β, in turn, slowly decreases with increasing temperature: from 16.3 (at 5°C) to 14.3 (at 45°C) for BSA, from 11.6 (at 5°C) to 10.9 (at 45°C) for ESA, from 8.91 (at 5°C) to 8.31 (at 45°C) for OSA and from 8.52 (at 5°C) to 7.75 (at 45°C) for RSA.

61. A glass-transition temperature for ovalbumin obtained from viscosity measurements and the Avramov's model

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All fully hydrated proteins undergo a distinct change in their dynamical properties at temperatures between 180 and 230 K. For temperatures above this range, anharmonic motions of bonded and nonbonded groups of atoms dominate, as in a liquid state. At lower temperatures, harmonic motions predominate, as in a solid state. For a given protein, the temperature at which its properties change from liquid-like to solid-like is called glass-transition temperature T_g. In the present paper T_g for ovalbumin has been obtained from viscosity measurements of aqueous solutions of ovalbumin and from the Avramov's model. According to the model, molecules in a flowing liquid jump from one equilibrium state to the other with different activation energy they have to overcome, and the frequency of those jumps follows a Poisson distribution. The model gives three-parameter dependence of liquid viscosity on temperature, and one of those parameters is T_g. The viscosity of ovalbumin aqueous solutions was measured at temperatures ranging from 5°C to 55°C and in the range of concentrations from 6 kg/m^3 to 430 kg/m^3. The measurements were performed with an Ubbelohde-type capillary microviscometer. Glass-transition temperature of a solution has been then obtained as one of the fitting parameters of the Avramov's relation to the experimental values of viscosity. Such obtained T_g of ovalbumin solutions increases with increasing concentration from about 127 K (c = 6 kg/m^3) to about 180 K (c = 430 kg/m^3).

The glass-transition temperature of a solution depends both on T_g for a dissolved protein $T_{g,p}$ and water $T_{g,w}$. To obtain $T_{g,p}$ for ovalbumin the modified empirical Gordon-Taylor equation was used. It gives the concentration dependence of T_g of a solution, and $T_{g,p}$, $T_{g,w}$ and a parameter describing the strength of the protein-solvent interaction (K) are fitting parameters. The numerical values of the parameters are as follows: $T_{g,p}$ = (220 ± 10) K, $T_{g,w}$ = (125 ± 1) K and K = (1.88 ± 0.39).

62. Molecular interactions between dl-α-tocopherol glycosidic derivative and DPPC in Langmuir monolayers

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The present work investigates the molecular behavior of dl-α-tocopheryl β-D-glucopyranoside (BG) at the air-water interface and the interaction between BG and a model lipid membrane (monolayer) composed of dipalmitoyl phosphatidylcholine (DPPC). The behavior of pure BG, DPPC and mixed BG/DPPC Langmuir monolayers was characterized by surface pressure-mean molecular area (π–A) isotherms.

The isotherm of BG monolayer showed surface pressure onset at ca. 60 Å^2/molecule and collapse at 26 Å^2 with surface pressure value of 58 mN/m. Inclination angle of the isotherm changed at mean molecular area of 40 Å^2, what corresponds to a phase transition from the liquid to the liquid condensed state. For the DPPC isotherm, a characteristic for this phospholipid plateau region appeared at about 5 mN/m, what is ascribed to a phase transition between the liquid-expanded (LE) and the liquid-condensed (LC) state. The isotherm collapse point occurred at 70 mN/m.

On the basis of the π–A isotherms registered for binary BG/DPPC monolayers, the plots of the mean area occupied by a single molecule (A_{12}) as a function of the BG molar fraction (X_{BG}) were drawn up. The study of A_{12}–X_{BG} dependences at surface pressures below the plateau region (~5 mN/m) indicates the presence of a negative deviation, corresponding to the existence of attractive interactions between BG and DPPC and only partial miscibility. The positive deviation observed for π above 30 mN/m indicates the repulsive interactions between the two compounds. Only for $X_{BG} < 0.3$ small negative deviations of A_{12} were found. This suggests that BG and DPPC are almost completely miscible in the monolayer at low BG concentration and the surface pressures above the plateau region.

The presented results may be used to describe and explain the phase transition phenomena in biological membranes where the surface pressure is in the range of 30–35 mN/m.

This study was supported by the interdisciplinary grant No. 12/782/WI/10 from Poznan University of Life Sciences and Poznan University of Technology.

63. Pretransition and the main phase transition of DPPC membrane with canthaxanthin – Monte Carlo simulation

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Canthaxanthin is a carotenoid pigment widely used as a component of food tanning agents. There is some evidence for adverse effects of high intake of canthaxanthin. The pigment, even at a concentration below 1 mol%, significantly influences structural and dynamic properties of lipid membranes [1].

The lipid pretransition occurs at a temperature a few degrees below the main (chain-melting) transition. The pretransition is linked to the formation of periodic membrane ripples. In this study the model membrane undergoing both the pre- and main transition is presented. The effect of canthaxanthin on a model dipalmitoylphosphatidylcholine (DPPC) membrane is studied in terms of intermolecular interactions included in the ten-state Pink model of lipid membrane completed by the processes of nearest-neighbor exchanges of molecules [2].

The main purpose of this work is to verify the parameters of intermolecular interactions within the model lipid membrane modified with canthaxathin. The proper set of the parameters should reveal the weakening of the pretransition as well as the broadening of the thermogram of the main phase transition in the presence of canthaxanthin [1].

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64. Free radical scavenging activity and membrane stabilization by polyphenols from the sumac plant

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Some of the most interesting organic compounds of plant origin are tannins, polyphenols with the molecular mass ranging between 500 and 3000 Da. Tannins are characterized by high chemical activity and a variety of biological effects, such as antitumor, antimutagen, antimicrobial, and antiinflammatory. These biological effects of tannins have been attributed to the presence of numerous phenol groups, which are thought to provide chemical basis for strong antiradical activity and ability to bind proteins, polysaccharides and some metals. A group of tannin-rich plants is widely used in traditional medicine, they are commonly known as sumac. In this study we show that the aqueous extract from leaves of the sumac plant (*Rhus typhina*), and its main component (3,6-bis-O di-O-galloyl-1,2,4-tri-O-galloyl-β-D-glucose) at a concentration 1–6 μg/ml exhibit antiradical activity against 1,1-diphenyl-2-picrylhydrazyl in solution and when incorporated into liposomes and measured by the EPR method.

We also found that polyphenols under study showed a strong antiradical activity in the reaction with ROS and exert protective effect against oxidation in erythrocytes induced by *tert*-butylhydroperoxide. Moreover the studied substances increase resistance of erythrocytes to hypotonic stress and decrease membrane fluidity as assessed by fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene. We conclude that protective effects of polyphenols from the sumac plant against erythrocyte damage can be explained, at least in part, by their direct interaction with membrane components, and their antiradical activity.

65. Electromagnetic radiation as a protective factor in osteoporosis prevention

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One of the factors affecting the metabolism of bone tissue are mechanical loads. Mechanical deformations induce electrical loads, which regulate metabolic activity of bone cells. The impact of electrical potentials on the rebuilding process of bone tissues provides the ability to control this process in physiological and pathological conditions using external electromagnetic fields.

The purpose of this work was to evaluate the role of microwave radiation as a protective factor for the bone tissue during a long-lasting corticotherapy.

The experiment was conducted on 40 mature female Wistar rats. Animals were divided into three experimental groups and one control group. Animals from experimental groups were injected intraperitoneally with *hydrocortisonum hemisuccinatum* for ten weeks in a single dose of 10 mg/kg/day. Two groups of animals have been irradiated with microwaves at frequency of 53.57 GHz during corticotherapy, one group were treated with impulse microwave radiation and the other with continuous wave electromagnetic radiation.

Estimation of changes in the bone tissue was performed on the basis of measurements of bone density and porosity, biomechanical parameters, microscopic examinations, analysis of bone composition and degree of mineralization of organic matrix.

An adverse impact of corticotherapy on the bone tissue was confirmed. In both groups of animals irradiated with microwaves during corticotherapy, bone density was significantly higher and porosity signifi-

cantly lower compared to animals treated with hydrocortisone only. The biomechanical parameters determined at the maximal load and at the elasticity limit showed better mechanical and elastic properties of bones of animals irradiated with microwaves.

The results of the experiment indicate that microwave stimulation is a protective factor for bone tissue during long-lasting corticotherapy.

66. Monitoring of changes of glycine structure under exposure to near infrared radiation – an ATR-FTIR spectroscopic study

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Our previous research on the influence of the NIR radiation on biological structures revealed that the primary process probably involves the dehydration of biological membranes [1–5].

The aim of this study was to determine the effect of NIR radiation and temperature on the structure of glycine and to check whether it induces aggregation of this polar amino acid. The experiment consisted of recording the ATR-FTIR spectra of aqueous solutions of glycine. The samples were irradiated using halogen lamp equipped with an appropriate filter (edge filter, range 700–2000 nm). Annealing of the samples was carried out; the temperature was varied in the range 20–90°C (each 5°C).

With increasing temperature the weakening of interaction between glycine and water molecules has been observed. As a result, interactions between two amino acid molecules have been strengthened, leading to the formation of dimers of the amino acid at temperatures above 75°C. This has been confirmed by temperature dependence of intensity, integral intensity and half width of main absorption bands and their shifts in the spectrum of unexposed aqueous solution of glycine. After exposure to NIR radiation a new absorption maximum from hydrogen-bonded COOH groups has been noted, probably reflecting the ongoing protonation of the $–COO^-$ group to the –COOH.

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67. Cytotoxic effect of 3-methoxyflavone and 3-hydroxyflavone in human adenocarcinoma cell lines

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Plant flavonoids belong to polyphenolic compounds. They are present in many vegetables and fruits, so they comprise a significant part of our diet. It is known that many of these compounds have the ability to induce apoptosis of cancer cells by modulation of cell signaling without causing overmuch damage to normal cells. Studies in cell lines have shown that some flavonoids (e.g. quercetin) inhibit cell proliferation and induce apoptosis in many types of cancer cells by inhibition a broad range of protein kinases including the phosphatidylinositide 3-kinase (PI3K). Furthermore, interactions of these compounds with biological membranes include not only interactions with protein components but also with lipid phase of membranes. Chemical structure of a flavonoid molecule (especially substituted groups) is an important factor determining their biological activity.

In these studies 19 flavonoid compounds, substituted at different positions of the ring with hydroxy- or methoxy- groups, were taken to study their effects on biophysical properties of lipid model membranes and to check the cytotoxic effect in cancer sensitive and resistant cell lines. To determine the influence of flavonoids on model membrane properties microcalorimetry and fluorescence spectroscopy techniques were applied. Cytotoxicity of selected compounds on doxorubicin-sensitive (LoVo) and doxorubicin-resistant (LoVo/Dx) human adenocarcinoma cell lines was investigated using a SRB microplate test. The results for only two flavones 3-methoxyflavone and 3-hydroxyflavone are presented because these compounds occurred to be active both as membrane perturbing agents as well as cytotoxic compounds for cancer cells. Flavone substituted at position 3 of the A ring with methoxy- group disturbs especially strongly the properties of lipid membranes in the liquid-crystalline state.

3-methoxyflavone and 3-hydroxyflavone exerted cytotoxic effect in both LoVo and LoVo/Dx cell lines and cytotoxicity of these compounds was strongly dependent on the kind of chemical group at position 3 of the A ring of the flavone molecule. The observed cytotoxic effect of both compounds may be in part related to their ability to change the properties of the lipid phase of membranes.

68. Model eye lens membranes – how are they permeable to vitamin C? A spin label study

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The permeability of vitamin C in model lipid membranes was studied. Unilamellar liposomes prepared from lipids derived from bovine and equine eye lens were used for experiments. The permeability was investigated by kinetic studies on reduction of membrane-impermeable spin label entrapped inside the liposomes resulting in a decrease of EPR signal. Liposomes made from bovine eye lens lipids were about three times more permeable to vitamin C than liposomes prepared from equine lens lipids.

The cholesterol content in both bovine and equine eye lens lipids was similar, but the sphingomyeline content in lipids derived from bovine eye was lower than in equine lens. The influence of alpha crystallin, the main protein of the eye-lens fiber cytosol, on this process was also studied. Alpha-crystallin significantly slowed down the permeation of vitamin C into liposomes.

69. Characterization of dendriplexes formed by dendrimers and anti-HIV oligonucleotides

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Current anti-HIV therapies are capable of controlling viral infection but do not represent a definitive cure. They rely on the administration of antiretroviral nucleoside analogues, either alone or in combination with vectors. The success of gene therapy is dependent on the development of carriers capable of delivering genes into cells. Dendrimers – branched, synthetic polymers, due to their architecture, are promising tool for gene delivery.

The aim of this work was to study the interactions between three anti-HIV antisense oligonucleotides (ODNs): SREV, ANTI TAR, GEM91 and carbosilane and polypropylene imine dendrimers (PPI) by monitoring changes in the fluorescence polarization of fluorescein attached to the ends of the ODNs, when increasing concentrations of dendrimers were added. Laser Doppler electrophoresis, dynamic light scattering (DLS) and transmission electron microscopy (TEM) were used to characterize, respectively, zeta potential, particle size and morphology of dendriplexes formed in different molar ratios. Antisense oligonucleotides interacted with cationic dendrimers in different molar ratios depending on type of carrier. After addition of carbosilane dendrimers, zeta-potential of ODNs was increasing from –25 mV to highly positive values for all studied ODNs. In case of PPI dendrimers, zeta potential of dendriplexes varied from –25 mV to –5 mV (for PPIG3 and PPIG4 complexes) and to zero (for PPIG2 complexes). The diameter of dendriplexes measured by DLS ranged from 50 to 240 nm, dependently on kind of dendrimer and on molar ratios of formed complexes. The structures studied by TEM presented a polydisperse size from about 50 nm to even 700–800 nm (PPI complexes). It means that instead of single dendriplexes, aggregates were also present.

Taking into account physicochemical properties of studied dendriplexes together with our previous results we can conclude that carbosilane and PPI dendrimers are good candidates for nucleic acid delivery.

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70. Combination of doxorubicin and docetaxel in free radical metabolism in rat liver – the effect of Pirolin

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Doxorubicin (DOX) and docetaxel (DTX) are broadly used in cancer therapy. The combination of DOX and DTX is clinically effective in therapy of solid tumors. Unfortunately, the drugs can also cause severe hepatotoxicity.

Pirolin (PL) as a nitroxyl derivative, can potentially provide protection against oxidative stress via its SOD-mimic activity and detoxification of carbon-, oxygen- and nitrogen-centered radicals or oxidation of reduced transition metals.

In this work we studied the effect of Pirolin on oxidative stress induced by DOX and DTX in rats bearing experimental breast tumors. The animals were divided into 6 groups, which received respectively: PBS; PL (10 mg/kg); combination of low doses of DOX (2.5 mg/kg) and DTX (3.75 mg/kg); PL with low dose of drugs; combination of high dose of DOX (5 mg/kg) and DTX (7.5 mg/kg) and PL with high doses of drugs. Four days after last injection the animals were anaesthetized and the liver was isolated and used for further analyses. The following markers of oxidative stress were determined in the liver: antioxidant capacity, hydroperoxides, thiol groups and lipid peroxidation.

We have found a dose-dependent increase in hydroperoxides and thiol groups in rats simultaneously treated with DOX and DTX. At the same time a decrease in antioxidant capacity of hepatocytes was

observed. Pirolin did not influence oxidative stress, induced by the drugs in the rat liver.

Our results show evidence of oxidative stress generated *in vivo* by combination of DOX and DTX and lack of a protective effect of Pirolin.

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71. DNA damage in MCF-7 breast tumor cells after treatment with doxorubicin and paclitaxel

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In Europe breast tumor is the most common type of cancer and the main cause of death due to cancer. It is supposed that the incidence rate will be increasing due to the growing number of people over 65, who are at the highest risk of developing the disease. Although there are several types of treatments offered to patients like surgeries, radiotherapy, chemotherapy and endocrine therapy, there is still need for further improvements in this area.

The most common chemotherapy for breast cancer involves doxorubicin (DOX) and paclitaxel (PTX), which are predominantly used in combination. However, the pending question is whether to administer the drugs simultaneously or in a specific sequence. Additionally, despite the fact that the combined therapy is used with a success in patients, *in vitro* studies indicate that DOX and taxanes might have rather antagonistic effects on each other when are used simultaneously.

Using the comet assay we determinated the effect of DOX and PTX on DNA of MCF-7 breast cancer cells. For the experiment the cells were incubated with each of the drugs or with their combination for 2 hours. Then, the drugs were washed out and the cells were incubated in fresh medium for 0, 24, 48, 72, 96 hours.

We have observed the highest amount of DNA in the comet tail at 24 h after DOX treatment. Under the same conditions PTX induced about two-fold lower damage to DNA. Surprisingly, when both drugs were used simultaneously no significant increase in the DNA lesions was found, which confirms that they may have antagonizing effect on each other. Further investigation is needed to assess the mechanisms of interactions between DOX, PTX and DNA.

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72. The effect of cholesterol on the phospholipid bilayer smoothness

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Cholesterol is found in relatively high concentrations in animal cell plasma membranes where it comprises 40–45 mol% of the total lipids. Furthermore, in ocular lens cells its concentrations is extremely high. In fibre cells cholesterol-to-phospholipids mole ratios are from 1 to 2 in the cortex of the lens to as high as 3 to 4 in the lens nucleus. The latter leads to the formation of immiscible cholesterol crystalline domains within the membrane.

Cholesterol plays a variety of important roles in membranes, among them increases mechanical strength, and regulates fluidity, phase behavior and permeability of the membrane. These cholesterol effects have been studied over the years and elucidated, partly thanks to molecular dynamic (MD) simulations. One of our current research interests is the effect of cholesterol on the diffusion of small molecules across the membrane. In order to understand this effect, we used MD simulations of atomic models of membranes that differ in cholesterol content. We have observed that the diffusion of small molecules depends on the state of the membrane outer surface i.e., its smoothness and mobility. Thus, in this presentation we investigate the effect of cholesterol on the membrane smoothness. To our knowledge, the "smoothing" effect of cholesterol on the phospholipid bilayer has not been studied so far so this study is certainly providing new results.

73. Effect of temperature of sterilization on free radicals formation in sisomicin

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Free radicals formation in sisomicin during thermal sterilization was studied. The aim of this work was to determine free radical properties and concentration in this drug sterilized at different conditions. This antibiotic was sterilized according to the norms at three temperatures: 160, 170, and 180°C. Times of heating of the sample were: 120, 60, and 30 minutes, respectively. Sterilization was performed at dry air.

Sisomicin is an aminoglycoside antibiotic used to treat various types of bacterial infections [1, 2]. Sisomicin acts by binding to a site on the bacterial 30S and 50S ribosome, preventing formation of the 70S complex. As a result, mRNA cannot be translated into protein and cell death ensues. Sisomicin use in urinary tract infections, meningitis and prostatitis, inflamma-

tions caused by *Pseudomonas aeruginosa*, *Enterobacter*, *Proteus*, *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* [1, 2].

Free radicals were studied by electron paramagnetic resonance spectroscopy at X-band (9.3 GHz) with magnetic modulation of 100 kHz. EPR spectra were measured by Radiopan (Poznań) spectrometer with Rapid Scan Unit (Jagmar Firm, Kraków). The measurements were done with microwave power in the range 2.2–70 mW at room temperature. Lineshape of the EPR spectra and their parameters were analyzed. Influence of microwave power on asymmetry, amplitude and linewidth of the spectra was shown. Spin-spin and spin-lattice interactions in the samples were discussed.

Complex character of the free radical system in thermally sterilized sisomicin was proved. Lineshape of the EPR spectra strongly depended on the microwave power. Free radical concentration and EPR parameters strongly depended on sterilization conditions. Optimalization of this process by EPR method was discussed. It was concluded that sisomicin after sterilization should be stored at ambient atmosphere.

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74. Interaction of selected natural polyphenol compounds of the anthocyanin group with biological and model membranes

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The subject of the study were compounds belonging to the anthocyanin group: *Callistephin chloride* – pelargonidin – 3-*O*-glucoside chloride and *ideain chloride* – cyanidin-3-*O*-galactoside chloride, which can be found e.g. in strawberries and chokeberries. The influence of those compounds on the shape of erythrocytes and changes in biological and model membranes were investigated. In particular the effect on the main phase transition of phosphatidylcholine was analyzed.

The effect of polyphenols on the main phase transition of phosphatidylcholine was studied with the help of the differential scanning calorimetry (DSC) and the fluorimetric method. The degree of order in the hydrophilic phase of the membrane was assayed by the fluorimetric method, using the laurdan and prodan probes. Lipid fluidity in the hydrophobic part of erythrocyte ghosts and liposomes was determined on the basis of the fluorescence anisotropy at various depths in the lipid bilayer, using DPH and TMA-DPH probes. The interaction of the compounds with lipids was also studied by nuclear magnetic resonance (^{1}H NMR). Changes of erythrocyte shapes were observed with the optical microscope.

On the basis of optical observations it was concluded that the polyphenols studied induce echinocytosis. It can thus be assumed that they concentrate mainly in the outer monolayer of the erythrocyte membrane and practically do not permeate into the inner monolayer of the membrane. These results are in agreement with results obtained with other methods. No substantial changes in the fluidity of the hydrophobic part of the lipid bilayer were observed.

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75. A twofold role of liposomes in the protection against free radicals by natural antioxidants

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Liposomes are small lipid vesicles with an aqueous interior of 50–200 nm in diameter. Liposomes are used extensively in basic research as a model of the biological membrane and are used as carriers and shield against free radicals. The vesicles are biodegradable, biocompatible and non-toxic. They have the ability to encapsulate drugs, reduce their toxicity and increase stability of the entrapped drug. Liposomes can entrap hydrophilic molecules into their interior, and hydrophobic molecules into their lipophilic membrane. In this study the protective properties of quercetin, epigallocatechin gallate (EGCG) and green tea extract with regard to PC liposome membranes subjected to oxidative stress were investigated. Lipid peroxidation was induced by UVC. Values of IC_{50} were the following: quercetin > EGCG (1.79×10^{-3} mM and 4.09×10^{-2} mM, respectively), and IC_{50} for green tea was 0.275 mg/ml. An examination of the possibility of association of the studied extract and flavonoids was also one of the aims. In this study we used fluorimetric method and DPH probe. The association constants were: quercetin (9259 M^{-1}) > EGCG (1462 M^{-1}), and for green tea it was 215 ml mg^{-1}. The process of encapsulation of quercetin, EGCG and green tea extract within DPPC, HSPC and PC liposomes was also studied. In the case of DPPC liposomes the percentage of encapsulation followed the sequence: EGCG > quercetin >> green tea. We have also determined the experimental conditions for nanocapsulation of extracts of plants of the *Rosacea* family.

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76. EPR studies of free radicals in thermally sterilized sotalol

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Thermally sterilized sotalol was studied by the use of electron paramagnetic resonance spectroscopy with microwave frequency of 9.3 GHz and magnetic modulation of 100 kHz. Sotalol is a drug used in cardiac arrhythmias and to treat hypertension in some individuals [1, 2]. Sotalol is a non-selective beta blocker. It is also a potassium channel blocker and is therefore a class III anti-arrhythmic agent. Sotalol is used to treat ventricular tachycardias as well as atrial fibrillation [1, 2].

Paramagnetic properties of heated sotalol and the others drugs were compared. Unheated sotalol was diamagnetic, similarly to other drugs which confirms the high purity of the sample examined. Thermal sterilization was performed according to the norms at temperatures of 160°C (120 minutes), 170°C (60 minutes), and 180°C (30 minutes). All the thermally sterilized samples were paramagnetic with a complex free radical pattern. Lineshape of the EPR spectra changed with increasing microwave power.

The first derivative spectra of sterilized sotalol were recorded by a Radiopan (Poznań) EPR spectrometer and Rapid Scan Unit (Jagmar, Kraków) at room temperature. Amplitude, integral intensity, and linewidth of the EPR spectra were analyzed. g-Factor was directly determined from the resonance condition. Free radical concentration in the samples was determined. Ultramarine and a ruby crystal were used as references. Continuous microwave saturation of the EPR spectra was used to examine spin-lattice interactions.

It was shown that paramagnetic properties of sotalol depend on sterilization conditions. Free radical contents in the drug changed during storage time. The best conditions of thermal sterilization and storage of sotalol were tested by EPR method.

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77. Phosphatidylcholine chlorohydrins induce caspase-3 dependent apoptosis

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Lipid chlorohydrins, generated as the result of the HOCl reaction with unsaturated bonds of lipid fatty acid chains, were found to be cytotoxic to some cell lines. Although cell death was attributed to decline of intracellular ATP level and extensive ROS production, the exact mechanism of cell death is not specified. Thus, our experiments were intended to decide whether lipid chlorohydrins lead to direct cell necrosis or induce apoptotic cell death. Phosphatidylcholine chlorohydrins were synthesized by treatment of unsaturated lipids (SOPC, SLPC, SAPC) with five molar excess of HOCl per one double bond. Mitochondrial potential was quantified with JC-1, the number of apoptotic cells with annexin-V/PI BD kit and the level of caspase-3 active form with FITC-conjugated monoclonal antibody. Fluorescence of cells was read with an LSR II Flow Cytometer. Lipid chlorohydrins appeared to be most cytotoxic towards HUVEC-ST cells leading to reduction of viability to about 63% after 6 h cell incubation with 100 μM SAPC-HOCl (and to about 76% for MCF-7 and A549 cells). All cell lines studied demonstrated decrease of mitochondrial potential after 6 h cell treatments and an augmentation of the number of cells in both early and late apoptosis. These effects were accompanied by an increase of the level of the active form of caspase-3 at concentrations over 50 μM for SAPC-HOCl indicating that lipid chlorohydrins initiate apoptosis in all investigated cell lines with caspase-3 as the executor. Moreover, we found that the intensity of the effects observed is correlated with the number of HOCl molecules incorporated into the fatty acyl chains of phospholipids.

78. Physical principles of drug transport through a skin barrier – proposed model and verification

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Studying the mechanism of drug transport through human skin is of considerable interest for understanding the barrier function of the stratum corneum as well as for many medical and cosmetic applications. In the work described here, PAS was applied to investigate the penetration of dithranol from semisolid vaseline formulations into artificial membranes. Usually, a mathematical model based on Fick's second law is applied to describe the process of drug diffusion into model skin. Here, we present a novel model of drug permeation through a skin barrier. We include other physical effects, characteristic for porous media, that could change an overall drug distribution in the membrane. These effects depend on chemical reactivity of the drug, medium structure and drug–matrix interactions. A mathematical equation, describing a permeation process, is more complicated than Fick's second law:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - q\frac{\partial C}{\partial x} - \mu C\,,$$

$$D = D_1 + D_2,$$

where: C is the drug concentration, D_1 is the diffusion coefficient, D_2 the dispersion constant, q – the Darcy flux and μ – the first-order decay rate constant. Taking into consideration all the mentioned above effects, the experimental data are better fitted to our model predictions than to Fick's second law model. Our drug transport model was built and tested in Wolfram's Mathematica 7 software.

79. Conformational changes in irradiated dehydrogenases determined on the basis of quenching of tryptophan fluorescence

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This work is a continuation of earlier studies conducted in the Division of Radiobiology, University of Łódź, devoted to the effect of ionizing radiation on some dehydrogenases: glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase (LDH). The studies revealed that these proteins, despite a very similar structure, show significant differences in radiosensitivity.

In the present work, we investigated the inactivation of the GAPDH and LDH by the products of water radiolysis in comparison with the changes of conformation which were estimated on the basis of fluorescence quenching studies. This studies were carried out using a neutral quencher (acrylamide), which can diffuse into the interior of the protein, and an anionic (iodide ion, I^-) and cationic (cesium ion, Cs^+) quenchers, which can quench the surface Trp fluorescence.

Protein preparations of a concentration of 1.4×10^{-6} mol/dm^3 in 0.02 mol/dm^3 phosphate buffer, pH 7, were irradiated with X rays (195 kV, 18 mA). The dose rate was 4.6 Gy/min. Quenching data obtained for all the quenchers used in this study were analyzed by the Stern-Volmer equation as well as by the modified Stern-Volmer equation. Fluorescence measurements were performed for native and 6 mol/dm^3 Gdn-HCl denatured proteins.

GAPDH was more sensitive to radiation inactivation (G_{inact} = 0.036 μmol/J) compared to LDH (G_{inact} = 0.004 μmol/J). Greater inactivation of GAPDH is associated with increased susceptibility of GAPDH to the conformational change in molecules determined from a value of the Stern-Volmer quenching constant (KSV). Conspicuous changes were observed mainly in the hydrophobic interior of the molecules, evidencing radiation-induced changes of the polarity of the microenvironment of internal protein Trp residues.

80. Induction of apoptosis by aclarubicin and doxorubicin in non-small lung cancer and liver cancer cells

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Aclarubicin (ACL) possesses many properties that distinguish it from other anthracycline antibiotics, e.g. in the structure of aglycone and in the trisaccharide moiety, consisting of rhodosamine, 2-deoxyfucose and cinerulose A. Thus, our study aimed at comparing some of the mechanisms of activity of ACL and DOX in HepG2 (hepatoma) and A549 (non-small lung) human cancer cell lines. We applied fluorescence microscopy and flow cytometry methods to analyze and evaluate: changes in cell morphology after cell staining with fluorescence dyes ethidium bromide and acridine orange, cell cycle distribution and percentage of apoptotic cells. Drug cytotoxicity was measured by NRU (neutral red uptake) and MTT microplate assays.

Our results showed that investigated cell lines were significantly more sensitive to ACL than to DOX. We identified numerous changes typical for apoptosis, but also for necrosis: alterations in the structure, size and shape of cell nucleus, profound chromatin condensation, cell shrinkage and nuclear fragmentation, formation of apoptotic bodies, impairment of the plasma membrane and cell disintegration.

In control cells a small number of cells (about 2%) with spontaneous DNA degradation were identified in the sub-G1 peak of DNA. In drug-treated cells a progressive increase in the percentage of sub-G1 DNA, dependent on the cell line, the drug used and the time of incubation was detected. DOX generated a block in the G2/M phase of the cell cycle, while ACL induced a block in the G1 phase. Pretreatment with verapamil increased both apoptotic and necrotic cell populations, but only in the case of DOX treatment. Summarizing, we have shown that both drugs can induce apoptosis and necrosis in cancer cells. ACL generated faster and more intensive apoptotic changes than DOX.

81. Influence of the environmental pollution on the redistribution of light energy absorbed by needles of Scots pine

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For the past few years we have been carrying on the research on the influence of the degree of the environmental pollution on plants. So far, we have used in our research the photoacoustic spectroscopy (PAS) – one of techniques of photothermal spectroscopy. It enables us to perform a simultaneous measurement of a num-

ber of the parameters which represent the physiological state of a plant (oxygen evolution, yield of photosynthesis etc.).

In the presented work an attempt was made to obtain the energy balance of pigments participating in the photosynthesis process by means of PAS and fluorescence techniques. For the examination we have used samples of *Pinus silvestris* needles, which were collected from sites differing in the degree of environmental pollution.

Registering the PAS spectra, at the beam intensity of approximately 25 μmol photons $m^{-1}s^{-1}$, we obtain spectra whose shapes and amplitudes are determined by the amount of the energy deactivated by pigments in a form of heat during the photosynthesis process. When applying additional strong nonmodulated light the obtained PAS spectrum corresponds in its shape to the absorption spectrum and its amplitude reflects the total energy absorbed by individual pigments. That results from the fact that any other channels of the energy flow are being closed by additional illumination. The fluorescence activation spectra provide supplementary data for the PAS measurements. Together with the measurements, the spectra enable the comprehensive tracking of energy flows among particular pigments during the photosynthesis light phase. The obtained results for the Scots pine needles demonstrate mainly the predominant influence of seasonal environmental changes and little impact of the level of the environmental pollution.

82. Micellization study of new synthesized sugar surfactants

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Sugar-based surfactants are of considerable research interest because they have improved surface and performance properties, reduced environmental impact, and have potential pharmaceutical and biomedical applications. These surfactants are made from renewable resources using the "green chemistry" methods, are easily biodegradable and increasingly used in washing agents, cosmetics, and drug carriers. Especially interesting are dicephalic surfactants which are composed of one hydrophobic chain and two hydrophilic groups. New synthesized dicephalic saccharide-derived surfactants (N-dodecyl-N,N-bis[(3-D-gluconylamido)propyl] amine [C_{12}DGA] and N-dodecyl-N,N-bis[(3-D-lactobionylamido)propyl]amine [C_{12}DLA] and their analogical compounds with one head group N-alkanoyl-N-methyllactitolamines [C_nMeLA] as well as common sugar-based surfactants N-dodecyl-β-D-glucopyranoside [C_{12}G1] and decanoyl-N-methyl glucamide [MEGA-10] were studied**.** The micellization processes were studied by means of isothermal titration calorimetry (ITC). The critical micelle concentrations (cmc), the enthalpies (ΔH_m) and the entropies (ΔS_m) of micellization as well as the contributions of the headgroups to the Gibbs free energies (ΔG^0_m(hy)) were calculated. Molecular modeling methods were used to relate the molecular properties of the compounds with their experimentally studied properties in solution.

83. Circular dichroism spectroscopy and isothermal titration calorimetry studies of interaction between G3.5 PAMAM and G4 polyamidoamine succinamic (PAMAM-SAH) dendrimers with human serum albumin

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Polyamidoamine (PAMAM) dendrimers are a specific group of polymers. They are intensively investigated as drug or gene carriers, imaging factors, chelators of metal ions and factors against aggregation of amyloid peptides. If the dendrimers are to be used in pharmacology, it is very important to study how they can interact with biopolymers presents in organisms (proteins, lipids, nucleic acids).

In our study the interaction between G3.5 PAMAM dendrimers and G4 polyamidoamine succinamic (PAMAM-SAH) dendrimers with human serum albumin (HSA) was investigated. Two experimental techniques were used to investigate the dendrimer-protein interactions. Spectroscopy of circular dichroism (CD) permits detection of changes in the secondary structure of protein. Isothermal titration calorimetry (ITC) allows to estimate how many molecules of dendrimers can be bound per one molecule of albumin.

For isothermal titration calorimetry studies, 5 μM concentration of HSA and 500 μM concentrations of G3.5 PAMAM and G4 PAMAM-SAH were used. For CD experiments 0.25 μM albumin and dendrimers at concentrations of 3, 4 and 5 μM (albumin/dendrimer molar ratio of 1:12, 1:16 and 1:20, respectively) were used. The results of our experiments clearly show that both kinds of dendrimers possess the ability to interact with molecules of human serum albumin. ITC demonstrates that approximately 3 particles of PAMAM G3.5 and 6 particles of PAMAM-SAH dendrimers can bind to a single HSA molecule. CD spectra show that both dendrimers change the secondary structure of albumin.

The main conclusion from our is that both dendrimers can interact with albumin and change its structure. PAMAM-SAH interacts with human serum albumin more strongly than PAMAM G3.5.

84. Simple models for the gene auto-repression and auto-induction

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I consider the two simplest schemes of regulation of the gene transcription by the protein encoded by the same gene. The schemes consist of a negative (auto-repression) or a positive (auto-induction) feedback loops, which are as short as possible. Ribonucleic acid mRNA is synthesized as a result of the gene transcription. Next, this mRNA is translated and a protein is produced. The binding of an aggregate of a few (n) molecules of the protein to the gene operator blocks (repression) or makes possible (induction) the gene transcription. In the case of auto-repression the system has one stable point of equilibrium corresponding to some nonzero values of the dynamical variables (protein and mRNA concentrations). The cell keeps the concentration of the regulatory protein constant. In the case of auto-induction the system has a more diversified phase portrait. With $n = 1$ the system has an equilibrium in the origin of coordinates (0,0). For some values of parameters this equilibrium is sole and stable. At some parameters values, an additional, nonzero, stable equilibrium point appears in the phase plane. At the same time the equilibrium point in the origin loses its stability. If $n > 1$, there is also a range of parameter values with the sole and stable equilibrium (0,0). However, in this case, a saddle-node bifurcation can take place in the system. Apart from (0,0), two additional equilibrium points appear, a stable node and saddle-point. The equilibrium (0,0) remains stable and the system becomes a bistable trigger. So short feedback loops can be realized in bacterial but not in eukaryotic cells.

85. Formation of AGEs in the presence of PAMAM dendrimers

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Incubation of proteins with glucose leads to their non-enzymatic glycosylation and formation of both "early" and advanced glycation end products (AGEs). This process is increased in diabetes mellitus due to hyperglycemia and leads to several complications such as blindness, heart disease, nerve damage and kidney failure.

Poly(amido)amine (PAMAM) dendrimers are a relatively new class of polymers with unique molecular structure predisposing them for to use as anti-glycation agents. The amine-terminated PAMAM dendrimers are known to easily react with other molecules, including glucose.

Based on the above-mentioned facts we aimed in the present study to verify whether: (a) PAMAM G2, G3 and G4 are effective in glucose scavenging and protection of a model protein (BSA) against non-enzymatic glycosylation and b) higher generation of PAMAM appears more effective as glycation inhibitors.

The ability of PAMAM dendrimers: G2 (12 and 120 μM), G3 (6 and 60 μM) and G4 (3 and 30 μM) to inhibit the modification of protein (BSA, 35 μM) by high glucose (500 mM) at 37°C, 72 h or 55°C, 48 h was investigated using a spectrofluorimetric assay.

The formation of AGEs was significantly increased in the systems tested by all dendrimer generations used at higher concentrations. Comparing two different incubations, we revealed that the incubation at 55°C (48 h) contributed to production of more AGEs. At low concentrations of PAMAMs, as used in the present study, dendrimers had no impact on AGEs level except the PAMAM G4 (3 μM) used at 37°C (72 h).

Our findings do not stand along with our hypothesis and theoretical considerations. The data clearly showed that PAMAM dendrimers regardless of the generation used and concentration were not able to inhibit the non-enzymatic protein glycosylation in *in vitro* studies.

86. Effect of non-modified and surface-modified nanodiamond powders on the viability and ROS production by human endothelial cells

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The use of Nanocrystalline Diamond Coatings (NCD) in medicine is increasing due to the high biocompatibility of NCD. NCD are produced by different methods: by MW/RF1 (Microwave/Radio Frequency Plasma Activated Chemical Vapour Deposition method), RF1 (Radio Frequency Plasma Activated Chemical Vapour Depositiona method), UDD (detonation method) and others. NCD are one of the best materials which can be used in medicine onto surgery tools and medical implants. The purpose of our study was to investigate the effect of diamond powders on the viability and production of reactive oxygen and nitrogen species (ROS/RNS) of immortalized human umbilical vein endothelial cells (HUVEC-ST). The cells were incubated with 2–100 μg/ml diamond nanopowders. Non-modified diamond nanopowder (< 10 nm particle size, SIGMA) and surface-modified nanodiamond (hydroxyl groups introduced by the Fenton reaction) were compared. The influence of the particles on the proliferation of HUVES-ST was determined by the MTT reduction assay. The highest cytotoxicity was found for modified nanopowder. The nanopowders enhanced cellular production of ROS

estimated by oxidation of 2',7'-dichlorodihydrofluorescein diacetate (H_2DCF-DA) and production of RNS estimated with the fluorogenic probe DAF-FM. The effects were most pronounced for the modified nanodiamond powder.

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87. Arthropod toxins modulating sodium channel function as insecticides

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Scorpion and spider venom contains toxins active on sodium channels. Some of them are highly selective for insects. They are considered to be potential bio-insecticides. Genes of such toxins can be introduced into the body of an insect using an insect pathogen, baculovirus. Co-application of two sodium channel modulators appears sometimes to be especially efficient both on the whole-body level and on a single channel level. A positive allosteric interaction between receptor sites on the sodium channel is one of the factor of such synergism. In our studies a new toxin (LqhTEE), of molecular mass of about 7 kDa has been isolated from *Leiurus quinquestriatus* scorpion venom. Preliminary results indicate that it shows some characteristics of beta toxins. Moreover, it modulates spontaneous activity of neurosecretory insect DUM (dorsal unpaired median) neurons. However it is not active on isolated insect axon. Determination of receptor for LqhTEE is in progress. Its interaction with classical pyrethroid insecticides is under studies using toxicity tests and electrophysiological methods.

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88. Oxidative stress in erythrocytes from healthy people subjected to cryotherapy

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Cryotherapy is the use of cryonic temperatures (from –100°C to –160°C) for triggering off and using organism physiological reactions to cold. It includes 8–10 treatments, one daily. Patients spend 3 minutes in a cryogenic chamber and then they are subjected to intensive kinesitheraphy. That kind of treatment is used in combination with pharmacological therapy, biologic revival and rehabilitation.

Blood cells are exposed to high oxygen concentration constantly. They are rich in polyunsaturated fatty acids so they are susceptible to peroxidation reactions. Products of lipid peroxidation modify physical properties of cells membranes. They can change membrane structures, lower the hydrophobicity of membrane interior and inhibit membrane enzyme and transport protein activities. In this study we determined the effect of cryotherapy on the activities of the antioxidant enzymes: catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD), on the total antioxidant capacity (TAC) and lipid peroxidation (TBARs level) in red blood cells of volunteers subjected to cryotherapy.

Changes in enzyme activities and TBARs level were measured in 20 healthy people. Blood samples were taken before the first cryotherapy treatment and after 8 days of treatment. ACD was used as an anticoagulant. All experiments were carried out the next day after taking the blood.

GPx activity was measured by the Rice-Evans's method (1991), the SOD activity was measured by the adrenaline test (Misra, 1985), the direct spectrophotometric method (Bartosz, 2003) was used for determination of catalase activity. Statistical significant increases in the activities of antioxidant enzymes (CAT, by about 18%, and SOD, by about 20%) were observed.

To determine the extent of lipid peroxidation, the Stock and Dormandy's method (1971) was used. There were no changes in TBARs level.

TAC was measured by Re's method with Bartosz's modification (1999). There were no changes in plasma total antioxidant capacity.

89. The filtration coefficients L_{pr} of isolated roots: a mechanistic description

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The present article has used for the description of results of measurements of filtration coefficients L_{pr} of isolated roots, obtained in the 1980s and 1990s by Steudle and others, with the use of the root pressure probe [1]. The objective was to explain the differences of values of the maize root coefficients L_{pr} depending on whether they were determined through "hydrostatic" or "osmotic" experiments. Detailed investigations have been based on the mechanistic equations of solute and solvent membrane transport across porous membranes [2] which are mutually compatible with Kedem-Katchalsky (thermodynamic) equations, and make allowances for the microscopic structure of the root membrane.

In the present paper, it has been assumed that the root – in the course of water uptake from the environment – acts as an osmometer with a porous membrane. Basing on this model, water root transport has been analyzed in both types of the above-mentioned experiments conducted with the use of the root pressure probe. It has been found that the coefficient L_{pr}^{hydro}, as measured in the hydrostatic experiments expresses the total hydraulic capacity of the root membrane and it is identical with the resultant root filtration coefficient L_{pr}. The coefficient L_{pr}^{osmo} in turn, as measured in the osmotic experiments, does not formulate the total volume of the water which flows through the membrane per unit of time and is equal to the coefficient L_{pr} only when the membrane is semi-permeable (i.e. when the coefficient σ_r of the membrane equals 1).

To conclude, according to the mechanistic approach, the total root filtration coefficient (L_{pr}) is determined in hydrostatic experiments, and not in osmotic ones.

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90. Cross coefficients in the Kedem-Katchalsky equations

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According to non-equilibrium thermodynamics (NET), stationary processes in a thermodynamic system close to a state of equilibrium are linearly dependent on all thermodynamic forces in the system. Proportionality coefficients in the equations linking processes and forces are subject to certain principles. In particular, the so-called cross coefficients of these equations fulfill the Onsager's principle.

Equations of this type are represented by the Kedem-Katchalsky (KK) phenomenological equations for membrane transport. However, Onsager's principle is not fulfilled by proportionality coefficients (L_p, σ, ω) in the so-called "practical" (i.e. experimentally convenient) KK equations, developed without observing certain NET principles.

In this paper, the phenomenological and practical KK equations have been obtained anew, in full agreement with the NET formalism. The cross coefficients of the new equations also fulfill the Onsager's principle. These equations should be treated as a complement to the KK formalism. They have been found to be not very clear in terms of interpretation, while their mechanistic interpretation, obtained on the basis of a porous membrane model, remains transparent. A membrane of that kind contains only pores which are either semi-permeable or completely permeable to the solute, and its total reflection coefficient σ ($0 < \sigma < 1$) depends on the relative quantity of pores of both types [1]. The mechanistic equations which describe that model are mathematically similar to the equations which we have obtained in the present paper. A comparison of proportionality coefficients in the mechanistic equations with corresponding coefficients of thermodynamic equations gives a clear physical interpretation to the latter. There exist correspondences between the coefficients of the KK equations in their "Onsager" and original forms, and the coefficients of the mechanistic equations. It has experimental significance since various equations determine the method of measurement of transport coefficients for various experimental conditions.

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91. The comparison of toxic effect of bromfenvinphos and chlorfenvinphos on human erythrocytes

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Bromfenvinphos – (*E,Z*)-*O,O*-diethyl-*O*-[1-(2,4-dichlorophenyl)-2-bromovinyl] phosphate is the insecticide elaborated in Poland, which was used against *Varroa destructor* causing honey bees disease called as varroosis. It was used for 4 years (1999–2003) under the brand name of Apifos. Bromfenvinphos was successfully used by majority of beekeepers to treat honey bees. It was easy to use and effective. It was removed from the market due to changes in legislation (lack an MRL – maximal residue limit). Furthermore, due to UE directive 91/414/EWG, registration of bromfenviphos as an insecticide requires full identification and toxicological evaluation for all its impurities present in commercial product, which content is above 0.1%.

Nowadays, many toxicological research is conducted in order to estimate the toxicity of bromfenvinphos and its impurities, which should finally allow to restart Apifos production.

This work describes the interaction of bromfenvinphos and its impurities (dihydro-bromfenvinphos, dibromo-bromfenvinphos, 2,4-dichlorophenacyl bromide; 2,4-dichlorophenacylidene bromide and 2,4-dichlorophenacylidyne bromide) with human erythrocytes as model cells. The effect of the above mentioned compounds on changes in acetylocholinesterase activity, size and shape of erythrocytes and changes in level of ROS was examined.

Bromfenvinphos and its impurities were added to the erythrocytes to obtain the final concentrations of

0.5, 5, 50, and 250 μM. The erythrocytes of 5% hematocrit were incubated at 37°C with the compounds studied for 1 h.

This work describes the interaction of low concentrations (0.5–50 μM) of the xenobiotics examined with human erythrocytes. We also investigated the concentrations, which may be present in human organism only as a result of acute toxicological poisoning (250 μM).

It was proved that chlorfenvinphos and 2,4-dichlorophenacyl bromide induced statistically significant changes in the parameters analyzed. It was also proved that bromfenvinphos was less toxic than chlorfenvinphos to human erythrocytes.

92. Changes in viability, morphological and apoptotic parameters of human peripheral blood lymphocytes exposed to bromfenvinphos and its impurities

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The varroosis is one of the most dangerous of honey bees diseases affecting honey bees *Apis cerana* and *Apis mellifera* almost all over the world with the exception of Australia. Varroosis is caused by *Varroa destructor*, an external parasitic mite, which attacks honey bees. Bee colony attacked by *Varroa destructor* cannot survive without human help and usually dies after 2–3 years. The most effective way to fight with that mite is the usage of chemicals, among which, one of the most effective was bromfenvinphos. Bromfenvinphos was registered in 1999 by the name of Apifos. In 2003 it was removed from the market due to changes in legislation. Now, registration of bromfenvinphos as an insecticide requires full identification of its impurities, which amounts are above 0.1% and then evaluation their toxicity.

This research was conducted on human lymphocytes, which were treated with bromfenvinphos and its impurities, i.e. dihydrobromfenvinphos, dibromobromfenvinphos, 2,4-dichlorophenacyl bromide; 2,4-dichlorophenacylidene bromide and 2,4-dichlorophenacylidyne bromide to analyze the induction of apoptosis, viability as well as their size and granularity. Moreover, the effect of chlorfenvinphos on the above mentioned parameters was assessed to compare its toxicity with the toxicity of bromfenvinphos.

All analyses were performed *in vitro*. The compounds were added to the lymphocyte suspensions to obtain the final concentrations of 0.5, 5, 50, and 250 μM.

The strongest toxic effect on the cells examined was observed for three bromefenvinphos impurities, i.e. dihydrobromfenvinphos, 2,4-dichlorophenacyl bromide and 2,4-dichlorophenacylidene bromide.

93. Biological effects of irradiation of rat blood vessel cells with 308 nm pulses of Xe-Cl excimer laser: Design of experiment and first results

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Ultraviolet laser pulses have been used in the ever-growing list of vascular therapies, especially in laser debulking of occlusions and revascularization. Despite successful clinical applications, up to now little is known about biological mechanisms and processes of therapeutic action of the laser UV pulses and of possible long-term negative side effects of procedures involving such a treatment.

The new experimental system designed and set up in our laboratory allows irradiating *in vivo* rat femoral arteries with pulses of 308 nm line of Xe-Cl laser, most commonly used in laser atherectomy procedures. At a first stage of the experiments, both the blood vessels and their surrounding tissues irradiated with a series of UV doses are examined for the presence and frequency of cellular DNA breaks using histochemical TUNEL (*Terminal* Transferase *dUTP Nick End Labeling*) technique. We hope that new information on both the frequency and spatial distribution of the UV damaged cells may provide clues as to biological background of clinically beneficial effects of laser-based vascular therapies.

94. ATP concentration changes in thermotolerant erythrocytes

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Hyperthermia is been used in the treatment of cancer in combination with radio- or chemotherapy. Thermal shock is a factor inducing development of thermal tolerance. These phenomena are responsible for tumor resistance to therapy. Two targets of heat interaction with cells are proposed – the nucleus and the cell membrane. Erythrocytes were used as a model in studies of membrane alterations caused by heat.

The human erythrocytes as enucleated cells are a convenient model to study the effect of heat on the plasma membrane. Our earlier experiments showed that erythrocytes are resistant to the second heat shock during fractionated hyperthermia. This conclusion was confirmed by studies of autohemolysis, osmotic fragility, internal microviscosity of cells, process of vesiculation and membrane ATPase activity.

In the present study, erythrocytes were suspended at 2% hematocrit in the incubation buffer.

The samples were incubated in following way:

A – at 37°C for 3 hours;
B – at 44°C for 15 min and then at 37°C for 3 hours;
C – at 44°C for 15 min, then at 37°C for 3 hours and at 48,5°C for 30 min;
D – at 37°C for 3 hours and at 48,5°C for 30 min.

In each experiment erythrocytes samples A, B, C, D were taken from the same individual. ATP concentration was measured in thermotolerant erythrocytes and in erythrocytes incubated at 37°C for 15 min; 1, 3, 5 hours after 44°C preincubation.

Increased ATP concentration was observed after 15 minutes of incubation at 37°C. Longer incubation at 37°C caused a decrease of ATP concentration. Lowest value of ATP concentration was observed after 3 hours of incubation at 37°C. Thermotolerance calculated from ATP concentration after 3 hours was also observed. These results show that development of thermotolerance in erythrocytes is correlated with cell energy metabolism.

95. Melatonin level and the antioxidative enzymes activities in the blood of coronary catheterization patients

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Myocardial ischaemia is one of the most common causes of mortality in highly developed modern countries. The main process underlying heart ischaemic disease is coronary artery disease (CAD), as a result of the accumulation of atheromatous plaques within the coronary artery walls. The intensified oxidative stress is supposed to play an important role in the pathogenesis of atherosclerosis of the coronary arteries. The aim of the study was to estimate the melatonin level and some antioxidative enzymes activities in the blood of patients undergoing diagnostic catheterization to detect CAD.

50 patients (aged 52–74 y; 23 males and 27 females) of Clinic of Cardiology of Military Hospital in Bydgoszcz participated in the experiment. Since these patients had the symptoms of heart ischaemic disease, they were recommended to have coronary catheterization. According to the obtained coronary angiograms, the patients were divided into two groups: one without any artery stenosis (30 persons) and the other with at least one artery stenosis (20 persons). The blood samples were taken from the cubital vein after overnight fasting before the catheterization procedure. The melatonin levels were measured in the blood serum, whereas the activities of Zn, Cu-superoxide dismutase (SOD-1), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione reductase (GR) were marked in the red blood cells.

The melatonin levels in serum were significantly decreased in the group of patients with CAD. The erythrocyte activities of CAT and GSH-Px were considerably increased in CAD patients. There were no differences in the activities of SOD-1 and GR in both examined groups.

The obtained results indicate the changes in the antioxidant defense in the blood of CAD patients. These changes may be due to the intensified oxidative stress in this group of patients. The decreased concentrations of melatonin in the blood of patients with at least one coronary artery stenosis point to the possible role of this antioxidant in the prevention of the CAD development. This finding may support the use of melatonin in prevention and therapy of heart ischaemic disease.

96. Application of photoacoustic spectroscopy in ecology

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Ecology is the study of the interactions between life and its physical environment. In the present paper we describe the application of photoacoustic spectroscopy (one of the photothermal spectroscopy versions where a quantity of energy deactivated into heat is evaluated) to quantify the environmental pollution effect on different plants. When a sample is exposed to modulated light, a part of the absorbed light energy is emitted in the form of modulated heat, resulting from thermal deactivation of pigments. The rest of the energy is predominantly dissipated in photochemical processes leading to modulated O_2 emission.

For the plants selected as bioindicators (green algae – *Scenedesmus armatus*, dandelion – *Taraxacum officinale*, Scots pine – *Pinus silvestris*) we describe the possibility of application of the photoacoustic spectroscopy to quantify a few key physiological parameters; the efficiency of photosynthetic oxygen evolution, photochemical energy storage, time of photothermal signal creation in a photoacoustic cell (heterogeneity of PS II), the coefficient of oxygen diffusion through the cell wall, the sample depth profiling, the light-saturation curve and kinetics nonphotochemical quenching.

97. Accumulation and cellular distribution of doxorubicin – transferrin conjugate in peripheral blood lymphocytes

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Doxorubicin (DOX) is one of the most potent anti tumor drugs with a broad spectrum of use. Unfortunately, numerous side effects such as severe cardiotoxicity and bone marrow suppression limit its use. To reduce this obstacle and improve DOX pharmacokinetics, we conjugated DOX to the transferrin, a popular human plasma protein. We observed the overexpression of transferrin receptors in cancer cells because they have huge demand for iron ions. Due to lack of transferrin receptors on the surface of peripheral blood lymphocytes we compared the interaction of doxorubicin – transferrin conjugate and free drug cells with normal cells.

The *in vitro* growth-inhibition test, XTT assay, indicated that DOX was significantly more cytotoxic to normal cells then DOX-TRF. The estimation of intracellular DOX-TRF level in human lymphocytes has confirmed a greater accumulation of DOX than of the doxorubicin-transferrin conjugate. Moreover, the microscopic observation of lymphocytes during drug treatment indicated a different localization of DOX and DOX-TRF conjugate inside the cells. We conclude that peripheral blood lymphocytes are less damaged by doxorubicin-transferrin conjugate than by DOX alone.

98. Induction of apoptosis by doxorubicin – transferrin conjugate in human erythroleukemia cells

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Leukemia, the most common malignancy associated with the hematopoietic system, is moderately sensitive to several cytotoxic agents. It has been demonstrated that development of multidrug resistance to the anthracycline drugs, doxorubicin and daunorubicin limits the success of chemotherapy in this disease. In our study, to decrease the resistance of cells to anthracycline, we used a doxorubicin – transferrin conjugate.

The aim of this work was to estimate the effect of doxorubicin – transferrin conjugate on the induction of apoptosis in K562 human leukemia cells. The sensitivity of cells to DOX-TRF and DOX alone was measured by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT assay). Apoptosis was detected by using DNA ladder assay and by measuring the activation of caspase-3. Moreover, morphological changes of nuclei connected with apoptosis were analyzed by acridine orange/ethidium bromide double staining. The results demonstrate that DOX-TRF is more cytotoxic to K562 cells than free doxorubicin. Morphological and biochemical cell changes were dependent on both the drug concentration and the time of incubation.

99. May the near-infrared radiation (NIR) destabilize the DNA molecule?

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The structure of bound water determines the conformation of biological macromolecules and controls their metabolic activity. In the further layer such molecules are surrounded by bulk water. The structure of water which surrounds the molecule can be changed by magnetic field, pressure, temperature, presence of ions or organic solvents [1, 2]. Another efficient structural modification factor is the near infrared radiation (NIR) [3, 4].

DNA molecule has been investigated in order to clarify processes that take place under the influence of NIR. Such choice is motivated by the fact that the DNA hydration layer has a first-rate influence on this molecule stability. UV-VIS spectroscopy results obtained for aqueous DNA solutions show that melting process of the macromolecules has three stages. The stage sensitive to NIR is the dissociation of bases of the molecule. Studies have shown that all used doses of radiation destabilize the system [5]. Opposite results can be obtained after introduction of modifiers to the solution of DNA.

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100. Model equations of the membrane transport of non-electrolyte solutions with concentration polarization

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Polymer membranes both natural and artificial are very sensitive on changes of physical and chemical outside