on the same set of images. Average correlation (mean value from correlation for each set of testing images) was -0.97. Designed model shows its suitability for image quality testing due to its correlation with subjective tests.

EQUATION FOR PROPAGATION OF MECHANICAL WAVE IN VISCOELASTIC TUBE

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Mechanical behavior of biological materials in dynamic loading depends on its elastic as well as viscose properties (Bia *et al.* 2007). Similarly, the propagation of pulse wave in viscoelastic materials depends on its elastic and viscose parameters (Šrámek 1995). Measuring of pulse wave velocity (PWV) in arteries is a noninvasive method of determination of mechanical parameters of arteries and may be used as independent predictor of cardiovascular mortality. Unfortunately, the satisfactory theoretical background for solution of corresponding inverse problem does not exist at present. Connection between PWV and mechanical properties of aortas is currently calculated on the bases of Moens-Korteweg equation which is derived from purely elastic model. The elastic model – elastic tube with incompressible fluid inside – inevitably leads to conclusion that PWV should be frequency independent and its propagation is without damping. It is in apparent contradiction with reality. Main inadequacy consists in neglecting viscose properties of tube wall and fluid inside.
To contribute to more exact solution of these problems we derived following partial differential equations describing pulse wave in viscoelastic tube

$$-\frac{\partial F}{\partial x} = M.\frac{\partial v}{\partial t} + N.v$$

$$-\frac{\partial v}{\partial x} = V.F + H.\frac{\partial F}{\partial t}$$

$$P = \frac{1}{2\pi y_0} \cdot \frac{dF}{dx}$$

$$v = \partial y / \partial t,$$

where F is the force effecting on the surface of segment of the tube, P is the net pressure on surface of segment, y_0 is the static radius of tube, y is the radius of tube.

For coefficients M, N, V, H holds:

$$N = \frac{4.\pi}{3}.\eta,$$

where η je is the viscosity of fluid.

$$M = \frac{2\pi\rho}{3} y_0^2,$$

where ρ je the density of fluid

$$V = \frac{1}{2\pi y_0 D.\eta^*}.,$$

where D is the thickness of tube wall, η^* is the viscose coefficient of wall.

$$H = \frac{1}{2\pi y_0 D.G^*},$$

where G^* is the elastic modulus of tube wall in shear stress.

For PWS holds:

$$v = \omega / \alpha.$$

$$\alpha = \sqrt{\frac{1}{2}(-N.V + \omega^2 M.H + \frac{1}{2}\sqrt{N^2 + \omega^2.M^2(V^2 + \omega^2 H^2)}}$$

The above presented theory and formulas where experimentally verified in our laboratory.

Conclusions:

1) The velocity of pulse wave depends on frequency. The higher is frequency the higher is velocity

2) The damping of wave depends on frequency.

3) The theory enables calculation of mechanical impedance of tube and, consequently solve the problems of mechanical matching of different tubes and reflection of wave.

4) The theory enables estimation elastic parameters (stiffness) as well as viscose parameters of wall (solve inverse problem).

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STABILITY AND CONFORMATION OF DNA IN DIVALENT CATION SOLUTIONS

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Interactions of DNA with divalent cations have been studied extensively for several decades due to their biological application, as an anticancer drug, probes of nucleic acid damage. Studies on DNA interactions with ions are of great interest owing to the crutial role which cations play in functioning of genetic apparatus *in vivo.* Transition metals have a strong base-affinity, they can chelate or coordinate directly to bases, resulting in a destabilization of DNA. Alkaline earth cations interact primarly with DNA phosphates can neutralize the negative charge and enhance the base stacking. The two bindings are both capable of altering the geometry of a nucleotide. Some investigations demonstrated that divalent ions not only can induce DNA condensation or aggregation, but also with water can alter its secondary or tertiary structure. In this present study, we investigate the effect of calcium ions on the parameters of the thermal transition of DNA at low Na^+ concentration. As a target of divalent ions interaction, calf thymus DNA (Sigma, molecular mass 1.9×10^7 Dalton) and DNA from chicken erythrocytes in aqueous solution was employed. The concentration of Na^+, taking into account the counter-ions introduced with DNA, was 4.2×10^{-3} M. The microcalorimetric investigations were made using a DSAM-4 differential scanning calorimeter. The temperature scanning rate was 1°C/ min. This enabled us to observe the complex nature of the process and evaluate the temperature and enthalpy of the transition in a wide range of concentrations of divalent ions. As the transition temperature T_m we took the value of the temperature of the maximum of the melting curve, width of the transition interval ΔT was defined as the halfwidth of the peak, the area bounded by the curve of the temperature dependence of the heat capacity $C_p = f(T)$ corresponds to the heat of the observed conversion process ΔH_{cal}. The maximum error of the heat capacity measurements did not exceed 1.5 % in the 0-100 °C. The helix-coil transition of deoxyribonucleic acid in presence of chloride salt of Ca^{2+} was studied at elevated temperatures in the range from 20 °C. to 100 °C. The Ca^{2+} concentration was varied between 0 and 20 $[Ca^{2+}]/[P]$. The secondary structure of DNA remained in the frame of the B-form family in the whole ions concentration range at room temperature. No significant DNA denaturation was revealed at room temperature even at the highest concentration of calcium ions studied. The dependence of the melting temperature of DNA, the width of its melting curve, and the enthalpy of the helix-coil transition on the molar ratio $[Ca^{2+}]/[PO_2^-]$ have been determined. The thermal stability of DNA is affected by the ion concentration and the nature of solvent. It increases at low $[Ca^{2+}]/[PO_2^-]$ ratios and the melting temperature T_m, ΔT increases relative to characteristic for DNA without divalent ions and ΔH_{vH} decrease. This implies than cations stabilize the DNA structure by reducing the charge repulsion between the phosphate groups. When the concentration Ca^{2+} increases, T_m passes through the maximum and ΔT decreases. It is due to elevating of cooperativity of the transition of DNA. With a further increase of the ion concentration T_m, ΔT and ΔH_{cal} changes very slightly, and decreases at high concentrations. DNA denaturation and a significant decrease of the melting temperature T_m, ΔT and ΔH_{cal} of DNA connected with a decrease of the stability of DNA induced by Ca^{2+} ions occurred and demonstrated sensitivity to DNA condensation and aggregation as well as an ability to distinguish between these two processes. No condensation or aggregation of DNA was observed at room temperature at any of the ion concentrations studied. This work was supported by VEGA grant 1/3403/06.

PHOTODYNAMIC EFFECT STUDY ON HUMAN LUNG CANCER CELL LINES AND OSTEOSARCOMA CELL LINES

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Photodynamic therapy uses the interaction of sensitizers and light to destroy cancerous cells and tumors. A potential benefit of PDT is that it causes minimal damage to healthy tissue. The photochemical interaction in the presence of molecular oxygen produces cytotoxic singlet oxygen ($^1O_2$) and other forms of active oxygen, such as hydroxyl radical etc. The tumor is destroyed either by $^1O_2$ (generated *via* energy transfer from excited sensitizer to triplet oxygen, type II mechanism) or radical products (generated *via* electron transfer from excited sensitizer, type I

mechanism). The selectivity of tumor damage depends on specific retention of a sensitizer in the tumor tissue after systemic administration combined with directed illumination. The cellular effects of PDT include plasma membrane, lysosomes and mitochondria damage leading to tumor ablation. The efficiency of sensitizers in situ is most likely to be dependent on their local accumulation and specific cellular uptake in the tumor site, stimulating research toward the development of water soluble and efficient in vivo sensitizer – delivery system with a high potential to target specific organs.We report the production of reactive oxygen species and the phototoxicity of photodynamic sensitizer palladium(II) *meso*-tetrakis(4-sulfonatophenyl)porphyrin ($PdTPPS_4$) on human lung cancer cell lines A549 and osteosarcoma cell lines HOS. The light emitting diodes (LEDs) were used as a source for evocation of the photodynamic effect. We investigate the concentration dose dependency of sensitizer in combination with LEDs irradiation on photodamage of cancer cells by in vitro methods. Viability of cells was determined by MTT assay. The quantitative changes of cell viability in relation to sensitizer concentrations and irradiation doses were proved by fluorometric measurement. A549 lung cancer cells and HOS osteosarcoma cells are sensitive to photodynamic damage and our results indicate decrease of viability and time-course of ROS production within cancer cells during photodynamic therapy *in vitro*.This work was supported by the Ministry of Education of the Czech Republic MSM 6198959216 and MSM 6198959215.

EVALUATION OF PHOTODYNAMIC AND SONODYNAMIC REACTION ON CANCER CELL LINES BY FLUORESCENCE METHODS AND ATOMIC FORCE MICROSCOPY

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Photodynamic therapy utilizes a combination of a photosensitizing chemical and visible light for the treatment of solid malignancies and is also showing promise as a treatment modality for many nonmalignant diseases including atherosclerosis deposits inside arteries. When the photosensitizers absorb light of an appropriate wavelength, it may cause their excitation with subsequent energy transfer to oxygen. Then the oxygen becomes highly reactive in cancer cells. The tumor is destroyed either by reactive oxygen species (ROS), type II mechanism, or radical products, type I mechanism, generated in an energy transfer reaction. The resulting damage to organelles within malignant cells leads to tumor ablation. Sonodynamic therapy is the use of an agent that is sensitive to ultrasound, allowing deeper penetration and destroying of abnormal cells. Ultrasound-induced cytotoxicity of sonochemical sensitizers inhibits tumor growth In this study disulfonated chloroaluminum phthalocyanine was selected for testing as a potential sensitizer for combination of sonodynamic and photodynamic therapy.We report the production of reactive oxygen species on G361 melanoma cells. The production of ROS was investigated by molecular probe CM-H_2DCFDA. The light emitting diodes (LEDs 670 nm, FWHM 15 nm, 10 $mW.cm^{-2}$)) were used as a source for evocation of the photodynamic effect. Ultrasound generator with transducer area 4cm^{-2}, frequency 1 MHz and intensity 2 $W.cm^{-2}$ was used for evocation of sonodynamic effect. Changes in cells were evaluated using fluorescence microscope and atomic force microscopy. The quantitative ROS production changes in relation to sensitizer concentration, irradiation doses and ultrasound intensity were proved by fluororeader. Ultrasound treatment can support the photodynamic effect because sensitizer can be relocalizated in the cells. Efficiency of photodynamic therapy and sonodynamic therapy is affected by a number of factors including absorption spectrum of the photosensitizer, wavelength of the activation light, depth of the light and ultrasound penetration in the biological tissue, tissue answer on singlet oxygen. Our results indicate a synergistic effect of chloroaluminium phthalocyanine, light and ultrasound on reactive oxygen species production in G361 melanoma cells. This work was supported by the Ministry of Education of the Czech Republic MSM 6198959216 and MSM 6198959215.

PROGRESS IN PACING TECHNOLOGY

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Pacing (CRM) system consists of a device and of one, two or three leads. Leads are implanted venously and device subcutaneously. The use of CRM systems, including implantable cardioverters defibrillators ICD and pacemakers PM, increase worldwide. In The Czech Republic, there were 1481 implants of ICD, and 8195 implants of PM in 2007. Devices can be divided according to treated arrhythmia. Pacemakers are used for bradycardia, ICD (shock and pacing) for tachycardia. Another type is cardiac resynchronization therapy consisting in the implantation of the third lead to coronary venous, provided synchronization of both ventricles and treating of heart failure. From current clinical studies and challenges, the next progress in the industry can be assessed. The use of wand-less telemetry and consequently remote device control is currently taking place. The passive version of regular programmer is placed in the patient household. It would load data from devices and send to evaluation center, in regular time intervals. This enables the physicians to be informed about major episodes immediately. Automatic algorithms enhance the options of devices with regard to inadequate therapy diverting (discrimination algorithm), battery longevity increasing (automatic setting of pacing output) or right ventricle pacing optimalization. Discrimination algorithms can compare intracardial morphology of ventricular tachycardia or normal sinus rhythm or tracked tachycardia from atrium. The sensitivity of these algorithms achieves 100%; specificity is about 90%, maximum 94%. Setting of appropriate pacing output can significantly save the battery power and prolong the longevity of device up to several years. An additional electronic sensing channel for determination of sufficient pacing impulse is used by one type of the automatic algorithms. The stimulation threshold can be measured for example once a day by progressive falling of stimulation output until lost of capture. Using new materials brings MR procedure to CRM patients. Recently the clinical study with MR resistive pacemakers is conducted. One Czech center is also participating. Miniaturization of LV leads allows proper LV stimulation and better resynchronization therapy. Active implantable medical devices are dynamically developing area of implementation of biophysical principles for benefit of the patients. The use still increases worldwide. This work was supported by the Ministry of Education of the Czech Republic MSM 6198959215.

THE MEASUREMENT AND EVALUATION OF THE ELECTRIC PARAMETERS OF A DUAL-CHAMBER PACE-MAKER

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The pacemaker is an electronic device assuming the primary function of myocardial muscle pacing by generating of electric impulses in patients with sinus node dysfunction or cardiac conduction system dysfunction. Pacing can be divided from various standpoints: into indirect pacing (through surrounding tissues) and direct pacing (performed in the heart cavity), or according to the duration of the pacemaker application into temporary (with the pacemaker outside the patient´s body) or permanent (the pacemaker placed under the skin), according to the dependence on the heart action into asynchronous or synchronous pacing, according to point of the pacing into single-chamber and dual-chamber pacing. The measurement on a dual-chamber pacemaker begins with the construction of a unit for the simulation of the hearth action in a patient´s body. It has an adjustable amplitude (0 – 20 mV), frequency (0 – 100 Hz) and mark-space ratio. The system output impedance should be within the range of 200 – 2000 Ω. It is possible to add a defined noise into the output channel. It is further possible to measure the tolerance of sensing of the atrial and ventricular channel of the dual-chamber pacemaker DDDR. The equipment under test is the Guidant

pacemaker, model 1294, type DDDR with attached IS-1 type electrodes. The next component is the Guidant programmer, model 3120, with accessories. The unit is connected through the Humusoft MF624 measurement card to a computer and to an oscilloscope. The values with low impedance around 180 Ω reach a certain voltage level, but they do not correspond to the setup values. The voltage value of the pulse drops when it it is too steep. The pacemaker tries to compensate the voltage drop by increasing the current to its maximum value which is 38 mA. The corresponding energetic value at the maximum setup reaches 248,5 μJ. The impedance values around 490 Ω correspond to setup values and their maximum current carrying capacity is 13 mA. The energetic value is 86,2 mJ. With the impedance of 2470 Ω, the lowest drop of steepness is obtained. The current at the maximum values setup is equal to 3 mA. The energetic value is only 17,2 μJ. When observing the mentioned parameters, there are no substantial differences between the programmed values and the real values. When seeking suitable working pacing impedance, we place the electrodes so that they do not exhibit low impedance values which would decrease the batteries lifetime, but we also avoid the electrodes placement resulting in high impedance values which do not enable a sufficient amount of energy to pass into the cardiac muscle.

STATISTICAL SURVIVAL ANALYSIS

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This abstract is focused on possibilities how to use incomplete censored data in survival analysis. Survival analysis is most frequently applied to patients with oncological diseases when a study is finished and a number of individuals are still alive. The first important paper on this topic was announced a few years ago as the most cited statistical study in the biomedical area. This methods tries to mine a maximum relevant information from studies finished at a time when collected data has not been completed yet. Only so called „low estimation“ for the data is available. Such situations can be covered by the class called survival. A typical example of these data is a study (situation) where the dependent variable is the survival (the length of life) of oncological patients. There are many cases known both from literature and biometrical experience when the survival analysis has been applied to various materials and machinery or electrotechnical products (material fatigue). Even in the situations where the observed event is not patient‘s death but any selected event survival analysis can be used. The statistical department of the Institute of Medical Biophysics has been applying survival analysis for more than two decades. The first impuls to start with the method was a requirement for an analysis of incomplete survival data obtained mostly from oncological patients. These data was collected by internal physicians and haematooncologists. Generally survival analysis is a collection of methods that process the variable of a “time to an occurrence of an event”. This variable is also often called “time of survival” or even shortly “survival”. The “survival time” means a number of years, months, weeks or days from the beginning of the patient’s observance till the occurrence of an observed event (death as the rule). Most studies on survival analysis are finished before the observed event occurred for all subjects. This situation is called in survival analysis „censoring“.

After finishing a clinic study we obtain input data for a consequent statistical processing. There are two items of data for every patient:

1. survival time (means a time of an individual observation; also used for censored data)
2. reason why the individual observation was finished – coded as 1 for the observed event, 0 for censored data.

The goals of survival analysis are:

1. to estimate and interpret survivor and/or hazard function from survival data
2. to compare survivor functions
3. to assess the relationship of explanatory variables to survival time.

In practise survivor function is estimated with the Kaplan-Meier method. Values of the function $S(t)$ are calculated in all times when the observed event occured for any patient. The horizontal axis indicates time. The vertical axis displays survival probability in a time which ranges from 0 to 1.

The statistical department of the Institute of Medical Biophysics uses statistical programme SPSS. However the SPSS application is not able to display 95% confidence interval for survivor functions. To solve this problem we cooperated with a programmer and designed and implemented a new software program in our department. The program is written in C++ language with using MFC libraries and was developed in Visual Studio environment from Microsoft. This work was supported by the Ministry of Education of the Czech Republic MSM 6198959215.

S-BLM ON PLATINUM SUPPORT – ELECTROCHEMICAL STUDY OF BILAYER/D-GLUCOSE INTERACTIONS

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In biomedical applications membrane surface provides biocompatibility and can reduce nonspecific surface processes. A widespread approach of the membrane formation involves the non-covalent self-organization of a bilayer on freshly formed surfaces of hydrophilic metals. In this study, we took a strategy of deposition of supported bilayer lipid membrane (s-BLM) prepared from egg yolk 1,2-diacyl-*sn*-glycero-3-phosphocholine on the tip of a Teflon coated Pt electrode. Electrochemical impedance spectroscopy (EIS) over the frequency range from 10^{-1} to 10^{4} Hz at 50 mV excitation sine wave was applied to characterize s-BLM. Smoothed EIS data were analyzed using complex non-linear regression least square (CNRLS) fit to a model represented by an equivalent electrical circuit. The average membrane capacitance CPE_m (the constant-phase element representing the capacitive properties of the membrane) of the data collected on twelve s-BLMs after gradual thinning and stabilization at 10 kHz sinusoidal signal was CPE_m=(1.4±0.3) nF and the membrane resistance R_m=(87.2±10.7) MΩ. The value of the specific membrane resistance R_{sm}~0.17 MΩ.cm^2 demonstrates high insulating properties of s-BLM. The specific membrane capacitance CPE_{sm}~0.72 μF.cm^{-2} differs to some extent from that expected for the well-organized lipid bilayer. To gain additional insight on the formation of the highly resistive lipid film, monitoring of the time evolution of s-BLM was carried out. Development of the insulating lipid film showed that the capacitance and the resistance of the s-BLM differe from that one prepared by gradual thinning at 10 kHz sinusoidal signal. The decrease of effective thickness of s-BLM producing an increase in the membrane capacitance can be a consequence of a disorder and a loose packing of the first adjacent monolayer of lipid molecules or the presence of a simple monolayer on the rough support. Even if parameters of s-BLM appear not to correspond with the well-defined bilayer, the electrode surface was covered with the stable and highly electrically isolating lipid film. Subsequently, s-BLM was exploited to examine the influence of D-glucose as a trial of purposefulness of membrane utilization as a sensor. As sugars in solution or covalently linked to membrane have an effect on most probably the stability of bilayers, the possibility of glucose recognition based on the changes of bilayer parameters is not excluded. A selective molecular recognition can be transformed into changes of macroscopic parameters of the lipid film. Actually, the addition of D-glucose resulted in a change in the electrical parameters of the lipid film. The observed alteration was time dependent. A semicircle diameter corresponding to the contribution of resistance of the lipid phase R_m to the impedance on Nyquist plot of EIS spectra decreased as the consequence of 100 mmol.l^{-1} glucose addition. Gradual decrease implies a lower membrane resistance, possible due to the decrease of compactness of the film as well as the formation of defects. The EIS spectra remained unchanged up to 60 min. Within this time scale, s-BLM treated with glucose tended to be stable. The analysis of the data revealed the increase of the membrane capacitance and a slight increase of the CPE_m power α as well. The effect of glucose on the lipid film may be similar to that of trehalose. The glucose by analogy can act as a spacer between the lipids, affecting the water permeability of the bilayer and increasing the area per molecule. Increased water permeability and area per phospholipid molecule than result in the change of the membrane resistance and the membrane capacitance. Entrapment of excess water changes the dielectric properties of the s-BLM as well.

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ELECTRIC PROPERTIES OF A MEMBRANE AND ADSORPTION ISOTHERMS

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At an interaction of a substrate particles with model lipid membranes the particles can adsorb onto membrane surfaces (peripheral proteins), can insert into membrane between lipid molecules (cholesterol), some with formation of various pores or defects (alamethicin), or can build in existing defects in membranes (integral proteins, lipoproteins). The usual consequence of the interaction is a change of membrane parameters. The amount of substance particles integrated to the membrane (adsorption, or absorption) is characterized by adsorption isotherm, generally rather binding or association isotherm. The aim of our work is to find, or to utilise a relation between adsorption isotherm and some electric properties of a model lipid membrane at its interaction with the investigated substance. In our model, the surface of a membrane is composed of a few areas. They are: area covered with the regular lipid bilayer, area corresponding to pores or defects, area covered with adsorbed particles to the membrane surface and the area of the membrane surface targeted with integrated particles. The membrane capacitance C_M and its conductivity G_M are the summation of contributions of all particular areas. After a concentration change Δc of an investigated substance in the working solution and its following interaction with the membrane, the relative contributions of particular areas change and so do measured C_M and G_M. The theoretical dependences $C_M = f(c)$ and $C_M = g(c)$ considering the adsorption isotherms for the investigated system substance – membrane are confronted with the measured dependences. Some conclusions concerning the isotherm and corresponding interaction can be constructed. For the simple case of irreversible adsorption, the new adsorption isotherm was suggested using the model of decay law. In the model, the decrease of defects in the membrane or receptors on the surface (the decrease of "active sites"), as the consequence of their occupation or filling up by interacting particles, is considered as the decay of corresponding "active sites" on the membrane. The measured dependence of capacitance on the HDL concentration in the working solution fits better with our "decay law" isotherm, than with the Langmuir isotherm. The result correlates with our presumption, HDL particles interact preferably with defects in model lipid membranes. If glucose interaction is concerned, the "decay law" isotherm is less convenient then in previous case, because of a desorption, which should be considered here and defects are less efficient. At very low concentrations, the change of capacitance with the concentration is proportional to the concentration in both models. Possible binding isotherms, or binding mechanisms, help to elucidate some irregular dependences $C_M = f(c)$. The work was supported by VEGA grant 1/3403/06.

PERIPHERAL PULSE WAVE ANALYSIS IN MEASUREMENT OF PAIN

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The results of peripheral pulse wave analysis with respect to the perception of pain are presented. Time records of peripheral pulsation were obtained by screening the volume pulse of the radial artery. A self-constructed device for measurement and record of peripheral pulse wave was used (1, 2). A group of 50 patients (with average age of 22.7 years) was examined in rest and when feeling pain caused by routine surgical procedures (group I, n=31, 62% of patients) or present in a post-op period (group II, n=19, 38% of patients). An eleven-grade visual numeric scale – a part of Universal Pain Assessment Tool (UPAT) – was used for subjective assessment of pain. The average value of the intensity of pain was 3.89 in group I and 4.51 in group II. The obtained curves were analysed visually and severe time parameters were analysed. The visual analysis was performed by comparison of each pulse beat of the painful curve with a pulse beat of rest curve. The beats were distended to 300 to 300 px to the same diameters. The changes in the time course of descending part of systolic peak were observed. These changes were labelled as splits. An aberrant wave in a short, pre-dicrotic intercept of time axis was recorded. The dicrotic wave itself was noted in 56% of rare waves. With respect of the split shape differences, specific names were created – the bicornual split, the left and right unicornual split. In the records with left unicornual splits only, where no exact time parameters were measurable, these were named as equalisations.
The splitted waves were measured on time axis of the curve. Two new time parameters were established – the time of peak split (TPS) and the relative time of peak split RTPS = TPS / TPT (TPT – Total Pulse Time). The long-lasting average value of TPS was 123,332 ± 24,330 ms, the long-lasting value of RTPS was 0,210 ± 0,045. The Spearman's correlation analysis shoved a middle-heavy dependency between frequency of aberrant waves and pain intensity (r = 0,574) and between frequency of splits and pain intensity (r = 0,536). There was no dependency between frequency of equalisations or time parameters changes. This work was supported by the Ministry of Education of the Czech Republic MSM 6198959215.

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THE PILOT STUDY OF BRONCHODILATATION INFLUENCE ON SPEECH FLUENCYIN BALBUTIES

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Stuttering is a serious health and social problem that can distinctively affect not only the mental development of an individual but also his life possibilities, including social fulfilment and his general life prospects. Etiology of stuttering is however unknown and that is why it is not possible to treat it causally in spite of research including. In the pilot study carried out in 6 centres in the Czech Republic in accordance with a united protocol for the period of 6 months 42 patients were included. The bronchodilatation substance of formoterol (Foradil®) was given to the patients. This substance works throuth β_2 receptors for time of 6 months. The medicine was administered once a day in the morning in a dose of 12 μg. During 6 months the evaluation of effectiveness on the basis of primary and secondary parameters was realized in every patient. The prime parameter „Extent of stuttering" was evaluated according to the ordinary scale (McGill Pain Questionnare). The extent of stuttering was evaluated by examining physician during the visits in centres and currently by a patient himself (in cases of the youngest with the assistance of a parents) and every day it was writen down in The patient's diary. As an introduction EEG and EMG examinations were realized (but not discussed further), in the beginning and in the end there were biochemical examinations and four times on the whole measurements and records of speech fluency. Nonparametric in dual test (Wilcoxon Sign Rank test) was used for the comparison of average marks in the whole group of patients. According to the records in a patient´s diary (i.e. evaluation of speech fluency by a mark from 1 to 5, which was done by a patient himself/herself) there were 3 average marks calculated in every patient: - an average mark that evaluated the speech fluency in the period without formoterol, - an average mark that evaluated the speech fluency in the first period of formoterol use, that is from. The position of a median of average marks is drawn by a fat horizontal line for each period. The bottom and the top of the box identify the positions of the 1st and the 3rd quartiles. The height of the box corresponds with the so called interquartile span, as the characteristics of the data variability. With each box there are marked positions of minimal and maximal non-distant values, distant values (so called outliers) are displayed as an asterisk or circles. Statistically

significant difference between average marks of selfevaluation was proved by the Wilcoxon Sign Rank test. The evaluation of speech fluency was conducted daily by 42 patients with stuttering according to the ordinary scale K in the period prior to the application of formoterol, in the first period of application (month 1-3), and in the second period of application (month 4-6) of the pilot study called Verification of bronchodilation influence on speech fluency at stuttering juveniles and adult . Evaluation of the speech by physicians (registered four times throughout the study (at the beginning and then in two-month intervals) confirms the positive effect of pharmacological treatment of stuttering and the hypothesis of upper airways obstruction involved in the origin of stuttering.

EXPERIMENTAL DEVICE FOR BLOOD TEMPERATURE MANIPULATION DURING EXTRACORPOREAL CONTINUOUS RENAL REPLACEMENT METHOD

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Authors´ concern is the testing of technical possibilities of temperature manipulation in an extracorporeal circuit with a final plan of development the medical device suitable for body cooling in sepsis, in acute myocardial infarction and testing biocompatibility of extracorporeal continuous renal replacement method (CRRT). The particular authors´ concern in this paper is the last application. CRRT requires continual systemic or regional administration of anticoagulancies. None of these methods is optimal because they are risky for patient or insufficient or too expensive. It is known that cooling of blood leads to inhibition of majority enzymatic reactions of coagulation cascade and to attenuation of haemostasis. Enough blood cooling may lead to stop coagulation processes. This was tested successfully on pig experiments during intermittent dialysis, when blood was chilled to 20°C and returned heated back to 37°C. The question is if simple cooling of blood will act as sufficient anticoagulation during CRRT. As far as authors know nobody tests this in CRRT and side effects of such procedure are unknown. Due to long time continual procedures the cooling method has to be robust to different blood flow and it has to resist sudden stop of CRRT. Hence simple ice cooling methods used in some former studies are not sufficient and authors decided to develop a more robust device based on computer control able to fulfill different protocol regimes. The device was assembled from medical proved parts. It has been able to cool and heat blood in wide ranges 15-40 °C on different flow 100 – 400 ml/min. It is known that thermal capacity of blood is 3.8 J/kg °C which value lies between thermal capacities of water and glycerol. This allows to make a mixture of these two liquids to get fluid which simulates thermal capacity of blood for purposes of laboratory experiments, later just "blood mimic fluid - BMF".Different types of heat exchangers (manufactured by Hotline and Level1, U.S.) were tested on the baseline BMF flow 200 ml min and temperature 37°C to get maximum heat exchange e.g. lowest temperature by use of 15°C cooling water. The best results were obtained by the exchanger Level1 DI100 - device intended for rapid fluid resuscitation in patients suffering by severe hypothermia and dehydration. The Exchanger was used contrariwise, to use coldest cooling water for cooling cold BMF first. Temperature in all parts of our system was measured by needle thermocouples (Omega, U.S.) inserted into the tubes of cooling and BMF circuits and were connected to a main computer. The System was controlled by flow and temperature of cooling water. Temperature was directed electronically by the digital communication between the computer and cooling device (Melcor, U.S.). Flow was directed by a peristaltic pump (Masterflex, U.S.) joined to the cooling circuit, also connected with the computer. The main computer was equipped by the Mat Lab software with author's program. Chilled BMF was passed into the CRRT monitor. After leaving the monitor, BMF was re-warmed to body temperature by the second exchanger connected to the warming circuit (Julabo, U.S.), ensuring the warming process similarly to the cooling one. The pilot experiment with anaesthetized pig shows the stability of temperature control and the whole system for all 6 hours period required by the protocol. SUPPORTED by grant MSM 0021620819.

STUDY OF THE CELL RESPONSE ONTO PHOTODYNAMICALLY ACTIVATED SENSITIZERS BY ATOMIC FORCE MICROSCOPY

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Cell lines can be study different microscopic technique such as fluorescent, confocal or electron microscopy. These methods delivers best resolution, but requires extensive sample preparation including thorough drying, which might lead to inhomogeneous shrinkage of the samples. The past decade has witnessed an emersion of the atomic force microscopy from solid-state physics into cell biology and even medical applications. This technique does not only record surface topography of the biological samples under physiological conditions, but also delivers micromechanical properties at high resolution. For these reasons, we used this relative new microscopic technique called Atomic Force Microscopy (AFM) to study of the morphology the cell lines A549, MCF7 and G361. Recognition of the cells and control of their surrounding during imaging have already been accepted as essential conditions for cell biological application of AFM. We imaged the cancer cells before and after photodynamic therapy (PDT) of photosensitizer $ClAlPcS_2$ and $ZnTPPS_4$. PDT was induced by efficient LED source with total light dose of 15 $J.cm^{-2}$. This method has been used to image the morphology of developing tumor cells and their processes. In some cases we could observe signs of apoptosis. Results show kinetic production of reactive oxygen species (ROS) within cells during PDT and modification of morphological features investigated by AFM. The combination of sensitizers and specific light source can lead to the loss of surface rigidity and eventually to dramatic changes of the cell shape or to formation of apoptotic body. This work was supported by the Grant Project MSM 6198959216 and MSM 6198959215.

STRUCTURAL CHANGES IN THE MEMBRANES OF HL-60 CELLS VISUALIZED BY FREEZE-ETCHING

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The membrane of a cells functions as a complex, dynamic structure which gives rise to various cell organelles that are unique for each kind of cell. The plasma membrane is very sensitive to physical stimuli and, of these, most to mechanical irritation, to which it responds with morphological changes. These can be studied by the method of freeze-etching that provides a view of two fracture faces, protoplasmic and exoplasmic, with imprints of disclosed membrane structures preserved as platinum-carbon replicas. The two fracture faces differ in the distribution of particles, the remains of trans-membrane proteins, and the environment they are adjacent to. The exoplasmic face borders on extracellular space and the protoplasmic face on cytoplasmic space. In this study ultrasound was used to investigate the effect of mechanical stress on HL-60 cells. The starting suspension of 10^7 cells per ml medium (450 ml DMEM with 50 ml bovine serum, 5 ml streptomycin/penicillin and 5 ml glutamine BIOTECH a.s., Praha CR) was used as follows: Experimental cells were treated by ultrasound in the horizontal direction in the near ultrasound field, at an intensity of 1 W/cm^2 and frequency of 1 MHz in a continuous mode for 10 min (BTL-07p BEUTILINE s.r.o., Praha CR; active transducer area, 4 cm^2) and subsequently fixed. Incubated cells were kept in an oven at 37°C for 90 min and fixed, and control (untreated) cells were fixed immediately. Fixation was carried out in a solution containing 2.5 % glutaraldehyde, 2 % para-formaldehyde and 0.05 M cacodylate buffer and, after a wash with 0.05 M cacodylate buffer, the cells were placed in 25 % glycerol. All three specimens were then frozen in liquid nitrogen at -210 °C (melting point), fractured and coated with Pt and C in a BAF 060 freeze-etching system (BAL-TEC). The protoplasmic face of the plasma membrane in treated cells showed, in some areas, mild undulation and was extended into membrane projections. The shape of particles was deformed and particle distribution was changed, while their number remained constant. The untreated incubated cells, which apart from

handling during the preparation were subjected to 90-minute standing, were either without any apparent changes or had membranes altered in shape, with particles that appeared sunken in relation to the fracture plane of the irregularly undulating membrane. These changes can be explained by the mechanical stress of centrifugation and the fact that the cell concentration was up to 17-time higher than in a routine culture. They take some time to manifest; in this experiment it was 90 min. The protoplasmic face of control cells showed none of these changes; membranes had even surfaces with a regular distribution of particles. Some particles were seen as aligned in rows or clustered in groups whose presence alternating with absence gave the membrane surface a net-like appearance. The exoplasmic face in both treated and incubated cells showed undulation similar to that of the protoplasmic face. Particles were distributed over the whole face with no cluster formation and their size varied, as it also did in the control cells, which made their exact evaluation difficult. It can be concluded that mechanical stress produced by either ultrasound or handling the cells has a similar effect on the cell surface, i.e., it causes a local undulation of the plasma membrane.

POSTURAL SWAY RESPONSE TO 30-SECONDS „ALL-OUT“ ISOKINETIC CYCLING AT DIFFERENT REVOLUTION RATES

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It has been found that fatigue induced by 30-seconds „all-out“ isokinetic cycling impairs the ability to produce power more profoundly at higher than at lower revolution rates. This is very probably due to more pronounced activation of fatigue prone fast twitch fibers during cycling at higher revolution rates. We were interested how such a different activation of muscle fibers would influence postural stability. To this end, a group of 16 cyclists (age 19.2 ± 2.9, height 180.0 ± 7.3 cm, weight 69.2 ± 8.4 kg) underwent in random order two 30-seconds “all-out” exercise bouts on the isokinetic cycle ergometer at revolution rates of 70/min and 130/min, respectively. Results showed that in an initial 5-second period of cycling there were no significant difference in maximal power produced at 130 rpm and at 70 rpm (723.5 ± 156.2 W and 669.7 ± 138.8 W, respectively). On the other hand, in the final period power produced at 130 rpm was significantly ($p < 0.05$) lower than at 70 rpm (355.5 ± 102.6 W and 474.5 ± 118.0 W, respectively). Consequently, fatigue index was significantly ($p < 0.01$) higher at 130 rpm than at 70 rpm (50.9% and 29.1%, respectively). In the first case, also slightly higher blood lactate after exercise has been found (12.3 ± 1.4 mmol/l and 10.8 ± 1.5 mmol/l, respectively). Thus, more profound respiration as a result of compensation the anaerobic acidosis caused by isokinetic cycling at higher revolution rates may be assumed. This factor likely contributed to significantly ($p < 0.05$) higher sway velocity in an initial 5-seconds phase of recovery after cycling at 130 rpm as compared to 70 rpm (33.9 ± 4.3 mm/s and 29.5 ± 3.8 mm/s, respectively). Such difference in velocity of the centre of pressure after both exercises has been observed during about 25 seconds of recovery. It may be concluded that rather than fatigue more marked ventilation is responsible for more profound impairment of balance after isokinetic cycling at higher than at lower revolution rates. Supported by the Scientific Grant Agency of the Ministry of Education of Slovak Republic and the Slovak Academy of Sciences (No. 1/0611/08)