Current Topics in Biophysics 2004, 28(Suppl A), 17-60

Posters

1. Relation between proapoptotic action of diphenyleneiodonium and redox homeostasis of human endothelial cells

A. Balcerczyk, M. Soszyński, D. Rybaczek, G. Bartosz

University of Łódź

Diphenyleneiodonium, DPI, significantly modulates oxidant-antioxidant balance of cells. It inhibits activity of NAD(P)H oxidase, but also affects other oxidoreductases. Changes in activities of enzymes under the influence of iodonium derivatives are markers of their radical mechanism of action.

Contradictory data have been published on the effect of diphenyleneiodonium on redox equilibrium of the cells; both inhibitory and stimulatory action of DPI on the production of reactive oxygen species has been shown.

We found that DPI (1-100 μM) does not stimulate ROS production in human umbilical vein endothelial cells. Oxidation of fluorogenic probes (dHR123 and H2DCFDA) was inhibited, after removal of the inhibitor from the reaction medium. These results do not confirm the finding of Li *et al.* (*Free Radic. Biol. Med*, 2003, **34**, 465) on HL60 cells. A reverse effect was observed when DPI was present in the analyzed samples. These results suggest an interaction between the probes and DPI or its metabolites. The level of nitrated tyrosine residues of cellular proteins, estimated by Western blotting, was generally parallel the production of ROS.

Suppression of the systems producing ROS/RNS by DPI caused an inhibition of proliferation of endothelial cells. Apoptotic changes of cells were observed 12h after incubation with DPI by DNA staining with acridine orange and activation of caspase 3. Condensation of chromatin was visible after Hoechst 33258/PI staining.

2. Assessment of potential agrochemical application of some new aminophosphine oxides and their equimolar binary mixtures with 2,4-D

T. Borowik[1,2], M. Langner[1,3]

[1]Wroclaw University of Technology
[2]Biophysical Scientific Students Union "Micela"
[3]Academic Center for Biotechnology of Lipid Aggregates, Wrocław

One of the major goals in modern pharmacokinetics is to target a drug directly to selected tissues and/or cells. Liposomes, due to their structure, enable the encapsulation of the biologically active compounds. In addition, their properties like electrostatics and steric can be precisely controlled. That makes them one of the major candidates for the twenty-first century drug carrier.

The aim of presented research is the determination of optimal lipid composition of liposome in order to optimize its association with carcinoma cells. The surface charge and surface polymer presence effect is examined. Experimental evidences that properly selected surface electrostatic charge and grafted polymers can improve the association of liposomes with colon cancer cells are presented. All measurements were carried out *in vitro* on human colon CX-1.1 carcinoma cells and the liposome association was evaluated with flow cytometry technique.

3. Effect of statin on erythrocyte membranes in patients with type 2 hypercholesterolemia

M. Broncel[2], W. Rożej[1], P. Duchnowicz[1], M. Koter[1], A. Aleksandrowicz[1], J. Chojnowska-Jezierska[2]

[1] University of Łódź
[2]Medical University of Łódź

Hypercholesterolemia is one of major risk factors since the excess of cholesterol affects the normal rheology of blood through its interaction with erythrocytes. The present study aimed at demonstrating changes in the plasma membranes of erythrocytes caused by high cholesterol concentration in the plasma and evaluation at the effect of statin therapy on the erythrocyte membrane. The study involved 30 patients with hypercholesterolemia and 20 healthy individuals as control group. The patients were given 10 mg atorvastatin or 20 mg

simvastatin per day. Laboratory tests were carried out before and after 4 weeks of pharmacology treatment.

The damage to plasma membrane of erythrocytes was measured on the basis of lipid peroxidation, content of membrane cholesterol, the fluidity of plasma membrane, ATPase activity, concentration of –SH group and changes in protein concentration in the fractions obtained on poliacrylamide gel.

Type 2 hypercholesterolemia causes changes in the structure and function of erythrocyte plasma membrane. The statins therapy reverses the alteration of erythrocyte plasma membranes.

4. Nitroxides induced increase in thiol groups in intracellular fluid of erythrocytes

S. Bujak, K. Gwoździński

University of Łódź

Text Nitroxides are stable organic radicals with unpaired electron on nitroxyl group (N-O). The derivatives of piperidine and pirroline are nonimmunogenic and relatively non-toxic for cells. The uncharged molecules easily penetrate plasma membrane. Inside the cells nitroxides are reduced to the corresponding hydroxylamines. They demonstrate protective mechanism in biological systems against oxidative damage on molecular and cellular level. As free radicals, nitroxides can react and dismutate superoxide radicals in catalityc way (SOD-mimic activity). Besides, these radicals induce catalase-like activity in hemoglobin and myoglobin as well as they can oxidase reduced forms of transition metals and eliminate their participation in Fenton's reaction.

The influence of pyrroline and piperidine nitroxides on the level of thiol groups in internal fluid of erythrocytes after incubation of erythrocytes with these nitroxides was investigated. Isolated erythrocytes (Ht 10%) were incubated for 1 hour with the following nitroxides: Tempol, Tempamine, Pyrrolid, Pyrrolin, Carboxypyrrolin at the final concentrations: 0.1; 0.2; 0.5; 1; 2 mmol/L.

The level of thiol groups in intracellular fluid of erythrocytes was estimated with 4,4'-dithiopiridine.

An increased level of -SH groups in hemolysate proteins under influence of all the investigated nitroxides was observed. The highest influence was observed for pyrroline derivative — Carboxypyrrolin, which caused increase in –SH groups approximatel

5. AChE activity in human erythrocytes incubated with 2,4-D and their phenolic metabolites

B. Bukowska

University of Lódź

Because many compound cause damage of the membrane's cell the investigations were performed concerning the effect on the activity of acetyltiocholinesterase by 2,4-dichlorophenoxyacetic acid and their metabolites: phenol, 2,4-dichlorophenol and catechol. The doses in the range of 10 µg – 1000 µg/ml of erythrocytes were used (the concentrations comparable to that accumulated in people exposed to action of those compounds) to estimate which is the lowest dose capable to provoke changes in the activity of acetyltiocholinesterase (*in vitro*). Human erythrocytes were obtained from whole blood taken from healthy donors at the Blood Bank of Lodz. Erythrocytes were separated from blood plasma and leukocytes by centrifugation (600g, 10min) at 4ºC and washed three times with phosphate-buffered saline (PBS; 150 mmol l^{-1} NaCl, 1.9 mmol l^{-1} NaH_2PO_4, 8.1 $mmol^{-1}$ Na_2HPO_4, pH 7.4). Isolated erythrocytes at a haematocrit of 5% were treated at a temperature of 37ºC for one hour with 10–1000 ppm of 2,4-D, phenol, 2,4-dichlorophenol and catechol. After this treatment acetylotiocholinoesterase activity was assayed by the method of Ellman *et al.* (1961, *Biochem. Pharm*., **7**, 88). The kinetics of acetylothiocholine iodide hydrolysis was recorded spectrophotometrically at 24ºC.

Statistically significant decrease of V_{max} and K_m in the activity of AChE incubated with catechol and 2,4-DCP was noted. 2,4-D decreases V_{max} but do not change the value of K_m Phenol did not significantly change AChE activity. A decrease of AChE activity was dependent on the dose of 2,4-dichlorophenol and catechol used. The differences were statistically significant begining at the dose of 500 ppm of catechol and 250 ppm of 2,4-DCP.

Strong toxic activity of chlorophenols is stem from presence withing their molecules chlorins which, powerfuly disturb protein structures, change the three-dimensional structure and thus enzymes activity.

It seems to be essential that in catechol toxicity special role plays damage of heme proteins and other protein molecule, and damage of lipids is not so important. It is known that oxidation of catechols leads to formation of semiquinone radicals. Semiquinones are able to bind to nucleophilic residues of proteins like -SH or -NH_2 and nucleic acids, what results in inactivation of these macromolecules.

6. Paramagnetic centres in DOPA-melanin-netilmicin complexes with Cu^{2+}

E. Buszman, M. Matuszczyk, B. Pilawa, D. Wrześniok, A. Grzegorczyk, T. Wilczok

Medical University of Silesia, Sosnowiec

Paramagnetic system of DOPA-melanin as the model eumelanin, and its complexes with netilmicin and paramagnetic Cu^{2+} ions were studied. The aim of this work was to determine the properties of paramagnetic centres in DOPA-melanin and their modification by netilmicin. The effect of paramagnetic Cu^{2+} ions on free radicals in DOPA-melanin-netilmicin complexes was examined. The behaviour of copper paramagnetic centres in DOPA-melanin-netilmicin complexes was tested.

Netilmicin — the antibiotic from the aminoglycoside group was chosen to our studies, because of the high affinity of this type of drugs to melanin biopolymers. The binding of copper ions to melanins is also known. Aminoglycosides ototoxic effects may result from their accumulation in the inner ear melanin. Paramagnetic centres in this biopolymer may be responsible for these negative effects.

DOPA-melanin was formed by oxidative polymerization of 3,4-dihydroxyphenylalanine (L-DOPA) in 0.07 M phosphate buffer at pH 8.0. DOPA-melanin was complexed with netilmicin and Cu^{2+} in the concentrations 1×10^{-3} M. The concentration of netilmicin in the studied complexes was determined by the use of UV/VIS spectrophotometer V-530 (Jasco).The amount of copper bound to DOPA-melanin was determined by atomic absorption spectrophotometer AAS 3 (Carl Zeiss, Jena).

EPR measurements were performed using an X-band (9.3 GHz) electron paramagnetic resonance spectrometer (RADIOPAN, Poznań) with magnetic modulation of 100 kHz. EPR spectra of both organic free radicals and Cu^{2+} ions were recorded. Concentrations of paramagnetic centers in the melanin samples, g-factors, linewidths ΔB_{pp} and amplitudes of the first derivative EPR spectra were determined.

For all the studied melanin samples single broad EPR lines (ΔB_{pp} ~0.5 mT) of o-semiquinone free radicals with g-value near 2.0040 were measured. In the EPR spectra of DOPA-melanin-netilmicin complexes with Cu^{2+} additionally the very broad (ΔB_{pp} ~20 mT) lines of copper ions appeared. Netilmicin increased the concentration of paramagnetic centres in DOPA-melanin. This effect is probably responsible for ototoxic properties of this antibiotic. It was shown that binding of copper ions to melanins considerably changes paramagnetic centres system in melanin. Cu^{2+} binding strongly decreased the amplitudes of EPR lines of melanin organic paramagntic centres. It was observed that slow and fast spin-lattice-relaxation processes are characteristic for o-semiquinone free radicals and paramagnetic copper ions systems in DOPA-melanin complexes with netilmicin and Cu^{2+}, respectively. Microwave saturation was observed for EPR lines of o-semiquinone free radicals. EPR lines of Cu^{2+} did not saturate in the studied range of microwave power.

7. Hemolysis study — effect of Near Infrared Radiation on erythrocytes

L. Chludzińska, A. Jarosławska, M. Dzik, M. Komorowska

Wrocław University of Technology

Human and animal erythrocytes up to 10 days after donation were studied. 10% hematocrit suspensions were irradiated using halogen lamp with 800–2000 nm filter. During irradiation procedure temperature of the suspension were controlled by water and air cooling systems. Static hemolysis curve: RBC were suspended in medias of different osmotic pressure. After centrifugation supernatant absorbance for 540 nm wavelenght were measured. Dynamic hemolysis curve: kinetics of hemolysis were studied using stopped flow method for erythrocytes suspended in different hipotonic NaCl solutions. After NIR irradiation osmotic fragility of RBC were changed slightly, but shapes of static hemolysis curves were significantly different. Rate of hemolysis for NIR irradiated erythrocytes were lower than for control cells. Swelling time estimated from dynamic hemolyss curve were also different. Osmotic fragility parameter is mean value for all studied population of erythrocytes and is inadequate for full description of hemolytic properties of RBC. Static hemolysis curve shape depends on distribution of osmotic fragility for erythrocyte population. This distribution for NIR irradiated erythrocytes is significantly different than for control samples, and suggests unification of osmotic properties of erythrocyte population. Lower rate of hemolysis and higher swelling time for NIR irradiated etrythrocytes suggest improved elastic properties of RBC membranes.

8. Red blood cells surface changes induced by ion strength and Near Infrared Radiation (NIR)

L. Chludzińska, J. Przybyłowicz, A. Jarosławska, M. Dzik, M. Komorowska

Wrocław University of Technology

Human erythrocytes up to 10 days after donation were studied. RBC were suspended in solutions of different ion strengths. For keeping osmolarity we added sorbitol to media. Effects of NIR irradiation were studied for hipo- and hyper-tonic media also. 10% hematocrit suspensions were irradiated using halogen lamp with 800–2000 nm filter in time period of 15 minutes. During irradiation procedure temperature of the suspension were controlled by water and air cooling systems. Elec-

trokinetic potential and electrophoretic mobility were measured for irradiated and control RBC. Agglutination process was studied by observation of antibody-antigen reaction for blood group tests in media with different antibody concentration. Electrokinetic mobility of irradiated RBC changes only in higher ion strengths. Electrokinetic potential of NIR treated erythrocytes was lower than for control samples for medium ion strengths. Observation of agglutination process indicates that NIR radiation affects antibody-antigen reaction. NIR changes surface structure of the erythrocytes. Lower values of electrophoretic mobility for irradiated erythrocytes suggest changes in volume charge density. After irradiation the accessibility of the antibody bonding sites decreases.

9. Effects of acetylcholine pulses on central and perypheral sinoatrial node cells

J. Cieniawa

Medical University of Lublin

The mammalian sinoatrial (SA) node is a heterogeneous structure. Cells from different regions of the sinoatrial node exhibit differences in their electrophysiological properties. Acetylcholine (ACh), the neural transmitter released from parasympathetic vagal nerve endings, causes a negative chronotropic effect leading to slowing of pacemaker activity. In this study we use the mathematical model of Zhang et al. of action potentials in the periphery and center of the rabbit sinoatrial node to simulate the effects of acetylcholine pulses on the phase sensitivity of the pacemaker cells. Phase-response curves of central and peripheral cells to acetylcholine pulses are simulated by delivering ACh pulses at various time points within the pacemaker period. Simulations suggest that cells from the centre of the SA node are more sensitive to acetylcholine pulses then cells from the periphery. The results of the simulations are in agreement with experimental studies.

10. The results of participation in the „3rd International Intercomparison on EPR Tooth Dosimetry"

B. Ciesielski, K. Schultka

Medical University of Gdańsk

In this study we present the results of dosimetry based on EPR signal induced in tooth enamel by ionising radiation. The research was performed as a part of our participation in international dosimetry programme organized in cooperation with IAEA. Each of thirteen laboratories from Europe, Asia and North America which entered for the programme obtained 22 tooth samples (11 molars cut into halves); 11 halves were irradiated with a dose range 0–900 mGy. The aim of the participants was to identify the irradiated halves and to determine radiation doses absorbed in these samples. The dosimetry procedure required separation and purification of the tooth enamel, determination of optimal parameters of EPR measurement and calibration of the radiation induced signal against the absorbed dose. The nominal, actual doses delivered to the samples in IAEA laboratory were 79 mGy (5 samples), 176 mGy (5 samples) and 704 mGy (1 sample), and the measured doses by us were 64 mGy, 155 mGy and 797 mGy, respectively. The correlation between actual and measured doses was very good, which was reflected by high values of correlation coefficients: r_2=0.726 for dose range 79-176 mGy and r_2=0.980 for whole dose range examined. A comparison of results obtained by participants in this programme with results of a similar intercomparison in 1999 shows significant improvement in accuracy of the applied dosimetric methods, in particular in the lower dose range (below 200 mGy).

11. The effects of day-light on stability of the dosimetric EPR signal in L-alanine

B. Ciesielski, K. Schultka

Medical University of Gdańsk

In this study the effects of exposure of irradiated L-alanine to day-light under normal laboratory conditions are presented. Gamma irradiated alanine powder was divided into three parts. One part was kept in darkness at 10°C. The second part was kept in darkness at room temperature, and the third part was placed in EPR quartz sample tube and left in normal day-light conditions at about 25ºC, protected from direct sunlight. EPR measurements were performed on each sample several times during the 43 weeks period. The obtained spectra showed variations in the EPR signal intensity i. e. double integral of the spectrum and amplitude of the central "dosimetric" line. Numerical decomposition of the spectra into three components reflecting contributions from three different alanine radicals (R1, R2, R3) to the total signal, allowed to attribute the spectral changes to variations in relative concentration of the radicals in the sample exposed to light. These variations were qualitatively similar to those observed previously for alanine samples exposed to strong artificial light from a fluorescent lamp. Despite of much lower intensity of the effects resulting for the day-light exposure, an increase in relative contribution of R2 radicals to the spectra, which was accompanied by a decrease of R1 component, indicates R1ŕ R2 free radical transformations. The decrease of the dosimetric signal was within 15-20%. Such a significant effect has to be taken in account by alanine dosimetrists.

12. Differences between collagen and elastin conformations studied by EPR spectroscopy — spin labeling method with respect to elucidate cross-linking effects in porcine pericardium

B. Cwalina, L. Dul

Medical University of Silesia

Collagen-rich tissues are usually fixed using glutaraldehyde (GA) reacting with ε-amino groups of collagen's lysine. In the pericardium tissue, GA should react also with ε-amino groups of elastin fibers interlacing with collagen fibers. These groups may bind a spin label isothiocyanato-Tempo (ITCTO). Thus, electron paramagnetic resonance (EPR) spectroscopy — method of spin labeling — may be used for study effects of proteins cross-linking in pericardium. Samples of insoluble collagen type I from bovine Achilles tendon (Sigma), elastin from bovine neck ligaments (Sigma) and porcine fibrous pericardium tissues, both native and modified (0.2% GA solution; 15min. or 2h), were spin-labeled (4-ITCTO; 4°C; 24 h) and an excess of the label was washed (10 times; 6000 r.p.m.). Between each of centrifugations, the samples were rinsed with phosphate-buffered saline (PBS; pH 6,5). Then the samples were measured (in capillary vessel of 0.8 mm diameter) using the EPR spectrometer (Radiopan-Poznań), at room temperature. The samples' spectra were recorded by means of EPR spectrometer type SE/X 2542 with cylindrical TM110 resonator. The microwave power nearly 100 mW with modulation frequency 100 kHz, modulation amplitude 0.08 mT, and scan time 16min with time constant 1 s were used. It has been demonstrated that differences between collagen and elastin conformations may be studied by EPR spectroscopy — spin labeling method, to elucidate cross-linking effects in pericardium tissue.

13. Collagen-rich tissues stabilization effects visualized by various staining techniques used in electrophoretic studies

B. Cwalina, A. Turek

Medical University of Silesia

Biomaterials derived from connective tissues are prepared through cross-linking of proteins (mainly collagen). Chemical and/or physical methods may be used for this purpose, including action of tannic acid (TA) and UV-irradiation. Electrophoresis SDS-PAGE is the method useful for monitoring structural changes in modified tissues since their susceptibility to proteins extraction is inversely proportional to stability. In the studies, the porcine pericardium (PP) samples: native and modified using 2% TA (4, 24 or 48 hours, 4°C) and/or UV-irradiated (3h, 14°C), non/or digested with pancreatine (1.5%, 3h, 14°C), were investigated by SDS-PAGE. Proteins were stained with Coomassie Brillant Blue R 250 (CBB) or silver, and analyzed using Biotec Fischer System. The tissue stability was evaluated through its resistance to proteins extraction with SDS/NaCl. Obtained the results shown that CBB-visualized were polypeptides of molecular weights 23-203 kDa, whereas smaller peptides were almost invisible. Silver-staining resulted in visualization of peptides of 11-201 kDa molecular weights. This technique makes visible also the negative-stained polypeptides extracted from tissues stabilized using TA and UV. The UV-irradiated tissues were less resistant to pancreatine action, as compared with both the native and the TA-treated tissues (with or without UV). The resistance to proteins extraction appeared to be not a time-dependent effect after the tissues treatment with TA and UV.

14. Tempace protects cells against oxidative stress induced by doxorubicin and hydrogen peroxide

J. Czepas, A. Koceva-Chyła, K. Gwoździński, Z. Jóźwiak

University of Łódź,

Nitroxides, the stable free radicals of well established chemical properties, constitute the class of chemicals of special interest owing to their potential antioxidant properties. It has been already shown that most of nitroxides is relatively noncytotoxic, nonmutagenic and nonimmunogenic. Unfortunately, despite of extended efforts, not all of them were found to have the desired properties. Thus, finding and establishing the nitroxides with strong antioxidant properties is a very important study. Considering the dependence of the properties of nitroxides on their structure, piperidine nitroxides were found to be potentially one of the most useful as antioxidants.

In order to evaluate the relevance of piperidine ring substitution on the nitroxide performance, we investigated Tempace, which is an acetamido piperidine nitroxide derivative. MTT cytotoxicity spectrophotometric test and TBARS assay were used to investigate the impact of Tempace on cell viability and membrane lipid peroxidation. The same tests were used to evaluate the effects of this nitroxide on cell damage caused by oxidative stress inducing agents doxorubicin and H_2O_2. All experiments were carried out on B14 cell line (Chinese hamster peritoneal immortalised fibroblasts). Tempace did not show any cytotoxic performance in these cells. Moreover, the nitroxide significantly protected cells against oxidative stress at 250-500 μmol/l concentrations following cell treatment with hydrogen peroxide. In TBARS assay, Tempace did not cause any lipid peroxidation at the concentration applied (20-200 μmol/l). In cells incubated with hydrogen peroxide, or dexorubicin, Tempace also revealed protective properties (40-200 μmol/l), which was siginificant upon the cell treatment with doxorubicin. In contrast to Tempamine, the nitroxide

mine, the nitroxide previously examined, Tempace shows more direct evidence for its possible therapeutic use as an antioxidant compound.

15. The use of mass spectrometry for estimation of radiation induced changes in cisplatin

T. Dąbrowski, B. Kwiatkowski

Medical University of Gdańsk

Cis-diamminedichloroplatin (cisplatin) is a well-known antineoplastic drug efficient in treatment of some types of neoplasms. Mechanism of cisplatin action, especially during concurrent chemoradiotherapy, has not been fully elucidated so far. Laboratory investigations using the neoplasma-cell lines as well as clinical trials (especially concurrent chemoradiotherapy trials) showed increased cytotoxicity of cisplatin modified by ionizing-radiation. This work presents a data concerning structural variability of cisplatin in aqueous solutions after gamma-irradiation (Co isotope 60). A mass spectrometry was used in the analysis. Polymeric molecules of cisplatin were observed in all samples. Complexes with 3 to 7 platinum atoms were found in water solutions of cisplatin, compared with 2 Pt atom complexes detected in dry samples. A small increase of intensity of individual spectral lines attributed to main wave was found in irradiated solutions. This effect was more evident for ions with 3 and 4 Pt atoms. Thus, the mass spectrometry revealed an increased complexity — polimerization of cisplatin molecules in irradiated solutions.

16. Electron spin resonance (ESR) as a method to estimate the time of blood extravasation

H. Domek, L. Sagan, J. Piątek, B. Gonet

Pomeranian Medical Academy, Szczecin

Electron spin resonance (ESR) was proposed as a dating method by Zeller as early as 1967, but its practical application began with the work of Ikeya in 1975. Since then, there have been proposed an interesting attempt of application ESR dating of organic substances utilising paramagnetic degradation products by Miki *et al.* (1987).

We have applied ESR spectroscopic method in order to estimate the time after a death or injury using extravasating blood. This method may be of importance as a practical test in forensic medicine.

ESR spectrum of the coagulated blood consists of the three signals at g=6, g=4.3 and the strongest at g=2.005. The sharp signal at g=2.005 is due to ascorbyl radical *in vitro* and it corresponds to the level of vitamin C *in vivo*. The investigation were performed using the blood of six healthy donors. ESR spectra were recorded as a function of ageing time about 20, 60, and 90 hours after extravasation.

The obtained results show a big individual variability among samples and ESR signal dependence on the condition in which the samples were stored (temperature, humidity) and small ESR signal differences in time. Thus, we can conclude the ESR spectroscopic method is not usable in forensic medicine application.

17. Changes on selected structural and functional parameters of red blood cells in elder human

P. Duchnowicz[1], K. Kujawski[2], M. Koter[1], K. Kędziora-Kornatowska[3], T. Kornatowski[3], J. Kędziora[2]

[1]University of Łódź
[2]Medical University of Łódź
[3]Medical University of Bydgoszcz

The free-radical theory of aging assumes that the older an organism is, the more its oxidative balance gets shattered. Both arterial hypertension and oxidative stress cause structural and functional changes to the area of erythrocyte.

The aim of this research was to evaluate membrane lipids peroxidation, membrane fluidity, membrane AT-Pase activity, inner microviscosity and concentration of –SH groups in erythrocytes obtained from elder people.

The research has been conducted on 42 patients divided into two groups. The first group was old-age patients with and without an accompanying hypertension. The second, control group consisted healthy patients.

Membrane lipids peroxidation was found stronger in the elder group. The activity of total and Na^+,K^+-ATPase appeared greater in the control group, and lower in the elder group. Changes in membrane fluidity and inner microviscosity were determined by EPR (electron spin resonance) study. The increase of membrane fluidity and inner microviscosity in elder group were observed. We also noticed higher concentration of free –SH groups in elder group.

The obtained results suggest that some, potentially pathological changes take place in erythrocyte membrane and seem to correlate with donor age.

18. Experimental studies of anomalous diffusion in membrane system

K. Dworecki[1], M. Konat[2]

[1]Świętokrzyska Academy, Kielce
[2]The State Vocational University in Nysa

We illustrate the experimental research results for the substance transport dynamics in a membran system

including gel solutions of different concentrations.In the studied system,the research of a temporal evolution of the boundary layers thickness δ (nearmembrane) were conducted (definied as a distance from the membrane surface to point in which the concentration is k times smaller then the concentration on the membrane surface), which shows a different dynamics of the changes as regards a normal and anomalous diffusion. The studies of a nearmembrane layers evolution in a membrane system consisting of two equal glass cuvettes separated by the horizontally located polymeric membrane were conducted by thee laser interferometric technique. One of the cuvettes from the membrane system was successively filled with an aqueous solution of glucose, saccharose and polyetyleneglycol 4000(PEC) in a gel form, the second of the cuvettes was filled with gel (1.5% aqueous solution of agarose). On the basis of the analysis results of interferograms relating to the nearmembrane region a temporal evolution of the nearmembrane layers thickness for individual dissolved substances in aqueous gel solution was determined. The results of these studies allowed to determine that the thickness of the layers in the studied membrane system grows in time according to the relation $\delta(t) \sim t\gamma$ where $\gamma<0.5$. Mechanism of glucose,saccharose and PEC transport in the given membrane system has a subdiffusion nature.

19. Review selected antioxidative enzyme activities in patients with diabetes mellitus type 2

D. Ertel[1], R. Błaszczak[1], M. Rutkowski[1], K. Kędziora-Kornatowska[2], E. Gendek[1], J. Kędziora[1]

[1]Medical University of Łódź
[2]Medical University in Bydgoszcz

The oxidative stress takes part in diabetes pathogenesis. The oxidative stress is a homeostasis disorder leading to the increase of free radicals concentration. The stress occurs when the balance between free radical production rate and concentration of protective enzymes is lost.

The aim of this work was to estimate selected antyoxidative enzyme activity in patients with diabetes mellitus type 2 in various periods of its metabolically balanced.

The examination was performed on 61 patients with diabetes mellitus type 2 who were divided into 2 groups according to the concentration of glicated haemoglobin (HbA_{1C}), concentration of plasma cholesterol triglyceride and the excretion of albumin in urine: the 31 patients with metabolically balanced diabetes mellitus type 2 (DM + C) (HbA_{1C}<6,5%Hb, cholesterol <3 mmol/l, triglicerydy < 1.7 mmol/l), and 30 patients with metabolically unbalanced diabetes mellitus type 2 (DM + AC) (HbA_{1C} > 6.5% Hb, cholesterol > 3.0 mmol/l, triglicerydy > 2.2 mmol/l). The patients from both groups had the right excretion of albumin in urine (< 30 mg/24h) and they did not suffer from hypertension. The controlled group included 40 healthy people (K).

In erythrocytes one marked: superoxide dismutase activity (SOD) and catalase (CAT) with spectrophotometer Beckman.

Contrary to K the radical decrease of SOD and CAT activities as well as were noticed in all examined patients with diabetes mellitus type 2. Both the SOD and CAT activities in DM + C groups were higher in comparison with the DM-AC groups.

The examination results confirm that the oxidative stress is reduced more intensively in patients with metabolitically balanced diabetes mellitus type 2 and dysfunction of antioxidative system is in the correlation with the metabolic balance.

20. Direct measurements of an electrosilent chloride/hydroxyl anion exchange transport with ion selective electrodes

A. Fogt, S. Przestalski

Agricultural University of Wrocław

The aim of this work was to measure directly the chloride anion flux across the lipid bilayer facilitated by organometallic compounds. In our previous investigations we proved that the organolead and organotin compounds can act like hydroxyl/chloride antiport within lipid bilayer. However, this was shown only indirectly. Here we tried to complete our results by measuring the chloride electrosilent flux with chloride-selective and pH electrodes.

Bimolecular lipid membranes (BLM macrovesicles) prepared from egg yolk lecithin were used in the measurements. One chamber was filled with NaCl and the other one was buffered. Organometallic compounds were added from ethanol solution to each chamber, up to the concentration in which these compounds did not influence the bilayer integrity, as it was checked by electrical conductance measurements. A combined chloride electrode was immersed in buffered chamber of small capacity to measure the chloride concentration changes and consequently the ion flux. As the measured ions were diluted to concentrations near to the lower edge of the electrode measuring range, we could only very roughly evaluate the chloride anion flux. Better results gave experiments in which the alkalization of the chamber where the hydroxyls were transported to was measured. The number of organometallic molecules engaged in the exchange transport and their affinity to the chloride and hydroxyl ions is discussed.

This work was supported by the Polish Commettee for Scientific Research grants no. 2 P04G 089 27 and 6 6261 92 03.

21. Prooxidative action *in vitro* of organotins on phosphatidylcholine and phosphatidylcholine/sphingomyelin membranes in presence of UV radiation

J. Gabrielska, S. Przestalski

Agriculture University of Wrocław

Organic compounds of tin find application in such areas of economy as the production of plastics, paints and plant protection substances. The organic derivatives of tin are in general toxic and constitute a serous menace to the natural environment, human species in particular. The toxic action of organic tin compounds on living organisms, on the cellular, level may consist in changes in properties of biological membranes, that may result from accumulation of toxins in the lipid phase of membranes, or from interactions during membrane penetration by the molecules. Our previous studies on the effect of phenyl forms of tin on membranes formed of phosphatidylcholine and sphingomyelin, also with cholesterol added, indicated at increased adsorption of the compounds in the phosphatidylcholine phase of membranes, compared to their adsorption in the sphyngomyeline/cholesterol phase. The lipid microdomains in some cell membranes containing sphingomyelin and cholesterol are thought to be, among others, responsible for signal transmission. We have also shown that phenyl tin compounds in the presence of UV radiation induce oxidation of phosphatidylcholine membranes. The aim of the work undertaken was to find out if the phenyl and butyl tin derivatives induce peroxidation in the presence of UV radiation in membranes that could have microdomains responsible for signal transmission to a degree comparable to that of phosphatidylcholine membranes.

22. Acoustics analysis of speech of persons with phonation disorders

H. Gawda, J. Warchoł

Medical University of Lublin

The study concerns the lack of unification for objective estimation of disorders speech. Searching for an optimal form of acoustic measurements supporting a speech diagnosing leads to explanation speech signals properties as a subtle structure of a spectrum or intercorelations between components of the spectrum. Conclusions presented in the paper are a results of investigations using a professional sound analyser SVAN 912 (Svantek). Sound samples in the form of polish vowels were analysed by FFT method (Fast Fourier Transform). The equipment enables to obtain dispersion of few herz`s. Investigations of correlations of harmonic tones enabled to univocally a specific influence of different speech disorders on a spectral structure of vowels. For example changes in distribution of secondary harmonic tones or beats as an result of a movement of a vocal canal were observed. The aim of the study is to approach a problem of diagnosing of patients with vocal organ pathologies.

23. Crystal structure of kinetin phosphate

J. Gierszewski[1], G. Ślósarek[1], A Pietraszko[2]

[1]Adam Mickiewicz Uniwersity, Poznań
[2]Institute of Low Temperature nad Structure Research, Polish Academy of Science, Wrocław

Kinetin is a plant hormone, an important factor influencing the development of a plant cell. At present it is a subject of intense study, since it influences a physiological processes in a human cells as well.

Molecular and crystal structure of kinetin has been studied previously (Soriano-Garcia & Parthasarathy, 1977, *Acta Cryst*. **B33**, 2674). We have made redetermination of crystal structure versus temperature (Krzaczkowska, Ślósarek & Pietraszko, in preparation) The structure of kinetin belongs to the triclinic system with the space group P^{-}_{1}. There are two molecules of N9H tautomer of kinetin in the unit cell. There are involved in a specific net of hydrogen bonds. A partial stacking of adenine residues has been also observed.

The other tautomeric form N7H is observed in a crystal of kinetin phosphate. In this crystal the unit cell size is doubled at the preserved symmetry. The conformation of the kinetin molecule is also preserved, but the system of hydrogen bonds is totally different. These is no stacking of adenine residues. A further analysis of the crystal structure shell deals with the biological aspects of intermolecular interaction between kinetin molecules.

24. Energy migration between FMN molecules in rigid PVA films

H. Grajek[1], A. Synak[2], M. Sadownik[2], G. Zurkowska[3], P. Bojarski[2]

[1]University of Warmia and Masury in Olsztyn
[2]University of Gdańsk
[3]Technical University of Gdańsk

Flavins acting as photoreceptors in plants can effectively exchange excitation energy. In biological membranes they are usually bound to proteins and are thus rigidified in their natural medium. It occurs that in rigid solution the fluorescent properties of flavins are significantly modified. Inhomogeneous orientational broadening of FMN energy levels manifests itself in rigid PVA films as a “red” shift of fluorescence spectra with the excitation wavelength. Moreover, at high dye concentrations FMN dimers exhibit their own fluorescence and they become imperfect traps for excitation energy. To investigate this process of energy transport between FMN

monomers and dimers emission anisotropy spectra have been measured at three different excitation wavelengths: 445 nm, 490 nm and 510 nm for several dye concentrations ranging from 0.0001 M to 0.684 M. As expected, at low concentrations emission anisotropy remained constant evidencing the lack of energy migration. However, at higher concentrations up to 0.684 M the anisotropy decrease was found with the observation wavelength due to the presence of inhomogeneous broadening leading to the excitation flow towards "red" FMN centers. The measurements indicate that the effect of energy migration between monomers followed by its transfer to dimers is strong. The anisotropy spectra recorded at highest concentrations show also that the fluorescence emitted originates probably both from H and J dimeric bands.

25. EPR spectroscopy of yeast iso-1-cytochrome c in denaturating solvents using proxyl-MTS spin label. Comparison between guanidinum hydrochloride and urea

P. Grudnik, J. Pyka, B. Turyna, R. Gurbiel, W. Froncisz

Jagiellonian University, Kraków

Cytochromes *c* play a central role in biological electron transport systems and, because of their small size and their stability have been a popular subject for study in the general areas of protein chemistry and redox reactions. In mitochondria they function as mobile electron carriers connecting the cytochrome *bc1* with the cytochrome c oxidase. In general cytochromes *c* are among the best characterized proteins. In this approach, the placement of a spin label at a specific position (Cys 102) within the protein allows us to monitor exclusively the chosen region of the protein.

In the present study iso-1-cytochromes *c* from *Saccharomyces cerevisiae* was modified with cysteine-specific spin labels (proxyl-MTS and MTSL). Continuous wave (CW) EPR was used to examine the effect of denaturating agents on the behavior of the spin labeled cytochrome c. Denaturant – induced, guanidinum HCl and urea, unfolding were performed in different concentrations of agents. Protein unfolding degree was qualified by using A_{-1}/A_0 calculation (vertical peak – to – peak amplitudes for the central line (A_0) and high – field line (A_{-1}) were taken).

The influence of the denaturans on the behavior of spin labeled cytochrome *c* used in this study shows that unfolding the protein causes the spin label to move more freely. Cytochrome *c* unfolds between 1-2,5 M guanidinum HCl which is equivalent to 1,3 – 3 M urea.

26. Effect of rivastigmine on erythrocyte membrane properties in Alzheimer disease

R. Hajduk, M. Popłoński, E. Poziomska-Piątkowska, A. Pieniążek, K. Gwoździński

University of Łódź

Numerous observations have emphasised the oxidative damages of central nervous system in Alzheimer disease (AD). Central nervous system is potentially vulnerable to damage by reactive oxygen species because it is rich in polyunsaturated fatty acids and there is high oxygen consumption of brain. Erythrocytes are also exposed to high concentration of oxygen and can be also injured by oxygen free radicals. Many drugs applied the treatment of symptoms of AD emphasise the use of cholinoesterase inhibitors. One of this is rivastigmine. The modifications in plasma membrane of erythrocytes in AD patients have been reported.

The aim of this study was determination of properties of erythrocyte plasma membrane components e.g. lipids and proteins after incubation of red blood cells with two different concentrations of rivastigmine. The spin label method was applied. We determined lipid membrane fluidity using three spin label fatty acids 5-DS, 12-DS and 16-DS doxyl derivatives of stearic acids located in different depth of lipid bilayer. For determination of the physical state of membrane proteins two covalently bound piperidine derivatives, maleimide (MSL) and iodoacetamide (ISL) were applied. Using spectrophotometric method osmotic fragility of erythrocytes was estimated.

The slightly increase in lipid fluidity of plasma membrane after rivastigmine-treatment was observed. Drug also induced slightly alterations in conformational state of membrane proteins. However the changes in membrane components were statistically insignificant. Rivastigmine does not affect osmotic fragility of erythrocytes. These finding suggest that this drug does not influence the plasma membrane of erythrocytes.

27. Absorption, fluorescence and photothermal properties of porphyrin dyes

I. Hanyż[1], T. Sarna[2], D. Wróbel[1], P. Głowacki[1]

[1]Poznan University of Technology
[2]Jagiellonian University, Kraków

Three porphyrin dyes: two negatively charged TPPS4 tetrakis(4-sulfonatophenyl)porphyrin and TPS 5,15 – ditolylo - 10,20 – di(4-sulfonatobifenyl)porphyrin and also positively charged TAP tetra(4-trimethylanilinium) porphyrin in phosphate buffer were studied. In this work the absorption, fluorescence emission and excitation, and photoacoustic spectra have been recorded at various temperatures. We also used time-resolved photothermal spectroscopy at various temperatures. The fluorescence

parameters: natural fluorescence lifetime and fluorescence quantum yields were estimated. The influence of temperature on porphyrin absorption properties in buffer solution was negligible in the temperature range studied.

By the use of the time-resolved photothermal technique the thermal parameters were determined. On the basis of Marti *et al.* (1996, *J.Photochem. Photobiol. A:Chem*, **97**, 11) and Rudzki-Small *et al.*(1992, *Biophys.Chem.,* **42**, 29) methods the prompt and fast nonradiative internal conversion and intersystem crossing transitions occurring in the dyes were established and the quantum yields of triplet state population as well as the triplet lifetimes were calculated. The knowledge of both triplet state population and triplet decay are essential in PDT.

The Poznan University of Technology grant BW 62-195/04 supported this research.

28. Effect of polyene antibiotic amphotericin B on proton transport across model lipid membranes

M. Hereć, W. I. Gruszecki

Maria Curie-Skłodowska University, Lublin

Mechanism of action of amphotericin B (AmB), is connected with changes in the natural permeability of cell membrane. Disintegration of biological membranes, caused by the molecules of the drug lead to uncontrolled leakage of monovalent ions and small molecules from the cell. In order to investigate the effect of AmB a fluorescence pH sensitive dye piranine trisulfonate (PTS), entrapped inside vesicles formed with EYPC was applied. Time dependencies of fluorescence changes of PTS, upon sudden acidification of the liposome suspension, were analyzed in terms of two-exponential kinetics. The highest concentration of the drug (3 mol% with respect to lipid) caused the decrease in the rate constant of the slow component of proton transport from 0.011 1/s to 0.005 1/s and the increase in the rate constant of the fast component from 0.06 1/s to 0.149 1/s. This increase can be interpreted as related to the formation of membrane channels by AmB. At low concentrations of AmB (0.1 mol% with respect to the lipid) we can observe the decrease in the rate constant of the low and the fast components of proton transport. The differences in the effect of AmB are interpreted in terms of different orientation of molecules of the drug with respect to the membrane at low and high concentration.

29. *In Silico* drug distribution – optimization of transport in the tumour tissue

R. Hrynyk[1], M. Langner[2]

[1]Wroclaw University of Technology
[2]Academic Center for Biotechnology of Lipid Aggregates, Wrocław

Numerical simulations of drug transport via passive diffusion from the vessels/capillaries in tumor tissue were used to optimize the concentration supply with liposomal carriers to intracellular regions of the solid tumor. Based on Finite Difference Method (FDE) and Crank-Nicolson scheme mathematical model links the effective drug concentration in the tumor cells with the molecular targets in a time- and concentration-dependent manner. Numerical model considers movements described by diffusion constant D and the influx and efflux contribution of liposomal accumulation from experimental study given by Fung et al. (2003, *Biophys. Biochem. Acta*, **1611**, 63). Reasonable parameter value estimation enables meaningful analysis of system behavior. As a result of simulations the importance of liposome lipid bilayer permeability and/or stability as well as tumor endothelium penetration are presented.

Proposed description is needed to improve the understanding of drug-target interaction and the ability to translate the theoretical findings to clinical application. Such approach may greatly assist the development of liposomal anticancer drug formulations with improved therapeutic properties.

30. Knoth theory in biology

A. Janiak-Osajca, A. Timoszyk, K. Walińska

University of Zielona Góra

A molecule of DNA may be thought as two linear strands intertwined in the form of a double helix with a linear axis. A molecule of DNA may also take the form of a ring and so it can be tangled or knotted. In the early 1970s it was discovered that an enzyme called a topoisomerase can facilitate this complete process, from the initial break to recombination.

Topology can help with the description and computation all of the possible configurations which can be attained from a given starting configuration. Topology can detect mathematical barriers which may exist between conformations. These barriers can only be overcome by the transient breaking and the reunion of macromolecules. The helical structure of duplex DNA imposes topological obstructions to life processes such as replication and transcription. It is the very existence of topological barriers which initiated the search for enzymes which can overcome them.

Knot theory is the study of entanglement of flexible circles and arcs in R3. Circles can become entangled with each other, forming links, and self-entangled,

forming knots. Knot theory is extremely important in the study of 3-dimensional topology. As knot theory grows and develops, its boundaries continue to shift. Now, in addition, they overlap with certain areas of mathematical biology and chemistry. One of the most interesting new scientific applications of knot theory is to use of it in the analysis of DNA experiments.

31. Clinical application of multi-leaf collimator in breast carcinoma irradiation

M. Janiszewska, I. Laskosz, G. Nowakowski, D. Dupla

Lower Silesian Oncology Center, Wrocław

Modern radiation therapy tools allow a precise delivery of a high dose to a target area (so-called planning target volume - PTV) and spare, at the same time, critical organs in the vicinity of cancerous lesions. One of the tools of conformal therapy is a multi-leaf collimator, which provides one with an opportunity to optimally adjust the therapeutic field to the tumor area. More difficult areas for radiation therapy include: mamma, after BCT, and chest after mammectomy with regional lymph nodes.

The objective of the study is to present technical and physical aspects of breast carcinoma irradiation when applying a multi-leaf collimator.

Lower Silesian Oncology Center has a Clinac 2100 CD- Varian accelerator equipped with a multi-leaf collimator composed of 80 leaves: 40 leaves on each side of the collimator. Each leaf is 1 cm wide in the isocenter.

All techniques were prepared for the energy of 6 MeV, due to the max depth of 1.4cm. The following techniques were applied:

Isocentric technique of tangent fields (from two to four) for the mamma after BCT.

Method of a common isocenter, for the areas of mamma and for regional lymph nodes.

Technique of complementary photon + electron fields, for the area of chest after mammectomy and lymph nodes.

In all the above methods, the input dose was measured on order to prove the compliance of treatment plans with their realization by the equipment.

The presented techniques were implemented as standard procedures in the preparation of breast carcinoma radiation treatment in the Lower Silesian Oncology Center.

32. Providing optimal radiotherapy of thyroid cancer by using conformal beam forming methods such as dynamic wedges and multileaf collimator

M. Janiszewska, I. Laskosz, G. Nowakowski, B. Izmajłowicz, M. Raczkowski

Lower Silesian Oncology Center, Wrocław

In treatment using ionizing radiation precise treatment planning has a fundamental significance. This is a necessary procedure in radiotherapy in order to carry out the treatment safely and obtain complete destruction of cancerous cells simultaneously protecting healthy cells as much as possible. Radiotherapy of thyroid area and its regional lymph nodes is a challenge for every medical physicist preparing dose distribution.

The aim of this paper is presenting the conformal method of irradiating thyroid cancer which has been worked out in Lower Silesian Oncology Center as well as comparing dose distributions obtained in the standard technique of two opposite fields and in the conformal method.

A patient with papillary thyroid cancer was chosen in order to present the technique of conformal irradiation. In the first stage, the irradiation area includes thyroid bed, local peritracheal lymph nodes, neck lymph nodes, supraclavicular lymph nodes, submandibular lymph nodes and upper mediastinum lymph nodes reaching tracheal bifurcation. The patient was planned in the technique of common isocentre. Because of big and irregular target shape, changeable distance SSD in different points of irradiated area and presence of critical organs like, first of all, spinal cord the target was divided into two parts. The first one includes thyroid bed, peritracheal lymph nodes, neck lymph nodes, supraclavicular lymph nodes, submandibular lymph nodes whereas the second one includes upper mediastinum lymph nodes as far as tracheal bifurcation.

The line of common isocentre was chosen on the border of divided target.

To compare dose distributions in standard technique and conformal one, isodose distributions were used, which were calculated in three perpendicular planes as well as dose-volume histograms, prepared in treatment planning system.

Using six-field technique made it possible to reduce the dose in spinal cord up to 60% of reference dose and to model therapeutic isodose into a hoof-shaped target in the area of neck and oval, deeply located mediastinum target. The traditional technique of two opposite fields determines using reference dose (100%) in the area of spinal cord, which definitely limits the final dose possible to use in a patient.

Monoisocentric six-field technique of irradiating thyroid is optimal from the point of view of both dose distribution and way and time of realization. Multileaf collimator and dynamic wedge, as tools fixed to the accelerator head, contribute to the improvement in the patient's comfort through minimizing the time of preparing exposition. Thus they minimize set-up error

paring exposition. Thus they minimize set-up error resulting from accidental movements of the patient.

33. Killing of bacteria by the photodynamic effect of dyes

A. Jankowski[1], S. Jankowski[2], J. Niedbach[1], A. Mirończyk[1]

[1]University of Zielona Gora
[2]Medical University of Wrocław

It is known that the survival of bacteria in the irradiated solutions of photosensitizers depends on the properties of dyes and the structure of bacterial cell wall (Bartosz, 1995 *Druga twarz tlenu*. PWN, Warszawa; Jankowski, Jankowski & Mirończyk, 2003, *Acta Mirobiol Polon.* **52**, 373). The aim of the present work was to recognize the interrelations between the kind of bacteria, the chemical properties of photosensitizers and the nature and concentration of reactive oxygen species (ROS) evolved in a given experimental conditions. Bacterial cultures (*Shigella flexneri* 2a, 1a, *Esherichia coli* K12, *Bacillus subtilis* 003, 0.5 – 1 cm^3) containing small amounts of dye solutions (10 μL) were incubated at various time intervals in the presence and absence of visible non mutagenic light(150 W/cm^2). The survival of microorganisms was determined by surface count method.

The concentration of OH^* radicals was determined in the solutions of dyes (Saphranine O, Hypericin, Eosin Y, Fe(II) sulfophthalocyanine, Malachit green) of analogous composition where instead the bacterial culture physiological salt solution was added The concentration of OH^* was determined by the spectrofluorimetric method using 4-((9acridinecarbonyl)amino)-2,2,6,6 tetramethylpiperidin-1-oxyl free radical) (TEMPO) (Haugland, 1996, *Handbbook of Fluorescent Probes* (VI ed) Eugene Molec. Probes)

The photodynamic action of dyes is expressed as a difference of survival percent between illuminated and not illuminated sample. A considerable photodynamic effect against Gram negative bacteria (Shigella flex. and E.coli) was found for safranine O and Eosine Y. These dyes are characterized also by considerable amount of OH^- produced (Tab.2). Well marked action of hypericin against E.coli may be due to production of singlet oxygen (Weiner & Mazur,1992, *J. Chem. Soc. Perkin Trans.* 1439).

34. Indolo[2,3-*b*]quinolines-liposome interactions: evidence for strong binding to negatively charged liposomes

A. Jaromin[1], A. Dychus[1], W. Peczyńska-Czoch[2], Ł. Kaczmarek[3], A. Kozubek[1]

[1]University of Wrocław
[2]Wrocław University of Technology
[3]Pharmaceutical Research Institute, Warszawa

Indolo[2,3-*b*]quinolines are a new class of highly potent antitumor agents synthesized at Pharmaceutical Research Institute, Warsaw, Poland. They are analogs of naturally occurring neocryptolepine, alkaloid isolated from roots of african plant *Cryptolepis sanguinolenta*, used in traditional medicine for treatment of infectious diseases, including malaria. Indolo[2,3-*b*]quinolines exhibit significant cytotoxic activity against several cell lines *in vitro* and strong antitumor activity against transplantable mouse tumors. They are also DNA intercalators and inhibitors of topoisomerase II.

Effects of indolo[2,3-*b*]quinolines upon model liposomal membranes were studied, since the therapeutic and toxic effects of agents are strongly influenced by their lipid affinity. The study of six indolo[2,3-*b*]-quinolines derivatives interactions showed strong binding to negatively charged liposomes in comparison to neutral vesicles. The apparent binding constants obtained for DMPC liposomes were in the range 6,67-370,3 M^{-1}, whereas for DMPC:DMPG (9:1 w/) 10000-117,6 M^{-1}. Our results indicated also the strong relationship between the presence and structure of the chain and the ability to incorporate into the carboxyfluorescein-trapped DPPC and egg PC liposomes. Compounds possessing the side chain stabilised the membrane after incorporation, contrary to agent without side chain, which completely disturb the membrane.

35. Determination of dielectrical and morphological parameters of eucaryotic cells by dielectric spectroscopy

W. Jaroszyński, B. Kwiatkowski

Medical University of Gdańsk

The electric properties of biological cells are strictly related to their structure and physiological state. These properties can be characterized by certain electrical quantities (e.g. relative electric permittivity and electric conductivity), pertaining to such morphological elements of the cell, as membrane, cytoplasm or nucleus. Thus a knowledge of these quantities is essential, not only for cognitive reasons, but also for purely practical ones.

In this study dielectric measurements were performed on the suspension of lymphocytes isolated from the spleen of mouse. Capacitance and conductance of cell suspensions were measured with Hewlett-Packard Im-

pedance Analyzer (model 4192A) and Hewlett-Packard Network/Spectrum Analyzer (model 4195A) in the 0.01-100 MHz frequency range.

The lymphocyte parameters were evaluated by applying nonlinear fitting of modified Irimajiri's equation to the measured data set, containing electric relative permittivity and electric conductivity of cell suspensions in a given frequency range.

The following dielectric parameters were estimated: relative electric permittivity and electric conductivity of the cell membrane, the cytoplasm, the nuclear envelope and the karyoplasm. Moreover, the following morphological parameters were estimated: the diameters of the cell and the nucleus, the thicknesses of the cell membrane and the nuclear envelope.

36. Separation of substances present in physiological fluids on semipermeable membranes studied by optical interference method

R. Jarzębińska, A. Jaroszewska, Z. Błaszczak

Adam Mickiewicz University, Poznań

The interference optical method has been applied to monitor transportation of physiological fluids through a semipermeable couprophan membrane placed between two chambers of a measuring vessel. The phenomenon of passive transportation (simple diffusion) of the substances studied through the membrane, induced by a difference in osmotic pressures on both sides of the membrane was studied by the interference method using a He–Ne laser beam ($\lambda = 632.8$ nm). The experimental apparatus consisted of a source of light, a Jamin interferometer, photodiode counting system, computer and a measuring vessel. The dynamics of the substance transportation was observed as changes in the optical density of the studied media. The time-dependent difference in the optical paths of two laser beams, passing through the media separated by the membrane, causes the appearance of interference fringes counted by a photoelectric system connected to a computer. During the transportation, changes in the refraction indices of both media were measured to get the information on the dynamics of transportation of molecules through the membrane. The permeability of the membrane for the solutes was determined from the Fick first law.

37. The twelve years of ASS-500 station situated in the area of Medical University of Białystok

J. Kapała, M. Tomczak, M. Karpińska, Z. Mnich

Medical University of Białystok

Air samples were collected at the early-warning sampling and monitoring radioactive contamination ASS-500 station situated at the Biophysics Department of the Medical Academy in Bialystok. This radioactive contamination monitoring station, designed and constructed at the Central Laboratory for Radiological Protection in Warsaw, forces large amounts of atmospheric air (400-800 m^3/hr) through a filter on which dust and aerosols are deposited, and measures and records changes in the global radioactive contamination deposited on the filter. The results of measurements are radioactive aerosols collected on filters over one-week cycles between 1992 and the end 2003. Over the period of nine years the air activity decreased from the mean values of 5.3 μBq m-3 in 1992 to the value 2.0 μBq m-3 in 2003. In the initial period, the good correlation between the Cs-137 activity in air and dustiness of air indicates that the prevailing source of Cs-137 activity is the dust containing soil and vegetation particles rising from the soil surface.

The presence of airborne Cs-137 leads to increased ionisation radiation hazard to the population from inhalation. The dose from radiocaesium absorbed by inhalation ranged from 1509 pSv in 1992 to 569 pSv in 2003. The effect of radiation hazard from inhalation is quite negligible (569 pSv in 2003), as it represents only a thousandth of a percentage of the total effective dose equivalent from environmental caesium contamination (value 2.9 mS).

38. Changeability in Radon concentration in one-family dwelling houses in the northeastern region of Poland

M. Karpińska, S. Mnich, J. Kapała

Medical University of Białystok

In 1988 International Agency for Research on Cancer considered radon to be the first class carcinogenic. Seasonal changeability of radon concentration in houses is a well-known phenomenon observed in various parts of the world.

Researchers have been interested in radon problems due to the complex character of the issue. They have tried to determine which regularities lead to elevated radon concentrations in dwelling houses when and how long detectors exposure should take place so that the results could determine the effective dose from radon. In order to examine the schemes of indoor radon concentration changeability in the northeastern Poland, all-year observation was performed in 4 chosen buildings.

Radon concentration measurements were performed simultaneously with observations of temperature and pressure outside.

All the buildings showed seasonal changes in radon concentrations. They had individual schemes of radon concentration changeability. Which varied together with the changes in outside weather parameters, such as temperature and pressure. Measurements based on several-days exposure give values ranged from 0.1 to 3.4 of the annual mean. Monthly measurements of radon concentration presented values from 0.3 to 1.8 of the annual mean. Half of the houses showed negative correlation between radon concentration and changes in atmospheric pressure.

39. Application of nonlinear dynamics in heart rate variability (R-R intervals) in children with diabetes type 1 with late vascular complications

A. Kitlas[1], E. Oczeretko[1], M. Kowalewski[2], M. Urban[2]

[1]University of Białystok
[2]Medical University of Białystok

We analysed the heart rate variability (R-R intervals) in diabetes type 1 children and healthy children by means of return map (Poincaré plot), approximate entropy (ApEn) and detrended fluctuation analysis (DFA).

The return map is a scattergram in which each measurement is plotted as a function of the previous one. Approximate entropy describes the complexity and irregularity of the signals. Detrended fluctuation analysis is a modified root mean square analysis of a random walk. It quantifies fractal–like correlation properties of the data.

We analysed two groups of patients: group A – 35 diabetic children with late vascular complications and control group B – 50 healthy children. For each patient 24 hour Holter ECG was recorded. ECG records were divided into two segments, day and night activity respectively.

Statistical analysis was performed by means of nonparametric sign test for paired and nonparametric Mann-Whitney test for independent data.

Return maps of a healthy child in his night and day activities are very complex. In the case of diabetic children we observe torpedo-shaped plots. The values of ApEn were lower in unhealthy children than in healthy children that indicated more regular heart rate in patients from group A. DFA method shows differences between healthy and diabetic children.

We concluded that return map, approximate entropy and detrended fluctuation analysis methods could provide useful information on R-R interval dynamics. Using these methods we can qualitatively and quantitatively study the heart rate variability and distinguish between healthy and unhealthy patients.

40. Impact of piperridine and pirrolidine nitroxides on cardiotoxicity of doxorubicin

A. Koceva-Chyła[1], A. Kochman[2], A. Stolarska[1], K. Gwoździński[1], Z. Jóźwiak[1]

[1]University of Łódź
[2]Medical University of Wrocław

Doxorubicin (DOX) is a powerful anthracycline antibiotic, widely used in treatment of a multitude of human cancers. The clinical usefulness of this drug is however significantly impeded by its high cardiac toxicity. It is believed that doxorubicin-induced cardiomyopathy is mainly caused by increased oxidant production generated by auto-oxidation of DOX and semiquinones in relatively unprotected heart myocytes. Hydrogen peroxide produced from the superoxide anion has been recently shown as the key oxidant that might be responsible for DOX-induced apoptosis in cardiomyocytes.

Different approaches, including development of less cardiotoxic application schedules and use of lower less toxic doses of the drug, or application of iron-chelating agent ICRF-187 have been developed in order to diminish this chronic side effect of DOX. Attention was also focused on researching and testing for natural and synthetic efficient antioxidants aimed at reducing and/or inhibiting free-radical reactions that mediate damage to cell. In this context a new class of emerging antioxidants nitroxide radicals has been intensively studied. These cell permeable stable radicals are non-toxic and non-immunogenic and are endowed with versatile antioxidant properties.

In this study we present our results obtained after the *in vivo* exposure of rats to doxorubicin alone or to doxorubicin in combination with pyrroline and pyrrolidine nitroxides – Pirolin and Pirolid. We tested the potential of these nitroxides to inhibit oxidant effect of DOX in rat myocardium on the basis of their impact on the level of the cell antioxidant defense system enzymes: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). We observed considerable increase in the activities of all of the investigated antioxidant enzymes in myocardium of rats injected with DOX. The most notable changes occurred in CAT activity and the least one - in SOD activity. Surprisingly the treatment of animals with Pirolin or Pirolid alone induced marked increase in both CAT and SOD activities — comparable to these caused by DOX. Contradictory to the effect of the nitroxides on CAT and SOD activities no detectable changes in activity of GPx were found under the same conditions. At the same time Pirolin and Pirolid given to rats in combination with doxorubicin reduced significantly an increase in SOD and CAT activities generated by DOX and entirely inhibited the prooxidant effect of this drug on GPx activity. The observed impact of piperridine and pirrolidine nitroxides Pirolin and Pirolid on the increase in the intracellular antioxidant enzyme levels as a response to oxidative stress induced by DOX suggest the protective antioxidant effect of these compounds against oxi-

dation processes initiated by doxorubicin in rat myocardium.

41. Effects of nitroxides on cardiac toxicity of doxorubicin *in vivo*

A. Koceva-Chyła[1], A. Kochman[2], A. Stolarska[1], K. Gwoździński[1], Z. Jóźwiak[1]

[1]University of Łódź
[2]Medical University of Wrocław, Wrocław

According to the prevailing hypothesis, the cardiotoxicity of anthracyclines is mediated by mechanisms that are distinct from those underlying the antitumor effects of these drugs and free-radical mechanisms and oxidative stress induced by anthracyclines in heart myocytes are considered as the main processes underlying their high toxicity towards these cells.

Glutathione (GSH) is the key regulator of intracellular redox status performing an antioxidant cell protection by cycling between its reduced (GSH) and oxidized (GSSG) forms. GSH acts directly as a free radical scavenger by neutralizing hydroxyl radical, restores damaged biomolecules by hydrogen donation, reduces peroxides, and maintains protein thiols in reduced state. Oxidative stress may cause changes in the glutathione redox state of different tissues and an increase in the intracellular level of GSSG and/or the rate of GSSG release from cells. GSH and its dependent enzymes work in concert with other antioxidants and antioxidant enzymes to protect cells against reactive oxygen intermediates. A marked decrease in cellular GSH levels in cells exposed to oxidative stress was well documented.

In the present study, the possible protective role of pyrroline and pyrrolidine nitroxides Pirolin and Pirolid against DOX-induced reduction of GSH intracellular levels in rat myocardium was evaluated. We have found that exposure of animals to a single dose of doxorubicin resulted in a marked depletion of intracellular level of glutathione in the heart tissue. Surprisingly nitroxides Pirolin and Pirolid themselves also caused a substantial decrease in the GSH level in cardiac myocytes *in vivo*, even greater by 50% than the decrease induced by DOX. In spite of this pro-oxidative outcome both Pirolin and Pirolid when injected together with DOX reversed its oxidative effects in cardiomyocytes and caused restoration of GSH level to the control value suggesting protective role of these nitroxides against doxorubicin cardiotoxicity.

42. Activity of ATPase and the induction and decay of thermotolerance

M. Koczmara, M. Koter-Michalak

University of Łódź

Defined conditions of hyperthermia leads to the increase in resistance of cells to high temperatures. Thermotolerance in erythrocytes exists and gains its maximum when the time of their incubation in the physiological temperature between thermal shocks is 3 hours and disappears after 7 hours of incubation in temperature of 37°C.

Plasma membrane of erythrocytes is one of the cell structures which may play some role in the induction of this phenomenon.

The aim of the work was to define the changes in the activity of the sodium-potassium pomp that can accompany the induction and decay of thermotolerance.

Erythrocytes with Ht=2% were incubated as follows:

15 min; 37°C – *X* h; – 30 min 37°C
15 min; **44°C** – *X* h; – 30 min 37°C
15 min; **44°C** – *X* h; – 30 min **48.5°C**
15 min; 37°C – *X* h; – 30 min **48.5°C**
where *X* = 0.25; 1; 2; 3; 5; 7, 16 hours at 37°C.

The measured parameters were:

the activity of the sodium-potassium pomp
the leak of the potassium outside the cell

The observed data showed that the incubation of erythrocytes at 44°C leads to changes in ATPase activity which increases together with the time of incubation at 37°C and gains its maximum after 3 hours. The activity of ATPase increases for all at the incubation times for which the increase in thermotolerance was observed. The maximum activity of the sodium-potassium pomp was observed for the time when the maximum thermotolerance of erythrocytes was also found. The potassium leak from cells increases together with the time and the temperature of incubation. For the process of the potassium leak the thermotolerance was not observed.

43. Thermotolerance in erythrocytes and structural changes of the proteins of their plasma membrane

M. Koczmara, P. Duchnowicz, M. Koter-Michalak

University of Łódź

High temperature treatment leads to structural changes in proteins of the cell plasma membrane. Human erythrocytes are enucleated cells and therefore they are used as the model cells for examining the role of plasma membrane in development of cell resistance to thermal shocks.

Preincubation in 44°C causes the phenomenon of thermotolerance, whose maximum is observed when the

time of incubation between the thermal shocks in the physiological temperature is 3 hours.

The aim of this study was to find the structural changes in the proteins of erythrocyte membrane before and after single and double heat shock.

Erythrocytes with Ht=2% were incubated as follows:

15 min; 37°C – *X* h; – 30 min 37°C

15 min; **44°C** – *X* h; – 30 min 37°C

15 min; **44°C** – *X* h; – 30 min **48.5°C**

15 min; 37°C – *X* h; – 30 min **48.5°C**

where *X* = 0.25; 1; 2; 3; 5; 7, 16 hours at 37°C.

The following parameters were determined:

the number of SH groups

the relation of strongly and weakly immobilized SH groups by the spin labels method

the protein components of the all membrane by the method of poliacrylamide gel electrophoresis (PAGE) in the presence of SDS

The obtained results showed the damage to the membrane proteins of heated erythrocytes. Together with the increase in the temperature the increase in the number of SH groups was observed. The decrease in the W/S parameter was observed, which W is weakly and S is strongly immobilized SH groups. Electrophoretical analysis of proteins of erythrocytes treated with the single and double thermal shocks, (when the time of incubation at 37°C was 3 and 7 hours) revealed the turn up of the two new low molecule protein fractions. Thermotolerance was observed for the number of SH groups and for the band 3 of electrophoresed proteins, when the incubation time between the shocks was 3 hours. The obtained results show that the treatment of erythrocytes with high temperature causes damage to the plasma membrane the proteins. Some of the changes accompany thermal resistance of the erythrocytes.

44. Optical properties of native human serum

M. Koralewski[1], J. Karoń[2]

[1]Adam Mickiewicz University, Poznań
[2]Karol Marcinkowski University of Medical Sciences, Poznań

Optical studies of the individual components of human blood serum (HBS) and the whole serum, have found some interest because of their possible clinical application. The methods of fluorescence, IR and Raman spectroscopy has been recently used for concentration measurements of multiple analytes in human serum. Reports by Surma *et al.* (2001, *Polish J. Med. Phys. & Eng.*, **71**, 25) indicate that a change in the quadratic magnetic field-enhanced optical activity of serum can be a marker of neoplasmic diseases. It has been also discussed that a change in the dispersion of the Faraday effect can be useful in diagnostics of sick headache (Ertel & Moskwa, 1991, [in:] M. Koralewski, *Magnetochiraloptical methods in biology*. A. Mickiewicz University Press, Poznań)

Systematic recognition of the fundamental optical properties of native blood serum in healthy normal individual is needed to provide adequate reference for comparison with malignant cases. The purpose of this work is to provide such reference results.

The paper presents results of a study on optical properties of native blood serum from healthy subjects taking into regard the effects of normal individual characteristics and laboratory specificity on the repeatability of results. The properties determined were UV/VIS absorption, optical activity, Faraday effect, light refractive index. The results obtained for HBS were compared with those obtained for their commercial replacements.

45. Complex regulation of ceramide synthase components *LAC1* and *LAG1*

M. Kołaczkowski[1,2], A. Kołaczkowska[1,3], B. Gaigg[4], R. Schneiter[4], W. S. Moye-Rowley[1]

[1]University of Iowa, Iowa, USA
[2]Wrocław Medical University, Wrocław
[3]University of Wrocław, Wrocław
[4]University of Fribourg, Friburg, Switzerland

LAC1 and *LAG1* are the two homologous genes involved in the essential ceramide synthase reaction in *Saccharomyces cerevisiae*. Inactivation of both genes leads to the synthetic growth defect and compromises production of ceramide. The biosynthesis of this key signaling molecule and its further metabolic conversion is tightly balanced to assure the proper control of proliferation and stress tolerance in yeast and higher eucaryotes. The regulatory mechanisms controlling this process are poorly understood. We will present data demonstrating the involvement of the pleiotropic drug resistance regulators in the transcriptional control of multiple steps of the sphingolipid biosynthetic pathway. Results of the detailed analysis of *LAC1* and *LAG1* expression by reporter gene, northern and western blot assays showing their differential regulation by several transcription factors controlling multidrug resistance and anaerobiosis, including Pdr3p and Rox1p will be discussed. Results of promoter mapping using reporter gene and *in vitro* footprinting assays indicating the involvement of a limited number of predicted putative binding sites in *LAC1* regulation will be presented. Our results show the extensive and coordinate control of multidrug resistance and sphingolipid biosynthesis in yeast.

46. $1/f^{\alpha}$ noise from electro-nanopores in BLM and sensitivity of the characteristics to ionic strength and cholesterol

M. Kotulska[1], S. Koronkiewicz[2], S. Kalinowski[2]

[1]Wroclaw University of Technology
[2]University of Warmia and Mazury, Olsztyn

Experiments on various types of nanochannels and nanopores, both biological and artificial, showed that random fluctuations of the channel conductivity produce two types of response, noise with Lorentzian spectrum $S(f) \propto f^{-2}$ and flicker noise (FN) with spectral density

$$S(f) = A\,\gamma_O^2 / f^{\alpha},\ A = S\,(1\ \mathrm{Hz})/\gamma_O^2,$$

where $\alpha \neq 2$, A is the Hooge parameter and γ_O is the open state conductance. The first characteristics can be modelled by a dichotomous Markov process in the pore. FN is more difficult to explain in terms of a simple Markovian model, without assuming the power-law distribution of the relaxation times in the two-state Markov process. Yet, the physical justification of the assumption has not been found.

There are indications that the stochastic process of the pore fluctuations may provide information about the chemical environment of the pore. Sensitivity of the PSD amplitude can be expressed by means of the Hooge parameter A and the FN exponent, α. It has been reported for various nanopores that higher concentration of diffusing molecules can be associated with larger PSD amplitude and, therefore, the Hooge parameter.

The discrete two-state Markov model has been tested on the nanopores generated in BLM by electrical current. The study showed that FN may result from fluctuations with continuous distribution of the conductivity states, which contradicts the two-state Markov models. Strong sensitivity of the characteristics to ionic strength of the electrolyte confirmed results from other nanopores, displaying correlations between various parameters. Cholesterol showed minor influence on the stochastic characteristics of the pore.

47. Thixotropic effect some of biochemical factors n ischaemic stroke

P. Kowal, A. Marcinkowska-Gapińska

Karol Marcinkowski University of Medical Sciences, Poznań

Yield shear stress (YSS) well characterizes a thixotropic status, that exemplifies a reversible loss of blood fluidity due to a low shear rate. At the stable haematocrit ratio YSS depends mainly on the fibrinogen level. Since the role of other biochemical factors has not well known in the YSS phenomenon in cerebral ischaemia, basically influenced by fibrinogen, we have undertaken this problem in the group of stroke patients. The study was carried out in 36 patients with acute ischaemic stroke and in 12 controls. YSS was estimated by means of microviscometric method. In all subjects the concentration of following biochemical factors were assayed: albumin, IgG, IgA, IgM, apolipoprotein A1 and b, cholesterol, triglycerides, LDL, HDL and fibrinogen. We found the positive correlations in relation to the following thixotropic effect: for all subjects and separately for patient's group: AlbYSS ($p<0.001$), ApoA1YSS ($p<0.001$), ApoBYSS ($p<0.05$), cholYSS ($p<0.01$), HDLYSS ($p<0.05$); for patients group without additional diseases: AlbYSS ($p<0.05$), ApoA1YSS ($p<0.005$), cholYSS ($p<0.05$), HDLYSS ($p<0.02$), LDLYSS ($p<0.05$). There were not any significant correlations in controls. Results of the study indicated that in the red cells and fibrinogen interaction may play a role some additional factors appearing during ischaemic process or have been presented in patient's blood but in a changed activity form.

48. Modification of yeast lipids and their susceptibility to oxidative stress and the action of antioxidants

A. Krasowska[1], A. Piasecki[1], A. Mróz[1], A. Prescha[2], K. Sigler[3]

[1]University of Wrocław
[2]Medical University of Wrocław
[3]Institute of Microbiology, Acad. Sci. CR, Prague, Czech Republic

Lipids in the plasma membrane of *Saccharomyces cerevisiae* yeast mutants lacking superoxide dismutases (cytosolic – SOD1 and mitochondrial – SOD2) undergo modifications that bring to higher unsaturation and elongation. These modifications enhance the susceptibility of the lipids to oxidative stress caused by paraquat (generator of superoxide radicals) and *tert*-butylhydroperoxide (TBHP – inducer of lipid peroxidation). Other alterations of the plasma membrane of these mutants include disappearance of free fatty acids (FFA) and an increase in the level of sterols

The antioxidative action of N-oxides of alkylamidoamines and alkylaminoesters, i.e., suppression of peroxidation of the tested lipids, depends on the length of alkyl chain, distance between amido and N-oxide groupings, and number of polar (amido and N-oxide) groupings in the antioxidant molecule of. This new group of antioxidants shows specificity against certain types of free radicals. Moreover, the presence of N-oxides in the plasma membrane brings about quenching of superoxide radicals (N-oxides with shorter alkyl chains) or lipid radicals (N-oxides with longer alkyl chains). Some N-oxides stimulate the growth of Δ*sod* mutants, indicative of protection against superoxide radical in the cells. These findings constitute a promising basis for intensive future research into the cell-protective properties of N-oxides of alkylamidoamines and alkylaminoesters.

The work was supported by grant 2483/W/IGIM

49. The influence of X radiation on the human erythrocyte membrane enzymes

A. Krokosz, Z. Szweda-Lewandowska

University of Łódź

We have studied the influence of X-radiation on the activities of two of human erythrocyte membrane enzymes — acetylcholinesterase (AChEase) and Na,K-ATPase.

Human erythrocyte suspensions in an isotonic Na-phosphate buffer, pH 7.4 with a hematocrit of 2% were exposed under air to X-radiation (200 kV, 20 mA) at a dose-rate of 23 $Gy \cdot min^{-1}$ at the dose range 40-600 Gy.

After irradiation the maximum reaction rate determined for AChEase did not alter to the dose of 200 Gy, and above that dose it decreased to 33% of control (unirradiated erythrocytes) for the dose of 600 Gy. The Michaelis-Menten constant (Km) reached the maximum at the dose of 200 Gy (159% of control) and then Km decreased to 53% of the control value for the dose of 600 Gy. The total ATPase activity slightly decreased after irradiation independently of a radiation dose. But the activity of Na,K-ATPase increased to the dose of 200 Gy reaching 114% of control for that dose. Above that dose the activity decreased to 94% of control for the dose of 600 Gy.

The osmotic fragility of erythrocytes irradiated up to the dose of 400 Gy was the same comparing to unirradiated erythrocytes. The cell stability decreased slightly after a dose of 600 Gy. These results revealed changes in the conformational state and function of membrane proteins preceding hemolysis. It may be suggested that these changes are responsible for keeping the osmotic stability and the integrity of the cell.

50. Magnetic centrifuge

K. Kubica

Agricultural University of Wrocław

One of the techniques for separating macromolecules and sub-cellular fractions is centrifuging. This techniques can be used for separation of particles that differ in their speed of sedimentation by at least one order of magnitude. Particles which do not satisfy that condition can be separated by centrifuging in density gradient (e.g., sucrose, glycerol, caesium chloride). However, separation with a centrifuge may often require a great rotation frequency for many hours. Also, when the particles density does not differ much from that of the medium, the time of swirling and centrifugal acceleration are large.

The subject of the project is a centrifuge whose rotor spins in magnetic field. Owing to such a solution the separation of charged particles can be accelerated, or made possible the separation of particles of density close to that of the medium.

A great majority of biological molecules or whole structures such as red blood cells are endowed with electric charge that follows from their chemical structure. Proteins in aqueous solutions possess amphoteric properties. The ionic groups of proteins originate from the groups $–NH_2$ and –COOH which are not bound with peptide bonds. The positive charges originate from the group NH_3^+ of lysine, arginine and histidine, whereas the negative charge – from dissociated groups $–COO^-$, mainly of aspartic and glutamic acids. Dissociation of those groups depends on pH of the medium, endowing a protein molecule with positive or negative charge, depending on its aminoacid composition. The magnitude of the charge is significant enough to allow separation in an electric field during electrophoresis.

In the device proposed a charged molecule placed in a centrifuge tube is subjected to an additional force of magnitude determined by its charge q, velocity **v** and magnetic induction **B**, aside of the centrifugal force and buoyancy. The new force is perpendicular to the vectors **v** and **B** and thus acts along the radius of rotation. Depending on the direction of **B** and value of electric charge that force can push the molecule along the radius in one or the opposite direction.

The magnetic centrifuge proposed offers a quicker and cheaper way of separation as compared with conventional ultracentrifuges.

51. Dielectric spectroscopy of human erythrocytes in the media containing hydroxyethyl starch

B. Kwiatkowski, W. Jaroszyński

Medical University of Gdańsk

Hydroxyethyl starch (HES) used as blood plasma substitute during acute controlled normovolemic hemodilution may cause erythrocyte aggregation. The aim of the study was to assess the degree and rate of erythrocyte aggregation in the media containing HES using dielectric spectroscopy.

The experiments were carried out on heparinized venous blood and erythrocyte suspension in 0.9% NaCl solution. The dependence of relative electric permittivity (determined for 100 kHz) of whole blood and erythrocyte suspension on time was applied to investigate the rate of erythrocyte aggregation.

The values of relative electric permittivity are differentiated by the degree of erythrocyte aggregation due to the presence of HES, both in whole blood and in erythrocyte suspension in 0.9% NaCl solution. The degree of erythrocyte disaggregation due to flow of the sample through a measuring chamber increases with the increase of flow intensity and correlates with decreasing relative electric permittivity value. The time constants t_1 and t_2 (which can be related respectively to the process of erythrocyte rouleaux formation and to the formation of 3-D network aggregates) indicate a difference between aggregation process taking place in whole blood

and after hemodilution with HES. Incubation of blood with HES (at the temperature 310 K) caused a decrease in aggregation degree. This effect was caused by the proces of hydrolysis of HES by endogenous alpha-amylase.

52. Effect of photodynamic therapy on free radicals in *human glioma GAMG* and *human glioma 42 MG BA* cells

M. Latocha[1], B. Pilawa[1], J. Dudek[1], T. Biniszkiewicz[2], L. Kozdrowska[3], A. Sieroń[2], T. Wilczok[1]

[1]Medical University of Silesia, Sosnowiec
[2]Center of Laser Diagnostics and Therapy, Bytom
[3]University of Zielona Góra

We tested *human glioma GAMG* and *human glioma 42 MG BA* cells, grown in monolayer cultures, in Dubelcco (Sigma) and RPMI (Gibco) medium respectively. Dubelcco, as well as RPMI medium, were supplemented with 10% fetal bovine serum (FBS; Gibco), 1% penicillin (10000 UI/ml) and streptomycin (10 mg/ml). The cultures were incubated at 37°C in a humid atmosphere containing 5% of CO_2.

Paramagnetic center systems in two different types of tumor cells treated by means of photodynamic therapy (PDT) were analyzed by electron paramagnetic resonance (EPR) spectroscopy. During PDT photosensitised cells absorb laser light of adequate wavelength and free radicals originate.

In the following paper the evolution of free radicals in irradiated *human glioma GAMG* and *human glioma 42 MG BA* cells were compared. *GAMG* and *42 MG BA* cells were treated by 5-δ-aminolevulinic acid (ALA) as photosensitizer and irradiated with 635 nm laser light. Free radical properties of the studied cells were tested 6 hours after PDT treatment.

EPR measurements were performed using spectrometer at X-band (9.3 GHz) with magnetic modulation of 100 kHz. Microwave power 0.7 mW was applied to avoid saturation of the EPR lines. EPR spectra as the first derivative of absorption were recorded by the use of Rapid Scan Unit. The experimental EPR lines were accumulated 300 times and analysed. Amplitudes, g-factors, integral intensities, and linewidths of EPR curves were determined.

EPR spectra of the studied tumour cells presented as very broad lines with linewidths higher than 1 mT. Strong dipolar interactions of unpaired magnetic moments and unresolved hyperfine structure are responsible for such line broadening. Organic free radicals with g-values close to 2.0040 are predominant in the studied cells. Photodynamic therapy (PDT) changed the number of paramagnetic centres in both studied groups of cells. The break of chemical bonds led to creation of free radicals. Effect of recombination of the formed free radicals may also appear. The number of free radicals in the studied cells after separate and simultaneous using of photosensitizer and laser light was discussed. The strongest increase of free radicals was observed for *GAMG* cells following the simultaneous application of photosensitizer and 635 nm light irradiation.

53. The effect of UV-C radiation on IAA-stimulated redox activity in maize coleoptile segments

H. Lekacz, W. Karcz, M. Kędziorska, P. Zawidlak

University of Silesia, Katowice

It is generally accepted that plants have a plasmalemma – bound redox system, which is involved in many physiological processes (for review sees Lüthje et al., *Biochim. Biophys. Acta*, 1997, **1331**, 81). It was previously showed (Karcz and Stolarek, *Physiol. Plant,* 1988, **74**, 770) that the exposure of maize coleoptile segments to UV-C radiation brought about the inhibition of proton extrusion and elongation growth. Karcz and Stolarek (1988, *Physiol. Plant.*, **74**, 770) have suggested that the suppression of both processes was caused by inhibition of plasma membrane proton pump. On the other hand, the H^+ export may also arise from plasma membrane redox reactions.

The experiments were carried out with 10 mm-long maize coleoptile segments cut from 4-day-old etiolated seedlings. Redox activity was determined spectrophotometrically in agreement with the method described by Carrasco-Luna at al., (1995, *Protoplasma*, **184**, 63). Redox activity and proton secretion were measured with or without auxin (IAA). Before experiments the maize seedlings were irradiated with UV-C radiation (300 μW cm^{-2}).

We have found that the inhibitory effect of UV-C on proton secretion and redox activity was partially abolished in the presence of indolilo-3-acetic acid (IAA)

54. Segmentation of multimodal MRI images using chossen clustering algorithms

K. D. Lewandowska, P. Boguś

Medical University of Gdańsk

In the last years one can find the large development of medical imaging methods (e.g. CT, MRI, PET, SPECT etc). This paper presents a new method of MRI images analysing which bases on multimodal medical images. A multimodal medical image is a set of images represents the same anatomical structure. The multimodal image can be obtain from one or more imaging techniques, for example a three-modal MRI image consists of three image modes represent the same slice characterized by proton density PD and relaxation times T_1 and T_2. The analysis of multimodal medical image gives us the

segmentation which integrates all information from the component image modes. This is done by performing a clustering in a multidimensional feature space which dimension is equal to a number of modes. The clustering algorithm automatically performs the process of division of the data set into clusters. In the paper we present some of our results of analysing a three-modal MRI image and some chosen clustering methods: classical hard c-means algorithm of clustering, fuzzy and possibilistic algorithms of clustering and algorithms based on statistical physic methods with using the maximum entropy principle. The final results are not perfect mostly because of applied pre-processing methods and using of imperfect clustering algorithms hence studies in this area are constantly performed.

55. Perturbation of model lipid membrane structure by resveratrol

B. Łania-Pietrzak, A. B. Hendrich, K. Michalak

Wrocław Medical University

Resveratrol (trans-3,5,4'-trihydroxystilbene) naturally occurs in pine trees, peanuts, grape skin and thus in red vine. This compound belongs to group of phytoalexins, a class of antibiotics produced as a part of a plant's defense system. It was shown that resveratrol contributes to the antioxidant properties of red vine and thereby may play a role in prevention of human cardiovascular diseases. Resveratrol is harmless to normal human cells but posses some anticancer properties. It appears to prevent or inhibit development of many types of cancers at different stages in many ways: from blocking estrogen and androgen binding to modulating genes.

In present study resveratrol was investigated as the agent potentially able to perturb the model phospholipid bilayers. We examined its influence on behavior of model systems made of various lipids: synthetic, zwitterionic DPPC and DMPC, anionic DMPG as well as on natural EYPC. In our studies we used differential scanning calorimetry (DSC) and fluorescence spectroscopic methods: determination of Laurdan generalised polarisation (GP) and DPH fluorescence polarization measurements. Calorimetric experiments have shown that resveratrol decreases enthalpy and temperature of phase transition, broadens the transition peaks but effects exerted on zwitterionic lipids were stronger than those exerted on the charged one. Measurements of DPH fluorescence polarization revealed that resveratrol may influence membrane fluidity and order but pronounced increase in DPH polarization were observed exclusively for bilayers in liquid-crystalline state. Resveratrol affects GP of Laurdan in DPPC membrane but only in temperatures above phase transition of DPPC. We conclude that resveratrol presumably penetrates the hydrophobic core of bilayer but its exact positioning in lipid membranes needs to be determined by further investigations.

56. Photogeneration of electric signal in electrochemical cell with porphyrin solvents

J. Łukasiewicz, A. Ptak

Poznań University of Technology

Porphyrin dyes are interesting objects of investigation because of their potential application in molecular electronics as optical switches or memory matrics, in traditional electronics (light diodes) and in conversion of light energy into electrical energy (solar energy devices).

Metal-free and complexed with metals such as: magnesium (Mg), zinc (Zn), lead (Pb), pallad (Pd) and platinum (Pt) tetraphenyl porphyrins (TPP) dissolved in DMSO and embedded in photoelectrochemical cell were studied. Absorption, fast and slow kinetics (micro second and second scale) of generated photocurrent and photovoltage were recorded. The energy of triplet states was calculated by quantum mechanics molecular simulations. It depends on molecular structure of the dyes, properties of solvent and temperature.

The wavelengths of applied excitations were adjusted to the Soret band maxima of the absorption spectra of porphyrin dyes. In a photoelectrochemicall cell porphyrin dyes are used to absorb light energy, what leads to electron injection from the porphyrins adsorbed to the semiconductor electrode. In terms of energy levels electrons from excited electron states of the porphyrins are injected to the conductive band of semiconducting electrode.

The kinetics of photosignal generation and decay indicate what electrical effects occur in photoelectrochemical cell, what mechanisms are responsible for electron transport between electrodes and finally which porphyrin dyes can be applied in technique.

The Poznań University of Technology (grant BW 62-195/04) suported this research.

57. Effect of temperature on the process of electric domains forming in the system of dipoles

D. Man, M. Podolak, A. Kluza, R. Olchawa

University of Opole

This work present the results of computer simulation of the electric interaction between polar heads of lipids in the surface layer of liposome membranes. In the investigated model the polar heads of lipids were represented by a flat electric dipoles system in form of matrix of rectangular or hexagonal centered geometry. The matrices contained 255, 1 nm long dipoles, which centers were separated by 2 nm space each other. The simulation algorithm was based on the Monte Carlo method. The aim of studies was determination of influence the temperature and random drawing arrangement of dipoles

system, at the beginning of the simulation process, on their final arrangement and potential energies. The system temperature was changed from 250 K to 350 K. Spontaneous forming of dipoles domain structures which shape depended on initial state drawing was observed. These structures and potential energy of the system final states changed suddenly with the temperature increasing. The simulation were repeated over hundred times for each of the matrix. The energies of the final states of the system in the case of rectangular matrix were close to each other but in the case of hexagonal matrix their values were arranged into two bands by energetic gap separated.

58. Effect of the organic tin compounds on the dynamic properties of lipid membranes by EPR method studied

D. Man, M. Podolak, G. Engel

University of Opole

The aim of the work was to test the influence of the organic tin compounds admixtures on the dynamic properties of the lecithin liposome membranes. Tested was three compounds with different alkyl chains length and one of them, the tin chloride (with the longest chain) to occur as a positive ion in the water solution. The admixture concentrations ranges from 0.5 to 15% in relation to the lecithin. The EPR spin probe method was used to the study. The results of studies indicate significant effect of tin chlorides on the membrane liposomes fluidity. The membranes fluidity increased with the compounds concentration increasing. Especially great activity in this process showed the tin chloride. In the case of this compound admixtures membranes fluidity increased to the 2.5% concentration but it not changed after exceeding this value.

59. Study of hemorheological properties by means of an oscillatory-rotary rheometer in patients after cerebral stroke

A. Marcinkowska-Gapińska, P. Kowal

Karol Marcinkowski University of Medical Sciences, Poznań

Blood is a systemic liquid of clearly non-Newtonian character and of distinct viscoelastic properties. Blood flow in the circulatory system depends on the physical and physicochemical properties of blood as well as on many phenomena resulting from the structure and properties of the circulatory system. Blood viscosity is one of the most important factors determining the blood flow and its value depends on the hematocrit, ability of the erythrocytes to deform and orient in the flow and on the plasma viscosity. In the studies we have utilized oscillatory methods known also as mechanical dynamic analysis. The principle of the technique lies in determining the amplitude and phase of the oscillations of the studied sample subjected to action of a harmonic force of controlled amplitude and frequency. The viscoelastic properties of the blood samples were measured, resulting in determining so-called complex blood viscosity. Standard rotary measurements of blood viscosity as a function of shear rate (flow curves) have been also performed as supplementary study. All the measurements have been performed by means of the Contraves LS-40 rheometer on blood samples taken from patients after cerebral stroke. The data obtained from the flow curve measurements have been analyzed in terms of rheological model of Quemada. Information obtained from both rotary and oscillatory measurements indicate in patients after cerebral stroke an increased ability of red cells to aggregate.

60. Influence of glutaraldehyde and idarubicin on the human erythrocytes

A. Marczak, Z. Jóźwiak

University of Łódź

A number of investigators have been focusing their attention on the encapsulation of antineoplastic drugs within erythrocytes. Due to the fact that erythrocytes play an active role in drug distribution, metabolism and elimination it was necessary to use the red cells as a carriers for anthracycline antibiotics including idarubicin. Glutaraldehyde is often used as crosslinking agent to entrapped the drug from the incubation medium. With this cross-linking agent much more of the drug was entrapped in the red cells, but the results suggest that glutaraldehyde in the drug-pretreated erythrocytes may also produce the significant perturbations in the structure and function of the cells. The aim of the study was determination of the glutathione content, and the alterations in the activity of glutathione related enzymes in human erythrocytes exposed to idarubicin (IDA) and glutaraldehyde. Measurements of reduced and total glutathione levels, and the activity of glutathione reductase and glutathione transferase were performed spectrophotometrically. We demonstrated that IDA at concentrations of 10 μg/ml small decreased the level of total and reduced glutathione. When IDA preincubated erythrocytes were treated with glutaraldehyde, the decreases in the determined parameters were observed. It was correlated with increased activity of glutathione transferase and decrease of the activity of glutathione reductase.

61. Spin-lattice relaxation in *Cladosporium cladosporioides* melanin

M. Matuszczyk, E. Buszman, B. Pilawa, T. Witoszyńska, T. Wilczok

Medical University of Silesia, Sosnowiec

Electron paramagnetic resonance spectroscopy (EPR) was used to examination of magnetic interactions in melanin existing in *Cladosporium cladosporioides* pigmented soil fungi. Mainly o-semiquinone free radicals are responsible for the EPR spectra of this biopolymer. Thermally excited multiplet states are also responsible for paramagnetism of these natural melanins. Paramagnetic properties of *Cladosporium cladosporioides* melanin are modified by bound metal ions. The binding of metal ions by melanin is very important for the environmental protection. EPR spectroscopy is the useful method to determine the ability of melanin in soil fungi to complex metal ions.

The aim of this work was to study the spin-lattice relaxation processes in *Cladosporium cladosporioides* melanin. The effect of diamagnetic zinc and paramagnetic copper ions on spin-lattice interactions in fungal melanin was examined. The comparative EPR analysis was done for model DOPA-melanin. Mycelium and melanin isolated from *Cladosporium cladosporioides* fungi, and DOPA-melanin were complexed with Zn^{2+} and Cu^{2+} ions with concentrations in the range 1×10^{-5}-1×10^{-3} M.

EPR spectra were measured by the use of and X-band (9.3 GHz) electron paramagnetic resonance spectrometer at room temperature. Magnetic modulation was 100 kHz. The EPR spectra were recorded in the range of microwave power from 0.7 to 70 mW. The evolution of resonance lines with microwave power was analyzed. The changes of amplitudes, integral intensities and linewidths with increasing of microwave power was discussed. Concentrations of paramagnetic centers in the melanin samples, and the parameters of their EPR lines (linewidths, g-factors) were compared.

The lineshape of the EPR spectra indicates that melanin *of Cladosporium cladosporioides* is similar to model DOPA-melanin (eumelanin). The single eumelanin EPR line dominates in EPR spectrum of the analysed melanin biopolymer. Only the low amount of pheomelanin exists in fungal melanin. EPR spectra of *Cladosporium cladosporioides* samples showed only a very weak signal of pheomelanin with unresolved hyperfine structure. This signal appeared at the higher microwave powers. Diamagnetic zinc ions increased the concentration of paramagnetic centers in the studied melanins. Paramagnetic copper ions decreased the concentrations of paramagnetic centres in the samples.

The continous microwave saturation of the EPR spectra of the melanin samples was applied. The changes of amplitudes of EPR lines with increasing of microwave power bring to light information about spin-lattice relaxation processes in the samples. The value of microwave saturation of EPR line depends on spin-lattice relaxation time. The power of microwave saturation increases with decreasing of spin-lattice relaxation time in the sample. For all the studied samples the saturation of EPR lines at low microwave powers was observed. It can be then concluded that slow spin-lattice relaxation processes exist in melanins. Metal ions increase the spin-lattice relaxation time in melanin. Fast spin-lattice relaxation processes occur in copper paramagnetic centers system in melanin. Microwave saturation of EPR lines of Cu^{2+} ions in melanin was not observed.

62. Microwave saturation of EPR spectra of DOPA-melanin-netilmicin complexes with Zn^{2+}

M. Matuszczyk, B. Pilawa, E. Buszman, D. Wrześniok, A. Grzegorczyk, T. Wilczok

Medical University of Silesia, Sosnowiec

High affinity of melanin for aminoglycoside antibiotics and metal ions is known. Stable paramagnetism characterizes melanin biopolymers. It was proved earlier (Pilawa, Buszman, Wrześniok, Latocha & Wilczok, 2002, *Applied Magnetic Resonance*, **23**, 181; Pilawa, Latocha, Buszman & Wilczok, 2003, *Applied Magnetic Resonance*, **25**, 105) that binding of drugs to melanins increases the concentrations of paramagentic centres in these biopolymers. The changes of magnetic interactions in melanin caused by drugs and metal ions are not well described so far. The aim of this work was to study the spin-lattice relaxation processes in DOPA-melanin complexes with netilmicin and diamagnetic zinc ions.

The exemplary aminoglycoside antibiotic - netilmicin was complexed with DOPA-melanin in concentration of 1×10^{-3} M. The same concentration of zinc ions 1×10^{-3} M was used. The complexes [DOPA-melanin-netilmicin]-Zn^{2+} and [DOPA-melanin-Zn^{2+}]-netilmicin were analysed by electron paramagnetic resonance (EPR) spectroscopy.

The measurements were done using EPR spectrometer produced by RADIOPAN (Poznań). The microwave frequency (~9.3 GHz) was measured by MCM 101 recorder. EPR spectra were recorded at microwave power in the range 0.7-70 mW at room temperature. Changes of amplitudes, integral intensities and linewidths with increasing of microwave power were analyzed. Concentrations of paramagnetic centers in the studied melanin complexes and g-factors were calculated.

Single dipolar broadened EPR lines were recorded for DOPA-melanin and its complexes with netilmicin and zinc ions. Netilmicin and diamagnetic Zn^{2+} ions increased the concentration of o-semiquinone free radcials in melanin. Homogeneous broadening for the all EPR lines was observed. Amplitudes of the EPR lines increased with increasing of microwave power, reached maximum value and decreased for the highest microwave powers used. Linewidths of the analyzed EPR lines increased with increasing of microwave power. The low values of microwave saturation of EPR lines were characteristic for DOPA-melanin, DOPA-melanin-

netilmicin complexes and the melanin complexes with Zn^{2+} ions. Similar effects were observed for complexes of DOPA-melanin with chloroquine, gentamicin and kanamycin (Pilawa *et al.*, 2002; Pilawa *et al.*, 2003). It indicates that paramagnetic centers systems of melanins and their complexes with drugs and zinc ions reveal the long spin-lattice relaxation times.

63. Various ways to explore processivity of Eg5 motor protein

M. Mazur[1,2], M. Korneev[3], C. Schmidt[3]

[1]Wroclaw University of Technology
[2]Biophysical Scientific Students Union "Micela"
[3]Vrije Universiteit Amsterdam, The Netherlands

Eg5 is a member of BimC kinesin super family. It's plus end-directed microtubule motor and pushes the centrosomes apart during mitosis. The biochemical cycle, biophysical properties and processivity of this motor aren't well known yet. (Processive motor can hydrolyze many molecules of ATP before detaching from the filament and non-processive motors interact with filament only once.) We can obtain information about processivity from gliding assays with using DIC microscopy and from 3-bead assay with Optical Tweezers. These kind of experiments were performed and the information about processivity was obtained. In gliding assays methylcellulse- highly elongated polymer was used to bring filament more down close to the surface and the speed of the Eg5 was measured.

64. The effect of the isomers of zeaxanthin on structural properties of lipid membranes

J. Milanowska[1], A. Kostecka-Gugała[2], K. Strzałka[2], W. I. Gruszecki[1]

[1]Maria Curie-Skłodowska University, Lublin
[2]Jagiellonian University, Kraków

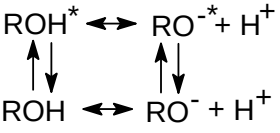
Zeaxanthin is a pigment that takes part in the xanthophyll cycle of higher plants under light stress condition. Zeaxanthin is also a pigment that is present in the membranes of macula lutea of the retina of primates. According to the current hypothesis zeaxanthin, present directly within the lipid phase of biomembranes, protects lipids against oxidative destruction, by quenching active oxygen species and threw modification of the physical properties of lipid phase. Zeaxanthin appears in vision apparatus in three stereochemical conformations: all-trans, 9-cis and 13-cis. Therefore it is interesting to learn the effect of the isomers of zeaxanthin on structral propertis of lipid membranes. We have compared the influence of isomers of zeaxanthin on the thermotropic phase behaviour of lipid membranes (multilamellar vesicles) formed with: dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphatidylethanolamine(DPPE), dihexadecylphosphatidylcholine (DHPC) by means of differential scanning calorimetry. Incorporation of isomers of zeaxanthin affects the thermal properties of examined multibilayers. The most pronounced changes of the thermotropic properties are visible in the vesicles formed with DHPC. This can be explained in terms of lack of the C=O bond in DHPC, involved in formation of hydrogen bonds between lipids and xanthophylls. Incorporation of zeaxnthin to the membranes formed with DHPC shifts of the main phase transition temperature to higher values by ca. 1°C.

65. Impact of acetic acid and ammonia vapor on the fluorescence spectra of 2-naphtholo-6sulfonamide of dodecylamine included in Langmuir-Blodget films

A. Mirończyk[1], A. Jankowski[1], A. Chyla[2], A. Ożyhar[2], P. Dobryszycki[2]

[1]University of Zielona Góra,
[2]Wrocław University of Technology

It has been shown (Mirończyk, Jankowski, Chyla, Ożyhar & Dobryszycki, to be published) that 2-naphtholo-6-sulfonamide of dodecylamine (NSDA) included in a Langmuir-Blodgett (LB) film can undergo excited state proton transfer (ESPT) to environment. Only a scarce information is available in the literature on the spectroscopy of compounds in LB films undergoing ESPT (Mirończyk, Jankowski, Chyla, Ożyhar & Dobryszycki, 2004, *J. Phys. Chem.*, **108**, 5308; Mirończyk A. & Jankowski A., 2002, *J. Photochem., Photobiol.* **A 153,** 89) (see Scheme 1).



Scheme 1. Relation between ground and excited state proton transfer

The scope of the present work is to explore the influence of gases on the electronic spectra of NSDA included in LB films. One may expect that these substances incorporated in LB films, will be sensitive to acids and bases. They may find application as chemical sensors and signal processors. We have found that acetic acid vapor inhibits ESPT, (as may be inferred from disappearance or a decrease of the fluorescence of RO^* form), greatly increases (up to 1 order of magnitudes) the fluorescence quantum yield of ROH^*(see Scheme 1) and shifts the fluorescence band maximum to the blue (5–20 nm). Analogous effects, though less marked, are evoked by HCl. Ammonia enhances ESDP and fluorescence quantum yield though this last effect is smaller than that induced by acetic acid.

The indicated phenomena may be explained by formation of a complex of acetic acid with NSDA (Mirończyk A. & Jankowski A., 2002, *J. Photochem., Photobiol.* **A 153,** 89), formation of a hydrogen bond

and de-aggregation of the chromophore molecules. The behavior described may be exploited in gas sensing and signal transduction

66. Photoelectrical reaction of *Nitellopsis obtusa* cells in the presence of aluminum

R. Mizerski, R. Kurtyka, W. Karcz

University of Silesia, Katowice

The main goal of the present study was to determine the effect of aluminum ions on the photoelectrical reaction of internodal cells of Nitellopsis obtusa. The electric reaction of plants cells in response to the action of visible light has been studied over many years by various investigators. It was found that the illumination of higher plant cells and giant algal cells induces transient changes of electric potential differences across the plasmalemma. So far there is no systematic data on the effect of Al^{3+} ions upon photoelectric reaction in plant cells. Membrane potential measurements were carried out using a standard electrophysiological technique. Before the experiments the cells were incubated within 12 hours in the dark in solutions containing different (10-5-10-3 M) $AlCl_3$ concentrations, pH of the medium was 4.4. We have showed that in the cells of Nitellopsis obtusa visible light induced hyperpolarization of the membrane potential, which value was dependent on aluminum concentration in the bath medium. In the presence of 10-3 M Al^{3+} the photoreaction wasn't observed, while in concentrations 10^{-4} and 10^{-5} M the amplitude of hiperpolarization was lower (32.1±3.4 and 26.8±2.5 mV, respectively) as compared to the control (45.4±4.5 mV). Our preliminary data suggest that aluminum ions may change the activity of plasma membrane H+-ATP-ase as well as may cause destruction of photosynthetic apparatus.

67. Radon concentration in multi-storey buildings at different age

Z. Mnich, M. Karpińska, J. Kapała, A. Birula

Medical University of Białystok

It was found that radon exhalation rate from concrete is connected with an aging process of this material (Martialay, 1987, [in:] *Concrete durability*, J. M. S. Detroit (ed.), MI: Amer. Concrete Institute; Roelofs & Scholten, 1994, *Health Phys.*, **67**, 266; Rogers *et al.*, 1995, *Health Phys.*, **68**, 832; Chan *et al.*, 1995, *Health Phys.*, **68**, 716). The relationship between buildings age and radon concentrations inside them was also observed (Fjeld *et al.*, 1990, *Health Phys.*, **59**, 217; Fujimoto & Sanada 1999, *Health Phys.*, **77**, 410; Gerken *et al.*, 2000, *Health Phys.*, **78**, 268; Gunby *et al.*, 1993, *Health Phys.*, **64**, 2; Leung *et al.*, 1998, *Health Phys.*, **75**, 303; Verger *et al.*, 1994, *Radiation Protection Dosimetry*, 5**6**, 225).

The aim of the study was to compare radon concentrations in neighboring hospital buildings which were constructed in different years during the period 1963–2000 and are located on areas with similar radon potential.

Radon concentration in buildings was measured using a method of registration of alpha particle tracks on synthetic foils (Dudney *et al.*, 1995, *Health Phys.*, **69**, 501; Fleicher *et al.*, 1980, *Health Phys.*, **39**, 975; Urban & Piesch, 1981, *Radiation Protection Dosimetry.*, **1**, 97). These foils register alpha particles coming from radon decay as micro-tracks. The CR-39 plastics, made of polyallyl diglicol carbonate, were placed in NRPB chambers and exposed in chosen rooms for one year. During exposition of the detectors the measured rooms were in normal living condition.

The value of AM radon concentration in soil gas amounted to 14464 Bq m^{-3}. In a hospital, built 40 years ago, the AM radon concentration in the cellar was 38.4±36.7 Bq m^{-3} and on higher levels it was 17.1±10.3 Bq m^{-3}. In a hospital, built 16 years ago, these values equaled 45.5±47.2 Bq m^{-3} and 20.1±12.5 Bq m^{-3}, respectively. In the newest hospital, built 3 years ago, radon concentration (AM) in a cellar was 32.3 ± 27.4 Bq m^{-3} and the respective value on higher levels amounted to 20.4 ± 12.6 Bq m^{-3}.

When comparing radon concentrations in the cellars, no statistically significant differences were found. Similarly, no statistically significant differences were observed between radon concentrations measured on higher levels in investigated hospital buildings.

68. Spectrophotometric analysis of interaction of amphiphilic substances with lipid bilayers

E. Moczko[1,2], A. Zań[1,2], M. Langner[1,3]

[1]Wroclaw University of Technology
[2]Biophysical Scientific Students Union "Micela"
[3]Academic Center for Biotechnology of Lipid Aggregates, Wrocław

Quercetin is a ubiquitous bioactive flavonoid. It is present in e.g. citrus fruit, onion, apples, red wine, tea. Quercetin appears to have many beneficial effects on human health, including cardiovascular protection, antiallergy, antioxidant and anticancer activities. We intend to analyze the interaction of quercetin with phenyltin compounds that are toxic and highly reactive. Both of the compounds used are amphiphilic and tend to aggregate. Our aim was to study the interaction of these amphiphilic compounds in methanol, aqueous solutions as well as in the liposomes. We performed quercetin titration with diphenyltin chloride. Phenyltin altered quercetin absorbtion spectra. Achieved data were used for further spectrophotometric analysis. We compared signals in different environments to determine the interaction in phospholipid bilayer.

69. The assessment of a selective inhibition of potassium channels and guanylate cyclase in the relaxation induced by exogenous nitric oxide in the human nonpregnant myometrium

B. Modzelewska, A. Kostrzewska

Medical University of Białystok

Nitric oxide is a multifunctional molecule that mediates a number of diverse physiological processes. The aim of our work was to study paths of nitric oxide induced relaxation of the human nonpregnant myometrium. We investigated the effects of specific blockers of potassium channels and the effect inhibitors of guanylate cyclase on the influence of exogenous nitric oxide on of the human non-pregnant myometrium. After preincubation of uterine strips with L-NNA, exogenous NO (donated by DEA/NO) inhibits their spontaneous contractile activity dose-dependently. Methylene blue and cystamine did not prevent nitric oxide -induced relaxation of the uterine strips. All the potassium channels blockers used significantly inhibited the effect of NO on the contractile activity of the myometrium (estimated by AUC value). Incubation with apamin did not significantly alter the DEA/NO-induced decrease of the amplitude of myometrial contractions. However, we observed the significant decrease in their frequency. Preincubation with CTX did not change the influence of DEA/NO on the amplitude of the contractions, but it inhibited the decrease in frequency caused by DEA/NO administration.

Our data suggest that exogenous NO relaxes the human nonpregnant uterus without involvement of cyclic guanosine monophosphate and beside calcium and voltage-dependent charybdotoxin-sensitive potassium channels, apamin-sensitive potassium channels are also present in the human nonpregnant myometrium.

70. The overall motion correlation time for some globular proteins in diluted solutions from viscometric measurements

K. Monkos

Silesian Medical Academy, Zabrze

The behavior of globular proteins in a streamline flow of a solution is determined by two effects: (1) the orientation of their principal axis parallel to the flow direction by the flow and (2) acting against this the rotational Brownian motion produced as a reaction of the proteins to the random heat motions of the solvent. As a result the orientation of the proteins principal axis is anisotropic in space and can be described by the distribution function of all possible directions, which fulfils a diffusion-type equation. When the external stress field disappears, the anisotropy in the principal axis space distribution vanishes because of the rotational Brownian motion of proteins. The time in which the initial orientation decreases by the factor of 1/*e* is called the overall motion correlation time. For diluted solutions it can be obtained if the intrinsic viscosity, molecular mass and the axial ratio of the proteins in solution is known.

The intrinsic viscosity has been measured by using an Ubbelohde-type capillary microviscometer immersed in a water-bath controlled thermostatically. The obtained numerical values of the overall motion correlation time are in the following range: from 622 ns (1°C) up to 111 ns (55°C) for human IgG immunoglobulin; from 121 ns (1°C) up to 33.6 ns (45°C) for human serum albumin; from 65.7 ns (1°C) up to 14.3 ns (55°C) for ovalbumin and from 13.2 ns (1°C) up to 2.4 ns (55°C) for hen egg-white lysozyme.

71. Hemoglobin response to Near Infrared Radiation

M. Mróz, A. Dyrda, M. Komorowska, T. Misiaszek

Wroclaw Uniwersity of Technology

We found that concentration of deoxyhemoglobin increased about 30% after exposition red cells to near infrared radiation (NIR) (Komorowska *et al.* 2002, *J. Photochem. Photobiol.B: Biology*, **68**, 93). However affinity to oxygen of this conformer is very low. During next few days erythrocytes after exposition to NIR contained unchanged amount of oxy-, deoxy- and methemoglobin. The transition of the oxyhemoglobin to the deoxy state is induced probably by dehydration of the protein followed to NIR radiation and energy of dehydration was estimated as 0.8 kJ/mol (Colombo *et al.*, 1990, *Science* **256**, 655). Thus long-standing conformer could be the same form of deoxyhemoglobin which was found by Shibayama et al [3] within wet porous silicate sol-gels. The equilibrium O_2 binding measurements of the encapsulated (encapsulation evidently changes the hydration shells around proteins N. (Shibayama & Saigo, 2001, *FEBS Letters* **492**, 50) deoxyhemoglobin samples showed that deoxyhemoglobin free of anions coexists in two conformations that differ in oxygen affinity by more than 40 times Eggers & Valentine, *J. Mol. Biol.* **314**, 911). On the other hand water/alcohol or organic compounds solvents strongly stabilize oxyhemoglobin before autoxidation. The stabilization can be explained in terms of the formation of a solvation shell contained water and alcohol molecules what could be treated as partially dehydrated molecules (Nedjar-Arroume *et al.*, 1991, *Biotechnol. Appl. Biochem.*, **13**, 303). Thus dehydration induced by NIR may stabilize both forms of hemoglobin towards oxidation. This communication reports the effect of NIR radiation on the Raman and fluorescence spectra of human hemoglobin prepared from erythrocytes exposed to 750 –2000 nm radiation.

Obtained results clearly show that low oxygen affinity and increased resistance to autoxidation are due to dehydration process. The increasing hydrophobicity after

exposition to NIR stabilizes the low affinity T structure for three species: oxy, deoxy and met – hemoglobins. Additionally in the reduction reactions the heme iron of methemoglobin is more readily reduced to oxyhemoglobin when in the tense conformation (T state) relative to the R relaxed conformation.

72. Molecular dynamic simulation studys of native and spin labeled cytochrome c. Comaprison with EPR spectroscopy results

K. Murzyn, T. Róg, W. Blicharski, W. Froncisz, S. Szytuła, J. Pyka

Jagiellonian University, Kraków

10 nanosecond molecular dynamic simulations of 5 models of cytochrome c were performed. The first model was a native cytochrome c, second and third cytochrome c labeled at residue 47, and fourth and fifth models cytochrom c labeled at residues 102. As a label MTSL spin label (SL) was used. Two different conformation of s-s bond in SL moiety are possible thus two models of both labeled cytochrome c molecule were investigated. The simulation were performed in octahedral periodic box under constant pressure (1 atm) and temperature (310K) using Amber 6. For nonbonded interactions Particle-Mesh-Ewald method was used. For protein OPLS and for water TIP3P parameters ware used.

Analysis of obtained trajectories reviled multi conformational behavior of SL moiety. Numerous trans-gauche isomerization of torsion angles around single C-C bond in SL were observed during simulation time. SL interact with protein via direct H-bonds and by hydrophobic interactions. However lifetime of H-bonds is short while hydrophobic interactions cover about 60% of simulation time. As a result of hydrophobic interactions SL over ~40% of time is exposed to the water and over ~60% of time interact with protein. This results agree well with our previous electron paramagnetic spectroscopy studies (EPR) were the EPR spectrum is composed of two component slower (~70% of signal) and faster (~30% of signal). The fast component could be interpreted as a SL fully exposed to water and slow as SL interacting with

73. Analysis of the influence of microwave irradiation and glicocortycotherapy on the bone tissue of animals

G. Olchowik

Medical University of Lublin,

The influence of electrical potentials on bone turnover gives the possibility to steer this process in physiological and pathological conditions by the means of external electromagnetic fields.

It this work the resalts of investigation sugested of posibilities microwave irrdiation applications as a protective agent for the bone tissue during long-lasting cortycoterapy. The investigations were made on the female Vistar rats. Estimation of changes in bone tissue were proceeded on the basis of density and bone porosity measurements, mechanical measurements, as well as measurements of microscopic structure and analysis of the bone composition.

The measurements confirmed a harmful effect of hydrocortisone on bone tissue. In group of animal influenced only by hydrocortisone, density of bones was significantly lower and porosity essentially higher than in the control group. Mechanical test of femoral shaft indicated a decrease of bone strength. Quantitative analysis of bone composition pointed out that posteroidal osteoporosis is characterised quantitatively by a diminish of bone tissue, without any changes of their quality.

On the basis of the results of analysis of density and porosity of bones and biomechnical parameter of femoral shaft allowing the estimation risk of the fracture in particular groups of animals it could be stated, that microwave irradiation may be used as a protective agent for bone tissue during long-lasting hydrocortisone application.

74. Protonation effect of L-phenylalanine under NIR radiation: ATR-FTIR study

S. Olsztyńska, M. Komorowska

Wrocław University of Technology

The effectiveness of red and near infrared radiation (NIR) on tissues has recently become the focus of extensive studies for medical (diagnostics, therapy) and biophysical reasons (König *et al.* 1995, *Nature* **377**, 20; Meesters *et al.* 1999, *Biol. Psych.* **46**, 239; Kawada *et al.*, 2002, *Science* **29**, 91). The light effects depend on the irradiation wavelength, dose and local conditions. The irradiation time and the irradiation mode seem to be also important factors. In spite of many results, observed effects are still controversial. Nobody knows exactly what kinds of molecular modifications and processes occur at the molecular level. It is suggested that the primary effect after NIR exposure is dehydration of surface, what has been observed on erythrocytes and liposomes (Komorowska *et al.*, 2002, *Colloids Surf. B* **26**, 223; Komorowska & Czarnołęski, 2001 *Colloids Surf. B* **20**, 309; Komorowska et al., 2002, *J. Photochem. Photobiol. B* **6S**, 93).

The effect of pH on L-phenylalanine (Phe) before and after exposition to NIR (15 min., 700–200 nm) was investigated by ATR-FTIR spectroscopy. Characteristic bands of Phe were described and the p*K*a values were retrieved from IR spectra by using an intensity ratio method according to our recent paper (Olsztynska *et al.*

2001, *Appl. Spectrosc.* **55**, 901). Ratios of relative intensity of bands characteristic of each expected ionizable groups were calculated. Precisely the I_{1600}/I_{1730} ratio connected with $\nu_{as}(CO_2^-)/\nu_{as}(C{=}O)$ and the I_{1560}/I_{1519} ratio assigned to $\nu_{as}(CO_2^-)/\beta_s(NH_3^+)$ relation were taken into account.

Basing on our results, NIR radiation weakens interactions between polar groups of Phe with a solvent. The thermodynamic equilibrium is shifted. The values obtained by ATR-FTIR spectra before light action are following: pK_1=2.31 and pK_2=9.28. They are in good agreement with the literature values (2.16-2.20, 9.18-9.31). The experiment was repeated for irradiated solutions of Phe. When NIR radiation was switched off, during 5 minutes spectra were recorded and new values were received: pK_1 + Δ_1 = 2.79 and pK_2 + Δ_2 = 2.79. Thus, $\Delta_{1\ NIR}$ (5) = +0.48 and $\Delta_{2\ NIR}$ (5) = –0.62. The modified p*K*a values upon irradiation are attributed to processes of deprotonation of the $-NH_3^+$ to the $-NH_2$ group and protonation of the $-CO_2^-$ to the –COOH group. In consequence, the concentration of the cationic and anionic form increases what promotes hydrogen bonded aggregates of Phe (Olsztynska *et al.*, 2003, [in:] K. Wilk (ed.) *Surfactants and dispersed systems in theory and practice*, Oficyna Wydawnicza Politechniki Wrocławskiej, Wrocław, 2003, p. 405).

75. Erythrocyte hemolysis kinetics measurements in the stopped flow regime as a method for analyzing liposome stability

G. Pazdzior[1,2], J. Wojewodzka[1], M. Langner[1,3]

[1]Wrocław University of Technology
[2]Biophysical Students Society "Micela"
[3]Academic Centre of Biotechnology of Lipis Agreggates, Wrocław

Lipid aggregates are considered to be promising carriers for macromolecules and toxic drugs. In order to fulfill this function, aggregates should posses certain properties that ensure the efficient delivery of their cargo to the desired location. One of these properties is their stability in blood for the time during which they accumulate in the targeted tissue. This stability may be affected by a number of factors, including nonspecific lipid exchange between the aggregate and morphological blood components. Since erythrocytes consist of mostly blood cells, therefore their interaction with aggregates should be carefully analyzed. We present a method that allows lipid exchange between liposomes and the erythrocyte plasma membrane to be evaluated. The extent of this exchange was measured in terms of the toxicity of cationic lipid (DOTAP) incorporated into the liposome lipid bilayer, as evaluated by plasma membrane mechanical properties. Incorporation of the toxic lipid into the erythrocyte membrane manifests itself in changes of the mechanical properties of the cell membrane. Such quantitative changes can be calculated from the time constant of the hemolysis process. Hemolysis was performed due to osmotic shock.

76. The relationship between accumulation of nutrient elements in plants of *Zea mays* L. and concentration of IAA in external medium

K. Pazurkiewicz-Kocot, A. Kita

Silesian University, Katowice

One of the most important factors determining the uptake and accumulation of nutrient elements in plant tissues is the interaction between the ions of some elements and the effect of some physiologically important substances.

The aim of this work was to examine the relationship between accumulation of some nutrient elements (K, Na, Ca, Mn, Mg, Fe, Zn and Cu) in leaves, mesocotyls and roots of maize and concentration of auxin (IAA) in the external medium. The experiments were carried out with eight- to nine-day old maize plants (*Zea mays* L. var K33xFa) grown on the Hoagland's medium containing the standard macro- and microelements and IAA in different concentrations, at temperature about 27°C, pH=6.5. IAA was supplied to the nutrient solution on the second day of incubation, in the final concentrations 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} mol·dm^{-1}. The concentrations of the metals ions in the tissues of maize plants were measured by emission spectroscopy using sequential spectrometer with excitation by argon inductively coupled plasma technique (ICP-AES) (frequency 27.12 MHz, power 1.1 kW, sample rate 1.0 cm^3·min^{-1}). The plant tissues were dried in temperature 105°C and mineralized by using deficiency pressure microwave technique. The samples, about 1 g of dry weight were mineralized with HNO_3 and 30% H_2O_2 with increasing strong mineralizer.

The results indicate of the relationship between the accumulation of nutrient elements in plants of *Zea mays* L. and concentration of IAA in external medium.

77. Contracitle activity of internal thoracic artery used as coronary artery bypass grafts after long term vasodiltatory treatment

A. Pędzieńska[1], L. Buzun[2] T. Kleszczewski[1], P. Oleksza[2], B. Modzelewska[1], A. Kostrzewska[1], T. Hirnle[2]

[1]Medical Academy of Bialystok
[2]Medical Academy Hospital, Białystok

The aim of the present study was to investigate the time of reaction of thearteries used as coronary artery bypass grafts to 80 mmol K+, depending on the incubation time. Distal segments of left internal mammary artery (LIMA) obtained from 33 patients aged 38–73 year at

the time of routine revascularisation surgery. The local ethical committee approved the study.

Patients preoperative long-term medications included: b-blockers (metoprolol, atenolol, bisoprolol), ACE-inhibitors (captopril, enalapril, quinapril, cilazapril), calcium channel antagonists (amlodypine, felodypine, diltiazem), nitrates (NTG i.v., izosorbide mononitrate).

Under a dissecting microscope arterial rings (diameter 1-3mm) were prepared. The rings were mounted in an organ bath containing the physiological salt solution of pH 7.4 and a temperature 37°C, and bubbled with carbogen. The preparations were allowed to equilibrate for 3-8 h. During the equilibration period the passive tension was adjusted several times until the resting tension became stable at 6 mN. Tissue reactivity was investigated depending on the incubation time. Responses of the arterial rings to 80 mmol K^+ were recorded under isometric conditions. Quantification of the responses was done by calculation of under the curve area of contractions. The area was measured from the baseline over 10 min. period after each stimulus.

The maximum response was obtained after three hours of incubation.

78. Effect of potassium on IAA and FC-induced elongation growth, proton extrusion and membrane potential in maize coleoptile segments

A. Piasecka, W. Karcz, Z. Burdach

Silesian University, Katowice

Potassium plays an important role in controlling the cell membrane polarization and osmoregulation. Transport of potassium ions is regulated by K+ channels, which are voltage and pH dependent. The aim of the present study was to determine the interrelation between potassium and both IAA and FC-induced elongation growth, proton extrusion and membrane potential in maize coleoptile segments. The elongation growth and pH changes of the incubation medium were measured simultaneously in long-term experiments according to the method descibed previously by Karcz and Burdach (2002, *J. Exp. Botany*, **53**, 1089). The standard electrophysiological technique was used for membrane potential measurements (Stolarek & Karcz, 1987, *Physiol. Plant.*, **70**, 473; Karcz & Stolarek, 1988, *Physiol. Plant.*, **74**, 770). It was found that: (1)the absence of potassium in the incubation medium brought about the decrease of growth rate of maize coleoptile segments; (2) increase of potassium concentration enhanced proton extrusion to the incubation medium; (3) at lower K+ concentrations the higher IAA and FC- induced membrane hyperpolarization was observed.

79. Growth and development of the root apex with an apical cell. A computer study using the growth tensor

A. Piekarska

Silesian University, Katowice

Plant organs grow symplastically. Symplastic growth means the coordinated growth of cells during which neighbouring cells do not slide or slip with respect to each other and the whole organ maintains its physical integrity in time. Such growth is described mathematically by the growth tensor (GT) that can be calculated from the displacement velocity of points obtained on the basis of anatomical data. The GT generates a tensor field of growth rates occuring within the organ. Inherent characteristics of the field are principal directions of growth (PDGs) which are directions along which the relative elemental rate of the linear growth attains extreme values. At a given point there are three mutually orthogonal PDGs unless growth is isotropic. Empirical data have shown that cells divide in planes defined by the PDGs.

On the basis of the concept of the GT a computer model for growth including cell divisions was worked-out (Nakielski, 2000, *Tensorowy model wzrostu w zastosowaniu do wierzchołka korzenia*, Wyd. Uniw. Śląskiego, Katowice). So far it has been applied to the root apex with the zone of mitotically less active cells that occurs in angiospermes. This paper shows application of the model to the root apex with the apical cell and merophytes, typical for lower plants. Such root apex shows a characteristic pattern determined by precisely defined sequences of divisions of the apical cell. The apical cell is tetrahedral in shape. It divides asymmetrically during one cycle in a particular order along each of the three proximal faces. As a result of 50-55 divisions the whole root apex is produced.

The model is two- dimensional, it relates to the axial section of the root apex. There are three components: the meshwork of polygons which describes cell pattern, the GT field which is assumed to generate growth and the algorithm for cell division — defining rules according to which new cell walls are built in. The sequence of the growth is obtained as a result of operational application of the GT field to the meshwork. Fates of individual cells are determined by their positions in the GT field. During growth the meshwork extends, deforms and new cells by cell divisions are formed. Cells divide in planes defined by the PDGs but which plane is chosen depends on a position of the dividing cell within the GT field. New walls are built in automatically. The example of the root apex of Azolla pinnata is considered. The applied GT comes from the previous paper (Hejnowicz, 1989, Environ. *Exp. Bot.*, **29**, 85), parameters are found heuristically.

The model provides a satisfactory description of the root growth: subsequent stages of the simulation show development of the root apex with the apical cell. The cell pattern obtained in the simulation is similar to the pattern observed in anatomical sections of the real apex.

80. Changes in the spectral properties of rapeseed (*brassica napus L.*) oil and evening primrose (*oneothera pradoksa H.*) oil during UVA irradiation and ozonization

H. Pieńkowska

University of Warmia and Mazury, Olsztyn

The effect of ozonization and electromagnetic radiation - ultraviolet and visible — on the oxidation of fatty acids contained in evening primrose oil and rapeseed oil was studied by absorption spectrophotometry. A comparison was made between changes taking place in the above oils as a result of a photochemical reaction caused by electromagnetic radiation, and a reaction of accelerated oxidation with an oxygen-ozone mixture.

The basic factors which decide about the kinetics of vegetable oil oxidation were determined in the oils examined, including the composition of fatty acids, the unsaponifiable matter content, the pigment content and the peroxide value.

81. Decrease in proteins thiol groups in erythrocytes of patients with chronic renal failure

A. Pieniążek[1], K. Gwoździński[1], J. Mudyna[2], M. Luciak[2]

[1]University of Łódź
[2]Medical University of Łódź

Oxidative damages due to free radical production increase in chronic renal failue during hemodialysis sessions. Contact of neutrofils and others fagocyting cells with dialysing membrane lads to production of toxic oxygen species (TOS). TOS plays a key role in oxidation of lipids and proteins of plasma components as well as the plasma membranes of red blood cell (RBC). Lifespan of RBC is 75% shorter in uremia than in healthy patients.

The aim of this study was to evaluate of the thiol proteins in erythrocyte components before and after hemodialysis of chronic renal failure (CRF). The level of –SH groups were determined in plasma membrane, hemolizate and hemoglobin.

Thiol groups in erythrocyte membrane proteins were measured by Ellman method using DTNB. Erythrocyte cytoplasmatic protein as well as hemoglobin thiol groups were measured by spectrophotometric method using dithiopiridine. All results were calculated as percent of control. Statistical analysis was performed using Tukey's test.

All experiments showed significantly lower level of thiol groups in uremic patients in comparison with control. The decrease in thiol groups in uremic patients before and after hemodialysis was also observed. Decrease in thiol groups might be considered as an evidence of deep modification of erythrocyte proteins in chronic renal failure. The decline in the level of thiol groups can be a consequence of oxidation and/or carbamylation.

82. Thermally excited multiplet states in melanins

B. Pilawa[1], M. Latocha[1], R. Krzyminiewski[2], Z. Kruczyński[1], E. Buszman[1], T. Wilczok[1]

[1]Medical University of Silesia
[2]Adam Mickiewicz University of Poznań

Electron paramagnetic resonance (EPR) spectroscopy was used to examination of paramagnetic centres in melanin biopolymers. EPR spectra of eu- and pheomelanins were analysed at temperature from 100 to 295K. The aim of this work was to determine the type of paramagnetic centres existing in melanins. Synthetic model eumelanin-DOPA-melanin, eumelanin isolated from black strain of *Drosophila melanogaster*, and natural eu- and pheomelanins existed *in Cladosporium cladosporioides* pigmented soil fungi, were studied.

EPR spectra were measured by BRUKER X-band (9.3 GHz) electron paramagnetic resonance spectrometer. Magnetic modulation of 100 kHz and 7-200 mW microwave powers were used. The spectra were analyzed by the use of WIN EPR program. Amplitudes, integral intensities, g-factors, and linewidths were calculated. The influence of temperature and microwave power on the EPR parameters were drawn. Concentrations of paramagnetic centers in the melanin samples were determined.

o-Semiquinone free radicals were found in DOPA-melanin, and in melanin from both *Drosophila melanogaster* and *Cladosporium cladosporioides*. EPR spectra of DOPA-melanin and *Drosophila melanogaster* melanin were single lines. EPR spectra of *Cladosporium cladosporioides* melanin reveal complex character. They were superposition of single EPR line of eumelanin and EPR line of pheomelanin with unresolved hyperfine structure. Mainly eumelanin exists in *Cladosporium cladosporioides* fungi.

It was shown that temperature dependence of EPR spectra of the studied eu- and pheomelanins did not fulfill the Curie law. Besides of o-semiquinone free radicals, thermally excited multiplet states exist in DOPA-melanin and the two tested natural melanin biopolymers. Linewidths of melanins EPR lines decreased with increasing of temperature. Microwave saturation of EPR lines of all the studied melanin samples appeared at higher microwave powers with increasing of the measuring temperature. Spin-lattice relaxation time decreased at higher temperatures. Linewidths of the all recorded EPR spectra increased with increasing of microwave power. EPR spectra of melanins are homogeneously broadened.

83. Interactions of DOPA-melanin-netilmicin complexes with paramagnetic O_2 molecules

B. Pilawa, M. Matuszczyk, E. Buszman, D. Wrześniok, A. Grzegorczyk, T. Wilczok

Medical University of Silesia, Sosnowiec

Magnetic interactions of melanin with oxygen molecules were studied. The aim of this work was to characterize the interactions of model eumelanin-DOPA-melanin samples with paramagnetic oxygen (O_2) molecules. The influence of paramagnetic oxygen on paramagnetic centers in DOPA-melanin and DOPA-melanin-netilmicin complexes were studied. The changes of melanin-oxygen interactions induced by binding of paramagnetic copper (Cu^{2+}) and diamagnetic zinc (Zn^{2+}) ions to DOPA-melanin and DOPA-melanin-netilmicin complexes were compared.

Electron paramagnetic resonance (EPR) spectroscopy was used as the experimental technique. EPR measurements were done using an X-band (9.3 GHz) spectrometer with modulation of magnetic field 100 kHz. EPR spectra were recorded for samples in air and for evacuated samples (10^{-4} Torr). The microwave frequency was recorded ($\pm$0.0002 GHz) with MCM 101 recorder. The concentration of paramagnetic centers in the melanin samples, g-factors and linewidths were measured. The EPR lines were recorded at low microwave power 0.7 mW to avoid their microwave saturation. Ultramarine was used as the reference of concentration of paramagnetic centers. Ruby crystal permanently placed in the resonance cavity was used as the internal reference. Double integration of the first-derivative EPR spectrum was performed to determine the area under the absorption curve. The influence of microwave power in the range 0.7-70 mW on EPR spectra was analyzed. The changes of amplitudes and linewidths of EPR lines with increasing of microwave power were drawn.

Interactions of melanin with oxygen caused the creation of quasi-chemical bonds between melanin paramagnetic center and one of unpaired electron of O_2 molecule. The creation of quasi-chemical bonds decreased the concentration of paramagnetic centers in melanin samples. This effect was observed in DOPA-melanin, DOPA-melanin complexes with netilmicin and metal ions (Zn^{2+}, Cu^{2+}). Quasi-chemical bonds were also found in complexes of DOPA-melanin with chloroquine and metal ions (Pilawa, Latocha, Buszman & Wilczok, 2003, *Applied Magnetic Responance*, **25**, 105). Paramagnetic oxygen changed the spin-spin and spin-lattice interactions in melanin samples. O_2 molecules modified dipolar interactions of unpaired magnetic moments in melanin. Differences in microwave saturation of EPR spectra of evacuated samples and melanin in air were observed.

84. Tin chlorides interaction with the model membranes

M. Podolak, G. Engel, D. Man

Opole University

The aim of the work was to examine the influence of selected organic or inorganic tin chlorides on electric properties of model membranes that were nitrocellulose filters impregnated with lauric acid. The membranes separated test chamber and reference chamber filled with KCl hydrous solution of 0.01 M concentration. Admixtures of tin compounds were introduced into the test chamber. Electric membrane voltage and resistance as a function of compound concentration and time were measured with the use of Keithley 6517 electrometer. In the case of inorganic compounds increasing of the admixture concentration results in a fast increase of voltage and next stabilization of its value. Potential of the solution in the test chamber in all of cases was negative. Such polarization of the potential may means that the positive tin ions penetrate into the membrane leaving of negative chlorine ions in the test chamber. The electrical resistance of the membrane did not depend on the admixture concentration and equaled approximately 50 M ohms/cm^2.

In the case of organic chloride admixture concentrations was ten times weaker. The measurements were conducted for four constant admixture concentrations: 1 mM; 10 mM; 50 mM and 80 mM. In this case sharpe maximum of memrane voltage was observed and then its monotonic fall down to a certain stable value. In the case of 50 mM and 80 mM concentrations the potential of the solution in the test chamber changed into the positive.

85. Spectrophotomoetric determination of the protonation equilibrium constants and the binding constatns of new phenothiazine derivatives to phospholipid liposomes

A. Poła[1], D. Mosiądz[1], T. Modrzycka[1], N. Motohashi[2], M. Kawase[3], K. Michalak[1]

[1]Wrocław Medical University
[2]Tokyo Meiji Pharmaceutical University, Japan
[3]Josai University, Saitama, Japan

In drug resistance reversal caused by phenothiazine derivatives (PDs) and other amphiphile compounds their nonspecific interactions with membrane lipid bilayer very likely play an important role. The state of protonation of the PDs is crucial for their interactions with different lipids and it influences binding constants of these compounds with membranes. The protonation equilibrium constants (pKa) for several new PDs were determined using a spectrophotometric titration method. The pKa values were obtained by fitting the experimental data to the theoretical equation. The binding con-

stants (K_b) of PDs for both phosphatidylcholine (PC) and phosphatidylserine (PS) liposomes were determined using method of first derivative of absorption spectra. The pKa values for each of the investigated PDs were about 6.3. At pH = 7.4 only 10% of PDs molecules was in protonated state. It might be a reason that K_b values of PDs obtained for PS and PC liposomes were very similar. For PDs with three carbon atoms in the alkyl chain, the K_b constant increased 1.8 and 2.4-fold for PS liposomes and 1.7 and 2.0-fold for PC liposomes when hydrogen at position 2 of phenothiazine ring was replaced by chlorine and $-CF_3$ group, respectively. For PDs with four carbon atoms in the alkyl chain, the K_b constant increased about 3-fold for both PS an PC liposomes following the same substitutions.

86. Spectrophotometric determination of the protonation equilibrium constants and the phospholipid/water partition coefficients of selected neuroleptic drugs

A. Poła, A. Poła, A. B. Hendrich

Wrocław Medical University

The protonation equilibrium constants (pKa) for five selected neuroleptic drugs (ND) were determined using a spectrophotometric titration method. The pKa values were obtained by fitting the experimental data (absorbance) to the theoretical equation.

The partition coefficients (K_p) of ND between phosphatidylcholine (PC) as well as phosphatidylserine (PS) lipid bilayers and water were determined using the second derivative of absorption spectra method. This method is used to eliminate the background signal present in absorption spectra due to light scattering on liposomes. K_p values for investigated drugs were calculated from the relationship between ΔD (intensity changes of second derivative of absorption spectra at appropriate wavelength) and lipid concentration.

Two values of pKa for each of ND were obtained. The second pKa values were greater than 8.0 for perazine derivatives and were lower than 8.0 for perphenazine and fluphenazine. This means that at pH = 7.4 perazine derivative molecules were protonated in greater degree than perphenazine and fluphenazine molecules, what may influece phenothiazine-lipid interactions. The K_p values for all studied ND were in the order of magnitude of 10^5, and they was several times greater for PS than PC liposomes. The substitution of –H atom by –Cl atom or $-CF_3$ group in position 2 of phenothiazine ring caused an increase of ND K_p values for PC in greater degree than for PS liposomes.

87. Changes in cathepsin B kinetics in the presence of model and natural membranes

W. Poręba[1], A. Marcinkowska[2], J. Gutowicz[1]

[1]Wrocław University
[2]Medical University of Wrocław

Cathepsin B (EC 3.4.22.1) is a lysosomal cysteine protease, involved in many physiological and pathological processes. Its activity has been proved to be altered in cancer tissues, where it plays a role in degradation of basement membrane, resulting in formation of metastases. Moreover, in many cancer cell types, cathepsin B is transported outside the cells and secreted to surrounding fluids or remains attached to exterior side of cell membrane. Pro-cathepsin B was found to be associated with cancer cell membrane via p11 of annexin II tetramer. However, the mechanism of mature enzyme interaction with plasma membrane and possible changes in the catalytic activity are still unknown. In this paper, we present bovine cathepsin B kinetics in the presence of phospholipid liposomes and A549 type cancer cells. We investigated the influence of liposomes of different lipid composition on the kinetic parameter, using different fluorogenic substrates. Liposomes composed of negatively charged lipids strongly reduced cathepsin B activity. Presence of those liposomes increased Km and kcat, but lowered Km/kcat parameter for Z-Phe-Arg-AMC as a substrate. We compared the effects with those of cancer cells, which gave similar results. In addition, we show that the presence of negatively charged liposomes changes cathepsin B sensitivity to natural, reversible inhibitor, namely cystatin from egg white. Possible mechanisms of cathepsin B activity changes are discussed.

88. The effect of triphenyl- and tributyl{2-[4-(dimethyloamino)phenylazo] benzoato}tin(IV) on the thermotropic phase transitions of phosphatidylcholine and phosphatidylcholine/cholesterol bilayers

H. Pruchnik, B. Różycka-Roszak

Agricultural University, Wrocław

Organometallic compounds show a wide variety of biological activities which includes bactericidal, caricidal, fungicidal and antitumor agents (Pellerito & Nagy, 2002, *Coordination Chemistry Reviews*, **224**, 111). Many tri-n-butyl-, and triphenyltin hydroxycarboxylates, oxycarboxylates and fluorocarboxylates display interesting antitumor activities (Pruchnik *et al.,* 2002, *J. Inorg. Biochem.*, **90**, 145; Pruchnik *et al.*, 2002; *App. Organometal. Chem.* **16**, 587; Pruchnik *et al.*, 2002, *Eur. J. Inorg. Chem.*, 3214). We present the interaction of the newly synthesized triphenyl {2-[4-

(dimethyloamino)phenylazo]benzoato}tin(IV) – TPT(r), and tributyl{2-[4-(dimethyloamino)phenylazo] benzoato}tin(IV) – TBT(r) with lipid bilayers. The complexes are very effective cytostatic agents Pruchnik *et al.*, 2002; *App. Organometal. Chem.* **16**, 587; Pruchnik *et al.*, 2002, *Eur. J. Inorg. Chem.*, 3214)

The toxicity may be in part due to their interaction with membranes and in consequence alteration of their structure. In this work, we studied the effect of phenyltin and butyltin complexes on phase behavior and on the structure of dipalmitoylphosphatidylcholine model membranes and of the mixed phosphatidylcholine/cholesterol (DPPC/chol) bilayers. Bilayers used, contained 2, 5 or 15-mol% of cholesterol. Molar ratio of a TPT(r) and TBT(r) to mixed DPPC/chol bilayer was changing from 0.01 to 0.2. The studies were performed by means of DSC.

The influence of tributyl{2-[4-(dimethyloamino) phenylazo] benzoato}tin(IV) and triphenyl{2-[4-(dimethyloamino)phenylazo]benzoato}tin(IV) on the thermotropic phase DPPC is slight. TBT(r) broadened the main phase transition but hardly affects, also the pretransition is affected only a little. The subtransition is not abolished within the investigated concentration range. TPhT(r) reduced the transition temperatures and at higher concentration abolishes the pretransition.

The presence of cholesterol causes cholesterol-rich and cholesterol-poor domains to appear. It is observed as marked asymmetry of the main phase transition. Under the influence of TBT (*r*) and TPT(*r*) e.g. in the presence of 5 mol% cholesterol the temperature of the main phase transition lowers while its coopperativity increases.

89. A method for determining amphiphilic compounds transfer through the model lipid bilayer

M. Przybyło[1,2], A. Olżyńska[1,2], J. Gabrielska[3], S. Przestalski[3], M. Langner[1,4]

[1]Wrocław University of Technology
[2]Biophysical Scientific Students Union "Micela"
[3]Agricultural University, Wrocław
[4]Academic Center for Biotechnology of Lipid Aggregates, Wroclaw

There are no simple and time-efficient method which allows to measure compounds ability to penetrate the biological membranes. This is a serious experimental limitation especially when the compound does not have distinct spectral and/or fluorescence properties. We present a method that allows to determine the permeability of charged, non-fluorescent compounds through the model lipid membrane. The method is based on the analysis of the kinetics of the process resulting from studied compound association and permeation through the lipid bilayer. The principle of such approach is presented on the example of Fluorescein-PE fluorescence intensity dependence on time as a result of organometalic compounds interaction with phosphatidylcholine liposomes when measure with the stopped-flow experimental setup. Obtained results shows that two studied compounds, diphenyltin and triphenyltin, adsorb onto the lipid bilayer surface, in a diffusion control meaner, within the very short time (0.05s). The membrane passing by those compounds was observed in the minutes time range. The adsorption process was easily fitted with the single exponential for both compounds, indicating a single process phenomena. The long time kinetics have a complex dependence on compounds concentration and the presence of cholesterol in the membrane, showing that the lipid bilayer crossing can not be considered as the compound's diffusion through the hydrophobic barrier alone.

90. A modified AFM system for mechanical identification of single macromolecules

A. Ptak[1], S. Takeda[2], C. Nakamura[2], J. Miyake[2]

[1]Poznań University of Technology
[2]Tissue Engineering Res. Cent., Ibaraki, Japan

Recent advances in atomic force instrumentation have allowed mechanical studies of single biological macromolecules. These studies yield new information and represent ultra high sensitivity that is not available from population-averaged measurements.

We report the use of a modified, magnetic force controlled AFM system to study the elastic properties of single peptide molecules directly from a mechanical test (Ptak *et al.*, 2001, *J. Appl. Phys.* **90**, 3095). Peptides of different sequence and length were investigated. A spring constant as well as a longitudinal Young's modulus for peptide secondary structures were measured. The experimental data were compared with the results of computational simulations based on quantum semi-empirical methods. Analyzing the stiffness behavior we could conclude about the conformational changes of a molecule subjected to mechanical stress.

The technique opens the way to scientists for the quantitative study of the mechanical properties of single macromolecules. We propose to apply the system for the mechanical identification of single macromolecules and the detection of structural defects, especially in biopolymers.

A. Ptak was supported by Poznan University of Technology, Grant No: PB 62-195/04-BW.

91. Interaction of yeast iso-1-cytochrome *c* with *bc*1 complex from *Rhodobacter capsulatus*

J. Pyka, A. Osyczka, S. Szytula, B. Turyna, W. Froncisz

Jagiellonian University

Cytochromes *c* are electron transfer proteins localized in the intermembrane space of mitochondria. They function as the mobile electron carriers between the cytochrome *bc*1 complex and cytochrome c oxidase.

In our studies, we have been using electron paramagnetic resonance (EPR) as a tool for probing the local dynamic structure of spin labeled cytochrome *c* and interactions between cytochrome *c* and *bc*1 complex. In this approach, the placement of a spin label at a specific position within the protein allows us to monitor exclusively the chosen region of the protein.

Iso-1-cytochrome *c* from Saccharomyces cerevisiae modified with cysteine specific spin label ((1-oxyl-2,2,5,5-tetramethyl-Δ3-pyrroline-3-methyl)-methanethiosulfonate) at position 102 was used. Cytochrome *bc*1 complex was isolated from *Rhodobacter capsulatus*.

The effect of the interaction of the spin-labeled cytochromes *c* with *bc*1 complex on the CW EPR spectra was observed. Comparison of the EPR spectra of the free spin-labeled cytochrome *c* to these of the spin-labeled cytochrome c in complex with *bc*1 demonstrated clearly that complex formation could be detected by EPR in each case. Also, the ionic strength dependence and the reversibility of complex formation are analyzed.

Supported by the grant 3 PO4A 043 23 from the State Committee for Scientific Research (Poland).

92. Complex dynamics of a spin label attached to a cytochrome c

J. Pyka, M. Dutka, K. Ptasiński, W. Froncisz

Jagiellonian University, Krakow

Electron Paramagnetic Resonance Spectroscopy represents a very useful technique for studying the protein dynamics. The information about the motion of the whole molecule, or its domain or even backbone is retrieved form the EPR spectrum of a spin label, attached to the protein. Advances in „site-directed spin labeling" enable introduction of a spin label, usually MTSL [(1-oxyl-2,2,5,5-tetramethylpyrroline-3-methyl) methanethiosulfonate], at a chosen site of the protein chain. The resulting motion determines the complex EPR spectrum of the spin label, and is the sum of the macromolecule tumbling, subdomain movement or backbone fluctuations and internal dynamics of the nitroxide chain. The aim of our investigations is to elucidate the motion of the spin label attached to the naturally occurring cysteine at position 102. The observed X-band EPR spectrum consists of two components and results from the simultaneous occurrence of the nitroxide sidechain fluctuations and movements of the macromolecule. We compare the spectra of the complexes which are free in solution and bound to the CM Cellulose gel, measured at room temperature. In the second case the Brownian diffusion of the macromolecule is reduced. Experimental EPR spectra were fit to the theoretical model MOMD (microscopic order, macroscopic disordered).

Supported by the grant 3 PO4A 043 23 from the State Committee for Scientific Research.

93. EPR examination of HaCaT cells cultivated in thermal waters

A. Pytel, A. Garncarczyk, M. Gosek, B. Pilawa, M. Matuszczyk, T. Wilczok

Medical University of Silesia, Sosnowiec

Electron paramagnetic resonance (EPR) spectroscopy was applied to the culture of human immortalized keratinocytes: HaCaT. The aim of this work was to determine properties of paramagnetic centres existing in HaCaT cells. The type of paramagnetic centres, their amount and magnetic interactions in biological structures were studied. Spin-spin and spin-lattice interactions in HaCaT were discussed.

The influence of different types of thermal waters on paramagnetic centres in the epidermal cells were compared. Thermal waters modify regeneration processes in HaCaT cells. The proliferation of skin cells changed in the presence of the analysed waters in cultures and proliferation of cells depend on presence of paramagnetic centres in the cells. Thermal waters used for the cultivations of epidermal cells effects the paramagnetic centres system.

HaCaT cells were cultured in KGM-2 Keratinocyte Basal Medium (Clonetics) supplemented with BPE (Bovine Pituitary Extract), hEGF, Insulin, Hydrokortisone, Epinephrine, Transferrin at 37ºC, humidity 95% with 5% CO_2 concentration. Cells were cultured in the standard medium for 3 days to obtain monolayer cultures and then supplemented with thermal water with high or low mineral substance contents. After 3 days number of cells were measured in hemocytometer.

EPR measurements for cells in Pasteur pipettes were done by the use of an X-band (9.3 GHz) electron paramagnetic resonance spectrometer with modulation of magnetic field 100 kHz. Microwave frequency was recorded. EPR spectra were collected by computer. The EPR spectra were recorded in the range of microwave power 0.7-70 mW. Because of the very low intensities of all the analyzed samples, experimental EPR spectra were accumulated 300 times by Rapid Scan Unit. After accumulation EPR lines with low level of noise were obtained.

For such first derivative EPR spectra the following parameters: amplitudes, integral intensities, linewidths

and g-factors were determined. Spectra were analysed by the use of Elf1 program prepared by Jagmar company (Kraków). Concentration of paramagnetic center in the samples are proportional to the integral intensities. The EPR integral intensities were divided by the volume of cells in the pipette. g-Factors were calculated from the resonance condition. The influence of microwave power on amplitudes, integral intensities and linewidths were studied.

The very low amounts of paramagnetic centres resulted from rupturing chemical bonds in molecular cell structures of HaCaT samples. g-Values were higher than those obtained for free electron. Low values of spin-orbit coupling constant were responsible for this effect. High broadening of the measured EPR lines resulted from unresolved hyperfine structure and from strong dipolar interactions of unpaired magnetic moments in the samples. Changes of EPR amplitudes and linewidths with microwave power indicate homogeneous broadening of the analyzed resonance lines. HaCaT cells treated with thermal waters differ in the amount of paramagnetic centers and in their magnetic interactions.

94. Membrabe voltage modulates the $GABA_A$ receptor gating

M. Pytel, K. Mercik, J. W. Mozrzymas

Wrocław Medical University

Fast GABAergic synaptic transmission in the adult brain is mediated by ionotropic GABAA receptors. Although these receptors are activated by ligand, it has been reported that their kinetics can be modulated by the membrane voltage. However, the mechanism of such modulation has not been described in details. Since the membrane potential in neurons is known to vary over a wide range, it seems interesting to explore $GABA_A$ receptor modulation by this factor. For this purpose, current responses to ultrafast GABA applications were recorded at membrane voltage ranging from -70 to + 70 mV. Using this technique the agonist can be applied within less than 70 μs enabling to describe the $GABA_A$ receptor kinetics with resolution adequate to the time scale of synaptic currents. We have found that the membrane depolarization enhances the rate and extent of receptor desensitization. The recovery process in the paired-pulse protocol has been slowed down by increase in membrane potential. We have also observed that current to voltage relationship shows a rectification (smaller slope) at positive membrane potentials. Moreover, the onset of currents elicited by non-saturating GABA concentrations is accelerated at positive voltages indicating an increase in the binding rate. Altogether, these observations are consistent with an enhancement of desensitization and binding rate at increasing membrane potentials. We conclude that physiologically occurring changes in the membrane potential may significantly affect the GABAergic synaptic transmission.

Supported by KBN grant 3P05A 080 24 to MP and JWM and by the Welcome Trust Senior Research Fellowship in Biomedical Sciences in Central Europe to JWM.

95. Effect of fenofibrate on therapy on erythrocyte membranes in patients with dislipidemia

W. Rożej[1], M. Broncel[2], P. Duchnowicz[1], M. Koter[1], A. Aleksandrowicz[1], J. Chojnowska-Jezierska[2]

[1]University of Łódź
[2]Medical University of Łódź

Hypercholesterolemia may decrease the deformability of red bllod cells, which impairs their hemorheological behaviour and promote atherosclerosis.

To determine the effect of fenofibrate on erythrocytes plasma membrane in patients with familial hypercholesterolemia we prospectively studied serum lipid concentration, red cell cholesterol content, lipid peroxidation, erythrocyte plasma membrane fluidity and activity of ATPase.

Laboratory tests were carried before and after 4 weeks of pharmacological treatment. The patients were given 267 mg fenofibrate (lypanthyl) per day.

Hypercholesterolemia induced changes in the basic properties of human erythrocyte plasma membrane including its fluidity, the intensity of lipid peroxidation, content of cholesterol and ATPase activity.

The fenofibrate therapy reverse the alteration of erythrocyte plasma membrane properties.

96. Interaction of non-ionic saccharide-based surfactant (Cn MELA) with model membranes

B. Rożycka-Roszak[1], K. A. Wilk[2], B. Jurczak[1,2]

[1]Agricultural University, Wrocław
[2]Wrocław University of Technology, Wrocław

Non-ionic saccharide — based surfactants show improved performance due to their favourable ecotoxical properties (ability to biodegradation) and potential pharmaceutical, biochemical, and biomedical application. N-alkanoyl-N-methyllactitolamines (alkanoyl: decanoyl [C10MELA], lauroyl [C12MELA], miristoyl [C14MELA]) are examples of newly synthesised non-ionic saccharide – based surfactants.

The aim of the present work was to study the interaction of the above mentioned sugar- based surfactants with model membranes.

We studied the influence of C10MELA, C12MELA, C14MELA on the thermotropic phase behaviour of multilamellar vesicles, formed from dipalmitoylphosphatidylcholine (DPPC) and DPPC/cholesterol mixtures, by means of differential scannig calorimetry (DSC).

The obtained results showed that C10MELA and C12MELA gradually suppressed the pretransition and subtransition at higher concentration while the suppression by C14MELA occurred at lower concentrations. With increasing concentration of the surfactants studied the main phase transition broadens and shifts progressively to a lower temperature (mostly in the case of C14MELA). The compounds also change the transition enthalpy and cause splitting of the main phase transition into two peaks. In the presence of cholesterol, the observed effects of CnMELA on phase transitions of DPPC were enhanced.

97. Selected trace elements concentrations estimation in blood plasma of patients with chronic inflammatory and pre-cancerous conditions and with throat and larynx cancer

M. Rutkowski[1], K. Grzegorczyk[1], A. Lipka-Kociszewska[2], D. Ertel[1], J. Kędziora[1]

[1]Medical University of Łódź
[2]Specialized Hospital in Łódź

Many extra- and intrasystemic factors influence formation and development of neoplasmatic diseases. They include reactive oxygen species (ROS) playing role of promoters and progressors of carcinogenesis and contribute to chronic inflammation conditions leading to formation of neoplasms. Antioxidative trace elements – selenium and zinc — take part in ROS deactivation and prevent their pro-carcinogenous activity. Other trace elements, such as iron and copper, significant pro-oxidative activity (oxidative stress) was found. Oxidative stress may induce neoplasmatic changes.

Aim of the study was estimation of selenium, zinc, iron and copper concentrations in blood plasma of patients with the below mentioned illnesses of throat and larynx and comparison of results with those elements concentration levels found in healthy people.

The study included 133 persons (both males and females) divided into 4 groups:

I (control) – 33 healthy people,

II – 33 patients with chronic tonsillitis,

III – 33 patients with pre-cancerous conditions of larynx,

IV – 34 patients with larynx cancer (histopathological diagnosis was a basis for qualification into groups III and IV).

Studied individuals were non-smokers, they hadn't also receive any mineral preparations.

Material were comprise blood samples collected in fasting state from cubital vein and using potassium oxalate as anticoagulant. After centrifugation of red blood cells 1 ml of separated plasma was taken, mixed with 1 ml of 30% H_2O_2 and 4 ml of 65% HNO_3, mineralized in Teflon vessels in microwave oven for 45 minutes. Cooled contents was quantitatively transferred into 10 ml calibrated flasks, filled with deionised water and analysed after thorough mixing. The analysis used method of atomic emission spectroscopy with inductive plasma excitation (AES-ICP) using PU 7000 spectrometer from Philips.

In course of the study the biggest concentrations of iron and copper were found in blood plasma of patients with larynx cancer. This was accompanied by the lowest concentrations of selenium and zinc in blood plasma (see Table).

Groups studied	Estimated trace elements concentrations (μg/dl)			
	selenium	zinc	iron	copper
I	57.3±8.3	26.8±5.3	78±10	56±12
II	21.4±3.7	19.4±8.0	136±14	118±8
III	24.3±4.1	21.9±4.1	132±9	122±11
IV	16.5±4.6	14.3±2.8	252±48	134±9

Obtained results may suggest presence of iron and copper in etiology and development of larynx cancer. Monitoring of those concentrations in blood plasma may be, thus, helpful in diagnostics of suspected cases of this type of cancer. Data suggest also purpose fullness of selenium and zinc supplementation in cancer prevention.

98. Biological activity new aminophosponates

J. Sarapuk, A. Dziamska, K. Bielecki

Agricultural University, Wrocław

Aminophosphonates have been studied for many years because they are an interesting class of herbicides. The bond between the phosphorus and carbon atoms is widespread in the nature.Their biological activity is correlated to the lipophilic character of compounds and is almost independent on their steric or electronic factors.

It is commonly thought that the activity of aminophosphonates is related to the disrupting and injuring plant membrane. This toxicity may promote the formation of free radicals, identified as being responsible for oxidative stress. Among oxidative defenses in the cell, the antioxidant enzymes, especially catalase appear to be the most sensitive to radical proliferation.

We investigated the influence of a few new aminophosphonates on catalase activity in cucumber leaf (Cucumis sativus) and on the stability of a planar lipid membrane (BLM).

Plant material growth conditions were constant. Cotyledons from 7-day-old seedlings of cucumber were used for experiment. Discs were cut from cotyledons floated on aminophosphate solution. Then enzymes extracts were prepared from the discs. Catalase activity was measured as speed of enzyme containing tissue to surface of solution. Reaction was started by adding H_2O_2. In the BLM experiments critical concentration (CC) of compound, that destroyed planar membrane in the time not longer 5 min, was studied.

Both effects depended on the structural features of aminophosphonates studied.

99. Assessment of potential agrochemical application of some new aminophosphine oxides and their equimolar binary mixtures with 2,4-D

J. Sarapuk, K. Bielecki, D. Bonarska, H. Kleszczyńska

Agricultural University, Wrocław

Potential biological activity of some aminophosphine oxides (APO) and their equimolar binary mixtures with the well-known herbicide 2,4-D was studied. The measure of this activity was the inhibition of growth of cucumber (*Cucumis sativus*), and hemolytic efficacy of compounds.

The toxic unit approach was applied to model joint toxicity of binary mixtures. In this model, the value of the toxic unit (1 TU) is assigned to the 50% effective concentration (E_{C50}) of a compound. The sum of TU values contributed by each component describes toxicity of a mixture as follows: $T_{Umix} = C_1/E_{C50a} + C_2/E_{C50b}$, where C_a and C_b are concentrations of particular components and E_{C50} are their effective concentrations (Pape-Lindstrom & Lydy, 1997, *Env. Toxicol. Chem.* **16**, 2415-2420). The empirically measured toxicity was compared with expected toxicity as predicted by T_{Umix}, which was generated using E_{C50} values determined by tests of individual compounds.

It was found that APOs studied exhibit satisfactory biological activities when used individually in micromolar concentrations. Their joint toxicity in binary mixtures with 2,4-D showed antagonistic or synergistic effect, depending on APO used.

This work was sponsored by the State Committee for Scientific Research, grant 6 P04 096 21

100. A cycle of enzymatic reactions that can behave as oscillator, trigger or excitable system

J. Sielewiesiuk

Maria Curie-Skłodowska University, Lublin

A kinetic model describing oscillations of concetrations of methoxyphenolic compounds in a culture of bacterium Rhodococcus erythropolis is proposed. The system is positively invariant and satisfies the postulate of detailed equilibrium. The cycle of interconversions of four substances consists of eight enzymatic reactions catalyzed by four enzymes. Each of the reagents acts as a corepressor or noncompetitive inhibitor of one of the enzymes. The corresponding dynamical system consists of four ordinary differential equations. There is an integral of motion equal to the sum of concentrations of reagents - C. The other parameter of the system is the ratio of rate constants for backward and forward reactions - k. At $k = 1$, the system has one equilibrium point. The equilibrium is stable at $C < 4$. Destabilization of equilibrium at $C > 4$ leads to limit cycle oscillations. At $k > 8.2068$ or $k < 0.12185$, two areas with three equilibrium points appear in the parameter plane (C, k). Two of these equilibrium points are stable and the third one is unstable. So, the system behaves as a bistable trigger in these areas. In a more asymmetrical case, at $k > 11.9$ or $k < 0.0840336$, two additional areas with three equilibrium points appear. The evolution of the system in these areas depends on initial conditions. It monotonicaly relaxes to the stable equilibrium or attains this equilibrium after generation of high spikes of variables.

101. Damage in cell membrane in human erythrocytes incubated with microcystin-LR and anatoxin-A (*in vitro*)

P. Sicińska, W. Duda

University of Lódź

In natural and artificially water reservoirs toxic blooms of phytoplancton (mainly of cyanobacteria) a have been observed. The cyanobacteria product substances, which are very toxic for many organisms.

In Sulejowski Lake, which is the source of drinking water for Łódź agglomeration cyanobacterial blooms mainly contain the hepatotoxins: microcistin LR and neurotoxins: anatoxin-A.

The toxins influence many types of cells like: hepatocytes, neurocytes, lymphocytes, fibroblasts causing their morphological and functional changes, including oxidative stress.

In work the influence of microcistin-LR (MC-LR) and anatoxin-A (AN-A) on the degree of cell's membrane fluidity at the level of 5. and 16. carbon of fat acid residue was assessed. The level of the lipid peroxidation and number of SH- groups were also determined.

The study was performed on human erythrocytes isolated from whole blood. Erythrocytes were incubated with a microcystin-LR and anatoxin-A in the concentrations of 1, 10, 100, 1000 nM for 1, 3, 12 and 24 hours.

The study showed correlation between the parameters describing the membrane fluidity (parameter S, correlation times τ_b and τ_c), lipid peroxidation, number of SH-groups and the concentration of toxins also time exposure. The investigation did not evidence the statistically significant changes of arrangement parameters S after MC-LR influence, and for AN-A the increase of this parameter was observed by 13% and 20% for doses of 1000 nM after 3 and 24 hours incubation. Both toxins provoked similar increase of correlation times τ_b and τ_c, which after 12 hours incubations with the dose of 100 nM increase by 37% and 25% for MC-LR and AN-A respectively. After the incubation with describing toxins the increase of lipid peroxidation was observed and it was correlated with the rise of individual dose used and incubation time, the changes in the number of SH-groups were also noted.

The described disturbances of membrane parameters after exposure to microcystin-LR and anatoxin-a, *in vitro*, could be used to predict changes of membrane *in vivo*, during long-term consumption of water from reservoirs where cyanobacterial toxins are produced.

102. Copper-Cyanidine interaction in water-methanol solution

B. Smyk, B. Pliszka, R. Drabent

[1]University of Warmia and Mazury, Olsztyn

Cyanidine can act as chelating agent of metal ions. Therefore it is important to describe metal ions interaction with cyanidine. The aim of this work was to investigate the kinetics of copper-cyanidine (Cy 3-Glc) complex disintegration in methanol-water solution. Concentration of cyanidine and copper was 1.4×10^{-5} M. Complex disintegration was monitoring by absorption spectra in the range between 200 nm and 750 nm. Absorption spectrum of cyanidine after copper addition was changed and revealed a new long wavelength band with maximum at 588 nm. This band was disappearing in time, indicating complex decomposition. Disappearance of complex was accompanied by an appearance of new bands in 330-460nm and UV region. New bands may be connected with either cyanidine dimer formation or new copper-cyanidine complex or changed forms of cyanidine under the copper influence.

Absorption spectra were used to calculate decomposition rate constant of the complex in a different spectral ranges. An average decomposition rate constant was calculated. Observed reaction was pseudo-first order.

103. Inhibition of sodium current inactivation affects differently action potential characteristics

M. Stankiewicz[1], F. Grolleau[2], E. Kiełbasiewicz[1,2], M. Pelhate[2], B. Lapied[2]

[1]N. Copernicus University, Toruń
[2]Université d'Angers, France

Fast inactivation of sodium channels is responsible for short (0.5-2ms) duration of sodium action potentials (AP). Generally, inhibition of sodium current inactivation results in transformation of fast AP into "plateau action potentials" (PAP) lasting sometimes several hundreds milliseconds. Scorpion, anemone and spider alpha toxins inhibit sodium channel inactivation. Inhibition induced by spider toxins does not exceed 30% (when measured at the end of 5ms depolarizing pulse) and this is not sufficient to obtain PAP in isolated insect axons. However, when axon is pretreated with blocker of gK^+ PAP is observed. Generally, to record PAP, about 50% of sodium current inactivation must be blocked. This corresponds well to typical scorpion and anemone alpha toxins effect tested, for instance, on isolated cockroach axons. By contrast, in isolated neurone cell body which displays multi-conductance pacemaker activity (i.e., cockroach DUM neurones), pretreatment with scorpion and anemone alpha toxins only produces few PAP among normal beating activity. Same toxins applied on *in situ* cockroach axon produce salvos of repetitive activity instead of single AP recorded under control condition. However, PAP can be sporadically observed only if axon is depolarized. In *in situ* DUM neurones, toxin treatment only transforms the regular firing pattern into a burstic electrical activity. In this case, PAP have never been observed, even using high toxin concentrations. Finally, in isolated insect axons, elevation of extracellular Ca^{2+} concentration (i.e., from 5mM to 20-30mM) and reduction of external sodium (from 200 to 170 mM) reduce the ability in transforming fast AP into PAP. The decrease of sodium current amplitude by 15% is sufficient to limit PAP generation after alpha toxin treatment.

104. The interactions of newly synthesized phenoxazines with phosphatidylcholine model membrane

K. Stańczak[1], A.B. Hendrich[1], M. Kawase[2], K. Michalak[1]

[1]Wrocław Medical University
[2]Josai University, Saitama, Japan

Multidrug resistance (MDR) of cancer cells could be reversed by agents that influence the activity of membrane protein transporting chemotherapeutics (like P-glycoprotein). MDR reversing compounds also interact and perturb the lipid phase of cell membrane. Alteration of membrane biophysical properties may influence both the active and passive transport of different molecules through the membrane. In the last years many phenothiazines were shown to interfere with multidrug resistance and to perturb lipid bilayers as well. Phenoxazines, the structural analogs of phenothiazines, are also recognized as potential multidrug resistance modulators.

In our study interaction of the novel synthetic phenoxazines:10-H-phenoxazine (WM1) and benzo[α] phenoxazin-9-one (WM8) were examined. Their capacity to penetrate the cell membrane and to alter the lipid bilayer properties was studied by microcalorimetry and fluorescence spectroscopy. In microcalorimetric experiments it was shown that WM1 decreases both the temperature and enthalpy of DPPC main phase transition, whereas WM8 increases the enthalpy and does not influence the transition temperature. Interaction of these compounds with lipid bilayer was confirmed further by measurements of Laurdan generalized polarization. The liquid-crystalline phase of bilayer was perturbed by both of studied phenoxazines, however the effect of WM1 was more pronounced. The incorporation of phenoxazines into lipid membranes increases permeability of

liposomes for calcein. WM1 caused a higher leakage of this fluorescent marker from vesicles than WM8. Though both compounds interact with lipid bilayer the less hydrophobic compound, 10-H-phenoxazine more strongly influence bilayer properties studied in this work.

105. Spectral properties of stilbazolium merocyanines in resting and stimulated lymphocytes

E. Staśkowiak[1], A. Dudkowiak[1], K. Wiktorowicz[2], J. Cofta[2], D. Frąckowiak[1]

[1]Poznań University of Technology
[2]Karol Marcinkowski University of Medical Sciences, Poznań

Three stilbazolium merocyanines, selected on the basis of their investigations in model systems were introduced into resting and stimulated lymphocytes (Staśkowiak *et al.*, 2004, *J. Photochem. Photobiol. A: Chemistry*, **163**, 127; 2004, *J. Photochem. Photobiol. A: Chemistry,* in press). All these merocyanines in solution exhibit high yield of generation of very photochemically active triplet states, but are characterized by different yields of fluorescence emission. The phytohemagglutinin-stimulated lymphocytes were the model of tumor cells. The course of the photodynamic reactions in stained und unstained cells were established. The efficiency of triplet states generation of the dyes incorporated into stimulated cells has been evaluated using time resolved photothermal spectroscopy. As follows from the photographs of stained cancerous cells taken by means fluorescence microscope, merocyanines are located predominantly in cell membranes. It has been shown that the dyes exhibiting high yields of triplet state generation in the stimulated cells usually are also efficient sensitizers of photodynamic reactions. The exceptions are the dyes undergoing fast photobleaching in the cells.

This work was supported by Polish Committee for Scientific Research (grant no. 3 P05B 073 24, 2003-2005) and by BW 62-195/04 (for A.D.).

106. Mechanistic equations of membrane transport of mulitcomponent solutions

G. Suchanek[1], M. Przestalski[2], M. Kargol[1], A. Kargol[3]

[1]Świetokrzyska Academy, Kielce
[2]Medical Academy, Wrocław
[3]Loyola University, New Orleans, USA

We have considered a thermodynamic system in which a heterogeneous porous membrane separates multicomponent non-electrolytic solutions (water + n solutes). Passive membrane transport is effected in this system by an osmotic pressure difference resulting from n- solutes, as well as a mechanical pressure difference. The substances only permeate across those pores whose cross-section radiuses are larger than the dimensions of the given solute molecules. Smaller pores are permeable to water only.

Apart from mechanistic assumptions concerning the membrane structure and transport mechanisms across pores, all the remaining assumptions pertaining to the system at issue are identical with those binding in the Kedem-Katchalsky (KK) formalism. Considering these assumptions and applying to our considerations a procedure analogous to the KK equations, we have arrived at the set of n+1 mechanistic equations, formally identical to the KK equations. These equations formulate the flows and define mechanistic transport coefficients, such as coefficients of reflection and diffusive solute permeability as well as the coefficients of mutual diffusion of any solutes. The mutual formal equivalence of mechanistic equations and the KK equations enables a comparison of membrane coefficients of both equation types.

107. Water transport route from the soil to the root xylem according to the mechanistic approach

G. Suchanek[1], M. Przestalski[2], M. Kargol[1], A. Kargol[3]

[1]Świetokrzyska Academy, Kielce
[2]Medical Academy, Wrocław
[3]Loyola University, New Orleans, USA

1-membrane models of the root radial transport route are based on the Kedem-Katchalsky equations which pertain to homogeneous membranes. In view of this, the membrane of these models is treated as homogeneous. Yet transport parameters of the root depend on the efficiency of its numerous structures which lie on the route between the soil and the xylem. Consequently, we believe that the model membrane ought to be ascribed with the properties of a porous heterogeneous membrane, described by mechanistic formalism. This formalism makes allowances for the internal structure of the membrane, which expands the model approach to a variety of root transport aspects. Mechanistic equations, applied to 1-membrane root models with a porous heterogeneous membrane, assume the possibility of simultaneous water flows across the root along two parallel (symplastic and apoplastic) routes, which differ in their transport parameters. This model also enables the determination of conditions to be met for the water transport along these routes to occur in both directions - from the soil to the axial cylinder and outside the cylinder. This is possible because in the mechanistic approach a zero global volume flow of the water across the membrane (root) does not exclude non-zero local flows of opposite directions.

The obtained results are of fundamental significance for the explanation of the problem of the root's passive

discharge of various substances (incl. metabolic products) to the surroundings.

108. Mechanistic interpretation of transport parameters of root endodermis

G. Suchanek[1], M. Przestalski[2], M. Kargol[1], A. Kargol[3]

[1]Świetokrzyska Academy, Kielce
[2]Medical Academy, Wrocław
[3]Loyola University, New Orleans, USA

We have proposed a 1-membrane model of a root radial transport route, in which the function of the model membrane has been assigned to the endodermis as a structure of key significance for the transport route efficiency between the soil and the xylem. Due to the structural and functional complexity of the endodermis, the model membrane has been assigned with the properties of a porous heterogeneous membrane, described by means of the mechanistic transport formalism. Until the present, membranes which represented root transport routes, according to the assumptions of the Kedem-Katchalsky equations, have been treated in membrane models as homogeneous. The application of mechanistic formalism enables, apart from the description of new significant aspects of root transport, a deeper insight into the structure of the model membrane (the endodermis). Since both of these formalisms are generally equivalent, it is possible (through the comparison of corresponding coefficients) to offer a mechanistic interpretation of the KK transport parameters. This enables the application and reinterpretation of these root parameters which have been so far defined and measured (by numerous researchers) on the basis of the KK formalism.

In the above situations, it has become possible to go considerably deeper into the analysis of root transport functions.

109. Computer modelling of the lateral root formation

J. Szymanowska-Pułka

University of Silesia, Katowice

The symplastic growth observed in plant organs is a coordinated growth of cells (Erickson, 1986, *Plant Physiol.*, **82**, 1153). During such growth neighbouring cells do not slide with respect to each other maintaining their mutual contacts in time. Mathematically a vector field of the displacement velocity of points (**V**) can be regarded as continuous. Differentiation **V** defines the so-called growth tensor (GT) – a second range operator, on the basis of which the most overall description of the organ growth can be obtained (Hejnowicz & Romberger, 1984, *J. theor. Biol.* **110**, 93). The GT field for the root apex with the quiescent centre (QC) is known (Hejnowicz & Karczewski, 1993, *Amer. J. Bot.*, **80**, 309). Basing on the field a steady growth of the root apex of radish was modelled (Nakielski, 2000, *Tensorowy model wzrostu w zastosowaniu do wierzchołka korzenia*. Wyd. U. Śl., Katowice). Modifications of the field concerning introducing instability characteristing for the lateral root growth allowed to apply the model to description of formation of this organ (Szymanowska-Pułka, 2003, *Proceedings of the Ninth National Conference on Application of Mathematics in Biology and Medicine* M. Markun, J. Stefaniak (eds.), Institute of Mathematics, Jagiellonian University, p. 95).

We know from anatomical studies (Barlow, 1992, *Ann. Bot.* **69**, 533) and computer simulations (Nakielski & Barlow, 1995, *Planta*, **196**, 30) that there is a link between endogenous gibberellin level and the cell patern of the growing organ which means a suitable specification of the GT field. Here the question how the GT dedicated for the lateral root apex acts on the points of the organ is discussed. The matter is that the GT field consists of regions of specific spatial and directional distribution of growth rates, thus depending on the way of the field operation on a cell pattern results can be different. For example, the forming lateral root can be differently shaped (Casimiro *et al.*, 2001, *Plant Cell* **13**, 843; Geldner *et al.*, 2004, *Development* **131**, 389).

110. Influence of aggregated amyloid beta(25-35) peptide on surface region fluidity of neuroblastoma SN56 cell membrane

S. Świrydowicz, B. Madziar, A. Jankowska, H. Bielarczyk, D. Kulawiak-Gałąska, N. Knap, A. Szutowicz, M. Woźniak

Medical University, Gdańsk

The amyloid hypothesis of Alzheimer's disease (AD) states that the neurodegeneration in AD may be caused by deposition of amyloid β (Aβ) in brain tissue. Aβ is polypeptide composed of 40-42 amino acids residues. Aβ(25-35) is an 11-residue peptide, which possesses the same neurodegenerative activities as full-length Aβ peptide. Aβ spontaneously incorporates into cell membrane, hence the interaction of Aβ with the cell membrane is probably the part of its mechanism of neurotoxicity.

In this study the spin-label electron paramagnetic resonance (EPR) method was applying to designate the alteration of surface localised membrane region fluidity caused by Aβ(25-35). The spin label 5-doxyl stearic acid (5-DSA) was used as the molecular fluidity sensor.

Aggregated Aβ(25-35) at concentrations of: 1, 2 and 5 microM was added to SN56 cell culture for 72h. Next the cells were suspended in phosphate buffer at pH 7.4 and than labelled by 5-DSA.

The effect of Aβ(25-35) on the fluidity of investigated membrane region is concentration dependent. Aβ(25-35) at concentration 1 microM strongly stiffs the surface

region, at concentration equal 2 microM the same effect is visible but weak. Aβ(25-35) at concentration 5 microM practically does not change the fluidity. There are no differences between the EPR spectrum shapes for control (untreated cells) and treated with 5 microM Aβ(25-35).

111. Application of the percolation theory tools to the modeling of the process of trabecular bone aging.

Z. Tabor

Jagiellonian University Medical College, Kraków

In the study it is show how the methods of percolation theory could be used in the description of age-related changes of mechanical competence of trabecular bone. Previously introduced stochastic model of remodeling of trabecular bone is applied to the simulated aging of pairs of 2D sections of trabecular bone matched for apparent density, structural anisotropy and the age of the donors. The critical density of the structures – defined here as the density below which percolating bone cluster disappears i.e. the structure is fractured – is estimated for each structure. It is showed that structures belonging to pairs matched for density (clinically used as principal determinant of fracture risk) lose mechanical competence at different rate, depending on the value of critical density. Thus it is hypothesized that the risk of fracture has to depend not only on density of the structure but also on its critical density.

112. The influence of 50Hz magnetic field on the human heart rate varablilty — linear and non-linear analysis

Z. Tabor, J. Michalski, E. Rokita

Jagiellonian University, Kraków

In the study the problem of the influence of 50 Hz magnetic field (MF) on human heart rate variability (HRV) was investigated. The exposure system was a commercial device for magnetotherapy, generating field of the strength of 500 μT at the center of the coil, 150-200 μT at the position of human subjects' heart and 20-30 μT at the position of subjects' head. The exposure protocols, applied randomly, were either "half hour MF-off/half hour MF-on" or " half hour MF-off/half hour MF-off". The phonocardiographic signal of 15 volunteers were obtained during exposure and used for calculation of time-domain HRV parameters (mean time between heart beats (N-N), standard deviation of time between heart beats (SDNN) and the number of differences of successive beat-to-beat intervals greater than 50ms, divided by the total number of beat-to beat intervals (pNN50)) and nonlinear HRV measures (approximate entropy, detrended fluctuation scaling exponents). The protocol MF-off/MF-on was applied in 9 subjects. Repeated measures ANOVA (RMANOVA) performed for Mf-off/MF-off protocol indicated no statistical difference between four 15-minutes intervals of HRV data (p-value>20% for all parameters except of N-N, for N-N p-value equal to 3.7%). RMANOVA followed by post-hoc Tukey test performed for Mf-off/MF-on protocol indicated statistically significant difference during MF-on for N-N (8% increase, p-value < 0.1%), SDNN (40% increase, p-value 1.1%) and pNN50 (110% increase, p-value < 0.1%). The results of the analysis indicate that the changes of these parameters could be associated with the influence of MF.

113. Fusion of 3D ultrasound and computed tomography for liver cancer diagnosis

G. Tatoń, E. Rokita, M. Sierżęga, J. Kulig

Jagiellonian University Kraków

Ultrasound (US) and computed tomography (CT) are treated so far as separate imaging methods for the diagnosis of liver cancer. Recently the advancement in computer technologies opened new frontiers in the diagnostic methods. An interesting possibility is a combination of images collected with the use of different diagnostic modalities. Usually the 3D CT images are combined with magnetic resonance results in order to gain diagnostic information while the 3D US is considered separately.

The usefulness of combining the 3D images from CT and 3D US in liver cancer diagnosis was investigated. Thirteen healthy volunteers and ten patients suffering from liver tumors were subjected to the CT and 3D US examinations. The 3D US investigations were fulfilled in B-mode and in Doppler mode. 3D image reconstructions and fusions were performed with the use of software developed in our laboratories.

The proposed method would be especially useful in the surgical treatment planning when the tumor geometry and the vascular supply should be taken into account. It is since CT allows the precise determination of tumor geometry and 3D US with Doppler mode gives the information about vascularization.

The automatic procedures used for the image combining are still not reliable and the best results were obtained with manual approach. Some afford should be directed in the future for the development of more efficient automatic procedures for the 3D images registration.

114. Copper ions inhibit the activity of Kv1.3 channels in human T lymphocytes

A. Teisseyre, J. W. Mozrzymas

Wrocław Medical University

The inhibitory effect of copper ions (Cu) on the activity of Kv1.3 channels in human T lymphocytes (TL) was studied applying the whole-cell patch-clamp technique. Obtained data demonstrate that application of Cu caused a reversible reduction of the current amplitudes to about 0.13 of the control value. The inhibitory effect occurred in the concentration range from 20 μM to 2 mM and was weakly concentration dependent. The inhibitory effect and the recovery from block was time dependent with an onset of about 1-2 minutes. The current inhibition by Cu was voltage-independent and it was accompanied by a significant reduction of the current activation rate. Activation time constants in the presence of 100 mikrom Cu were about 3 times as high as under control conditions. The slowing of current activation rate was voltage-independent. Application of Cu did not affect significantly the current inactivation and deactivation rates. Also the steady-state activation of the currents remained unchanged upon application of Cu. The inhibitory effect of Cu was not altered when raising extracellular potassium concentration from 4.5 mM to 150 mM. It also remained unchanged when pH of the extracellular solution was lowered from 7.4 to 6.4. Possible physiological significance of this inhibitory effect is discussed.

This work was supported by CSR Medical University Grant No 452.

115. The inhibitory effect of genistein on Kv1.3 channels in human T lymphocytes

A. Teisseyre, K. Michalak

Wrocław Medical University

The whole-cell patch-clamp technique was applied to study the inhibitory effect of a tyrosine kinase inhibior genistein on the activity of Kv1.3 channels in human T lymphocytes (TL). Obtained data demonstrate that application of 80 μM of genistein caused a reversible reduction of the current amplitudes to about 0.225 of the control value. The inhibitory effect was weakly concentration dependent. The half-blocking concentration of genistein was in the range from 10 to 40 μM. The inhibitory effect was voltage-independent. This was accompanied by a significant reduction of the current activation rate. Activation time constants in the presence of 40 mikrom genistein were about 3 times as high as under control conditions. The slowing of current activation rate was voltage-independent. Application of genistein did not affect significantly the current inactivation and deactivation rates. The steady-state activation of the currents also remained unchanged. Upon a co-application of 10 mikrom genistein with 1 mM sodium orthovanadate, a tyrosine phosphatase inhibitor, the current amplitudes were reduced to about 0.62 of the control values, compared to about 0.5 obtained upon appcation of 10 μM genistein alone. Altogether, the obtained results may indicate that the inhibitory effect of genistein is due to direct actions on the channels rather than inhibition of tyrosine kinases.

This work was supported by CSR Medical University Grant No 452.

116. Effect of acyl chain composition on the interaction of cardiolipin with mammalian and bacterial lactate dehydrogenases.

G. Terlecki, E. Czapińska, K. Rogozik

Wrocław Medical University,

Cardiolipin is the typical, acidic phospholipid of bacterial membranes and the most important, lipid component of mitochondrial membranes in animal cells. Molecules of this phospholipid isolated from different sources differ from each other with acyl chain composition. The aim of our experiment was to study how important is the length and the saturation of acyl chains of cardiolipin molecule for its interaction with mammalian and bacterial lactate dehydrogenase (LDH), one of key enzymes of glycolytic pathway. Three forms of cardiolipin were used for the studies: (1) natural cardiolipin (NatCL), isolated from bovine heart and containing mainly (90%) 18:2 unsaturatred fatty acids; (2) synthetic cardiolipin containing only 18:1 unsaturated fatty acids (tetraoleoyl cardiolipin, TOCL); (3) synthetic cardiolipin containing 14:0 saturated fatty acids (tetramyristoyl cardiolipin, TMCL). The phospholipids were used in the form of unilamellar vesicles, approximately 100 nm in diameter. In the experimental conditions all the cardiolipins formed only lamellar structures. Activity of the enzyme was measured spectrophotometrically, after 5 min preincubation with the phospholipid. The influence of cardiolipins on activity of two cytosolic forms (muscle and heart) of swine LDH and cytosolic form of bacteria *Lactobacillus leichmannii* LDH at different pH, ionic strength and temperature was examined. All examined forms of cardiolipin were strong inhibitors of the studied enzymes at low pH (5.5), but only TMCL at pH 7.5 (except of bacterial LDH). CLTO and NatCL were found to be the weakest inhibitors of mammalian and bacterial enzymes, respectively. The most resistant on ionic strength was the interaction of TMCL with all the studied enzymes, particularly with muscle LDH. The resistance of the interaction of NatCL and TOCL with muscle LDH increased considerably when temperature was raised from 25 to 37ºC. In our opinion the fact that the TMCL:LDH interaction is the most effective compare to the other studied cardiolipins should be interpreted as the result of the presence of relatively short (14C) acyl chains in TMCL molecule. The presented

data clearly show that the saturation of acyl chains is not as important for the interaction as their length and suggest that the influence of the bilayer structure fluidity on the interaction is also significant.

117. The ihfluence of polysialic acid on phospholipid liposomes studied by 2D–NMR

A. Timoszyk, A. Janiak-Osajca, K. Walińska, L. Latanowicz

University of Zielona Góra

The two cellular processes appear to be affected by polysialic acid during the formation of tissues, namely of guidance and targeting of growing axons, and the separation and migration of cells. It appears to depend on the physical properties of this large polymer, which bears multiple negative charges and is heavily hydrated. Model phospholipid (1,2-Diacyl-sn-glycero-3-phosphocholine) liposomes were modified with the cationic octadecylamine. We will show full specification of 1H-NMR spectra of model phospholipid liposomes, modified lipid liposomes and polysialic acid. To descriptin of respective signals we used 2D Correlated (COSY) and 2D Total Correlation (TOCSY) Spectroscopy. Both of methods are appropriate to characterize the chemical structure of phospholipid liposomes and polysialic acid. We applied Rotating Frame 2D Overhauser Effect (ROESY) experiment for determining possible structure of 1,2-Diacyl-sn-glycero-3-phosphocholine in bilayer state. We have also stiudied polysialic acid sequence and glycosylation sites by ROESY. By ROESY we try to qualify position of polysialic acid in relation to liposome.

This work was supported by the State Committee for Scientific Research, grant No. 2P03B 08625.

118. Changes of ultrasound velocity in trabecular bone with partially demineralized bone tissue

H. Trębacz, H. Gawda

Medical University of Lublin

Disorders in mineralization of bone tissue as well as changes in trabecular structure of bone lead to bone pathologies and to increased fracture risk. The aim of the present study was to evaluate changes of ultrasound velocity in trabecular bone after a gradual demineralization of bone tissue. Anisotropy of velocity in bone samples and its changes after demineralization were also evaluated. A transmission method of 1 MHz ultrasonic pulses in three perpendicular directions was applied for bovine trabecular bone samples. Ultrasound velocities in axial direction of long bones were 2450-3000 m/s. After demineralization by, on average, 50%, velocity in this direction decreased by 19–39%, depending on the initial density of samples. Velocities of ultrasound in directions perpendicular to the bone axis were initially 2045–2700 m/s and after demineralization decreased by 10–20%. On the basis of the results of the study it was stated that ultrasound provides quantitative information on both degree of mineralization and the spatial arrangement of bone trabeculae. The important role of collagen fibers in mechanical properties of bone tissue was also proved.

119. Thermal analysis of collagen in mineralized and demineralized bone tissue and in tendon

H. Trębacz, K. Wójtowicz

Medical University of Lublin

In the present paper differential scanning calorimetry was applied to study thermodynamic processes in strongly cross-linked bone collagen and Achilles tendon. Powdered samples (37-38 mg) were placed into closed steel capsules and heated to 340°C at scanning rate 1.2°C/min. The measurements were performed on mature and immature collagen in bovine and rat bone samples and tendon. In order to evaluate influence of mineral on processes of collagen degradation, fully demineralized (in EDTA) bone samples were also studied. Two endothermal processes (T1 and T2) were found in each sample. T1 occurred in 145-170ºC in bones (depending on kind of the sample and its maturity), 130-145ºC in demineralized bone and 118-120ºC in tendon collagen. Energies of activation for processes T1 calculated from thermograms were between 400-680 kJ/mol and corresponded to dissociation energy of covalent bonds in organic compounds. Therefore, we attribute T1 to the degradation of inter- and intramolecular crosslinks in collagen. The endotherm T2 occurred between 300–340ºC and represents melting of collagen.

120. The formation of superstructures of modified lipid membranes — the toplological model

K. Walińska, A. Janiak-Osajca, A. Timoszyk

University of Zielona Góra

Liposomes have been described as artificial cells or organelles and have also been used to examine properties of membranes. Polyprenols occur in biological membranes of living cells, mainly as phosphoryl derivatives, and function as carriers of glycosyl units in the glycolysation reactions. Lipid bilayers modified by polyprenols change mechanical and structural properties. Lipid vesicles were prepared from mixture of pre-

nol-10 and DOPC and their structure were investigated using transmission electron microscopy techniques (TEM).Among several studied liposomes there are multibudding structures which can be lipid model of cytosis. Cytosis is known as the process of vesicle formation and their subsequent movement. Cells can use vesicles to transport solids or liquids across the plasma membrane either into, or out of the cell. The process of importing material in vesicles is called endocytosis and the process of exporting material in vesicles is called exocytosis. The description and explanation of the mechanism of budding secretory vesicles is possible by the application of the topological model.

The mechanism of the cytosis process can possibly be explained by the application of the theory of homeomorphic transformations of topological manifolds, the operation of the connected sum of the manifolds. The analysis shows succeeding topological stages of bilayer transformations during this process and it can be useful to understand some aspects of the cytosis.

121. Trifluoperazine promotes phase separation in raft containing model membranes

O. Wesołowska, A. B. Hendrich, K. Sidorska, K. Michalak

Wrocław Medical University

Lipid rafts are membrane domains enriched in cholesterol, sphingomyelin and glycosphingolipids that play a major role in many cellular processes, e.g. signalling. Many proteins are specifically associated with rafts including drug transporters involved in multidrug resistance (MDR) of cancer cells. The influence of trifluoperazine, well known MDR modulator, on model DPPC membranes containing various amounts of cholesterol (Chol) and sphingomyelin (SM) was studied by means of fluorescence spectroscopy. The use of Laurdan, fluorescent probe sensitive to phase state of lipid bilayer, allowed us also to determine if phase separation occurs within membrane. Studying Laurdan generalised polarization parameter (GP) we demonstrated that lipid domains existed in DPPC membranes containing more than 10 mol% of cholesterol, spingomyelin or both these components. Thus DPPC/Chol/SM mixtures turned out to be good models of raft containing membranes. Addition of trifluoperazine to liposomes caused enhancement of the observed phase separation effect. Experiments performed at different temperatures allowed as to follow the influence of the drug on lipid bilayer in gel-like and liquid crystalline phase states. Effect of TFP was the most pronounced in gel-like bilayers. Additionally main phospholipid phase transition temperature was significantly lowered in the presence of the drug. We concluded that trifluoperazine could influence the lipid lateral organisation in the membrane promoting microdomains formation.

122. Fluorescence investigantions of enzyme-ligand complexes: indentification of tautomeric forms of the purine substrates bound to purine-nucleoside phosphorylase

J. Wierzchowski[1], A. Bzowska[2], K. Stępniak[2]

University of Warmia & Mazury, Olsztyn
Warsaw University

Purine nucleoside phosphorylase (PNP) is an ubiquitous enzyme of purine metabolism, and its deficiency is related to severe immunological disorders. It is also a target for the antineoplastic and antiparasitic therapy (Bzowska, Kulikowska & Shugar, 2000, *Pharmac. Therap.* **88**, 349). Numerous purines and purine analogues exhibit substrate or inhibitory activities with PNP, and several fluorescent enzyme-substrate or enzyme-inhibitor complexes can be observed. In the present work, fluorescence excitation and polarization spectra of PNP-ligand complexes were investigated, and shown to be useful for identification of ionic/tautomeric forms of the bound ligands.

In particular, fluorescence difference excitation spectra of the fluorescent PNP-guanine complex show that the base exists most likely as the N(7)H tautomer. Fluorescent complexes of PNP with 8-azaguanine (8-azaG) and its 9-(2-methyloxy-phosphonoethyl) derivative were also examined, the former showing marked differences between fluorescence excitation and absorption of the free ligand. Also in this case a tautomeric shift towards the more fluorescent N(7)H tautomer is proposed. The foregoing results support the concept of protonation of the nucleoside substrate at N(7) as an initial step of catalysis.

Polarized fluorescence studies of the PNP complex with nonfluorescent inhibitors were carried out with the aim to determine the binding constants, and to better charcterize protein-ligand complex.

123. Phase transition of DMPC, DPPC and DSPC bilayers. Calorimetric and acoustic study

K. Wójtowicz

Medical University of Lublin

Water suspensions of phospholipid bilayers of different thickness containing either 7-dehydrocholesterol or cholesterol were studied by means of ultrasound absorption technique and calorimetric analysis. Bilayers of different thickness were obtained with phosphocholine (PC) of varying acyl chain length: dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC).

The aim of this paper is to examine the effect of length of phospholipids alkyl chain on the properties of lipid bilayers containing either 7-DEH or cholesterol.

Monotonic decrease of Tt with sterol amounts was observed. The decrease was being affected by PC acyl chain length. 7-DEH and cholesterol behave in a different way. At small sterol concentration the response of the lipid bilayer to 7-DEH increases on increasing chain length; the opposite happens when cholesterol is considered.

The enthalpy associated with the main transition and cooperativity decrease approximately linearly with sterol amount in both 7-DEH and cholesterol contained in bilayers. However 7-DEH is more effective in reducing transition enthalpy when dissolved in short chained lipids (DMPC). Cholesterol effect is almost independent of alkyl chains length.

Such a behaviour of 7-DEH should be associated with a lower perturbation of lipid bilayer structure than in the case of cholesterol. 7-DEH molecules are more flexible and adapt itself to alkyl chains movements.

124. Single molecule measurements

K. Zabrocka[1,2], C. Schmidt[3]

[1]Wroclaw University of Technology
[2]Biophysical Scientific Students Union "Micela"
[3]Vrije Universiteit, Amsterdam, The Netherlands

Kinesin is dimeric motor protein involved in intracellular transport of organelles and vesicles along microtubules. As the source of free energy it catalyzes the hydrolysis of ATP to ADP and inorganic phosphate. The goal of this project is to study about kinesin using fluorescent kinesin (the chemomechanical cycle and 16 nm step of kinesin, using FRET). In he future we want to observe single enzymatic ATP turnovers catalyzed by knesin by fluorescence. In order to measure single ATP tornovers on moving kinesin we will use single-molecule fluorescence techniques. In particular to reduce the excitation volume we will make use of fluorescently labeled kinesin that is bound to microtubules as light source to excite by FRET (Forster Resonance Energy Transfer), fluorescent ATP analogues only when they are bound to kinesin. Using 532 nm light the Alexa 555(donor) bound to kinesin will be excited and if Alexa 647ATP(acceptor) is present the excitation will be transferred and red fluorescence will be emitted instead of orange. An interesting aspect will be to try to observe the connection between the kinetics of the two heads by observing the correlation in ATP binding to both heads.

125. Influence of an artificial heart valve on erythrocyte membrane fluidity after their incubations with native and modified LDL

I. Żakowska[1], M. Szyller-Tracz[1], K. Jóźwik[1], K. Gwoździński[2], J. Olejniczak[3], E. Szajdzińska–Piętek[4]

[1]Technical University of Łódź
[2]University of Łódź
[3]MSW, Łódź
[4]Technical University of Łódź

Permanent interactions between artificial heart valve (AHV) surface and morphotic elements especially erythrocytes can influence on plasma membrane fluidity and affects processes occurring in the membrane such as transport and enzyme activities. The blood flow through the artificial heart valve (AHV) is determined by higher values of gradients of velocity and pressure at the valve outlet. Higher values of these gradients and their non-uniformity lead to shear stresses acting on the surface of blood cells as well as on plasma lipoproteins.The blood flow through the artificial heart valve is determined by higher value of the shear stress on the cell surface and in consequence by changes in the structure of membrane lipids and proteins.

Changes in erythrocyte plasma membrane fluidity from artificial heart valve patients (AHV-RBC) after interaction with LDL: native, oxidized and those from AHV patients using EPR spectroscopy were studied. Changes in lipid membrane fluidity of red blood cells were studied by EPR spectroscopy using spin labels: 5-, 12-, 16-doxylstearic acids. Red blood cells and low density lipoproteins were separated from patients with an artificial heart valve (AHV).

The interaction of physiological concentrations of native, oxidised LDL, (ox-LDL) and AHV-LDL (from artificial heart valve patients) with AHV-RBC from these patients was studied by EPR spectroscopy.

The results showed significant increase in AHV-RBC plasma membrane fluidity after the interaction with native, ox-LDL and IHD-LDL as measured by 5-DS and 16-DS. We showed that incubation of AHV-RBC with native and oxidized LDL induced the increase in the RBC plasma membrane fluidity. LDL isolated from AHV patients decreased lipid fluidity monitored by 12-DS. Significant changes (12-DS) for AHV-RBC after interactions with ox-LDL were observed. These findings suggest that LDL influenced AHV-RBC plasma membrane properties. Changes in lipid membrane fluidity induced by LDL may affect AHV-RBC deformability in blood flowing through artificial heart valve. The changes in the plasma membranes of red blood cells (RBC) can decrease their deformation and increase their aggregation and in consequence lead to alteration in the rheological properties of blood.

This work was supported by grant No. 7 T11E 013 21 (State Committee for Scientific Research, Poland).

126. The role of cholesterol and quercetin in organometallics interaction with phosphatidylcholine and phosphatidylcholine/sphingomyelin liposomes

R. Żyłka, J. Gabrielska, S. Przestalski

Agricultural University, Wrocław

The organic forms of metals are ubiquitous in the environment and thus constitute a menace to human health. The amphiphilic character of the compounds enables them to intercalate and penetrate cell membranes and thus disturb the life functions of cells. The degree of the molecules adsorption in membranes depends both on their properties and membrane composition and its state. The lipid bilayer of a cell membrane is a dynamic structure endowed with surface heterogeneity, with complex local and global architecture that is closely related to many cell functions. That is why the selective incorporation of toxic molecules to a specific membrane region may interfere with some processes that occur in that cellular region. So we have undertaken studies to obtain knowledge on the structural changes induced by adsorption of n-phenyltin molecules in the bilayer of membranes formed to mimic the various areas of the plasma membrane. The investigation of structural changes of the bilayer of membranes formed of phosphatidylcholine and phosphatidylcholine/ sphingomyelin with differentiated content of cholesterol (so-called rafts) induced by adsorption of n-phenyltin, quercetin and equimolar mixtures of n-phenyltin and quercetin were conducted using the microcalorimetric and fluorimetric methods. The results indicate at an essential role of cholesterol that affects the degree of membrane perturbation by the compounds studied, the equilmolar n-phenytin/quercetin mixtures including.

127. Merocyanine-copper complexes in polyvinyl alcohol

G. Pajchrowski[1], R. Cegielski[1], M. Niedbalska[1] H. Manikowski[1,2]

[1] Poznań University of Technology,
[2] State Vocational College, Piła

Merocyanine of stilbazolium betaine type (1-(12- hydroxydodecyl)-4-[(3-hydroxy-4-oxocyclohexa-2,5-dienylidene)-ethylidene]-1,4-dihydro-pyridine) is sensitive for pH of environment. In acidic conditions (pH<7) merocyanine exist in protonated form whereas in basic environment free base form of merocyanine dominates.

This free base merocyanine creates complexes with transition metal ions (Cu^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+} Cegielski, *et al.* 2001. *Dyes and Pigments*, **50**, 35-39; Cegielski *et al.* 2003, *Dyes and Pigments*, **58**, 19-25). Merocyanine – transition metal ion complexes are soluble in methanol and in methanol – water mixture. Stability of these complexes is different and spectral properties too. As anisotropic medium we have used polyvinyl alcohol stretched 0% and 300%. Complexes in polyvinyl alcohol used for experiment were stable more than 1 year. EPR spectrum of merocyanine – Cu^{2+} complex in methanol solution is observed in room temperature and is more pronounced in liquid nitrogen temperature. Merocyanine – Cu^{2+} complex in polyvinyl alcohol has also EPR spectrum with shape similar to that in methanol solution frozen to 77 K (Manikowski *et al.*, 2000, *Molecular Physics Reports*, **28**, 85-88.).

From UV-Vis absorption spectra it was calculated that one Cu^{2+} ion create complex with two free base merocyanines. Merocyanine in polyvinyl alcohol has EPR signal only when sample is illuminated by visible light in the absorption bands (400-600 nm). Situation is similar to merocyanine in methanol solution. Also for mixture of merocyanine (5×10^{-4} M/l) and Cu^{2+} (5×10^{-5} M/l) in polyvinyl alcohol similar light induced EPR signal is observed on the top of stable EPR signal of paramagnetic complex. Light induced EPR signal is originating from excess of free merocyanine. Ilumination of the complexes in polyvinyl alcohol causes partial disoociation of the complex too. As a result additional rise of signal from merocyanine is observed and decrease of paramagnetic complexe simultaneously. These changes are clearly seem in UV-Vis difference absorption also. 300% stretching of polyvinyl alcohol samples is orienting merocyanine and mero-cyanine-copper complexes. It is observed both in visible absorption (Cegielski, 2004, *Dyes and Pigment*, **62**, 213-219) and in EPR. Degrees of orientation of complexes is higher then merocyanine alone. Two merocyanines and one Cu^{2+} create complex.

This study are financially supported by Poznań University of Technology (PB-62-199/04-BW)

128. Kinetic studies of ethanol transfer across the lipid bilayer

M. Przybyło[1], A. Olżyńska[1], K. Weron[1], M. Langner[2]

[1] Biofizyczne Kolo Naukowe "Micela",
Politechnika Wrocławska
[2] Instytut Fizyki, Politechnika Wrocławska

The interactions of short chain alcohols such as ethanol with the lipid bilayer have been intensively studied especially in the context of their enhancing drug permeability properties. Although most of the investigations provide the structural information and focus on the mechanism of the interactions, no studies have been performed as yet that demonstrate in the direct manner the ethanol penetration through the lipid membrane. In the present work the dynamics of the ethanol transfer through the lipid membrane was examined using the sopped flow technique and the kinetics of the process

were obtained. Since the addition of ethanol to lipid membranes results in an increase of the dielectric constant the NBD fluorescent dye was used as the probe monitoring the process of EtOH transferring across the membrane. The kinetics obtained suggests cooperative transfor-mation in the lipid bilayer when exposed to the ethanol.