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International Congress of YOUNG SCIENTISTS

Yerevan State Medical University | April 13-14, 2010

ABSTRACTS

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Dear friends and colleagues,

With great pleasure I welcome you to the Yerevan State Medical University. We are celebrating the 90th anniversary of our University foundation and within the frameworks of this event we launch the International Congress of Young Scientists. The mentioned Congress is of titanic importance in sense that students, clinical residents and young devotees to medical sciences are granted an exquisite and momentous opportunity to communicate, share experience being a lifelong one and obtain innovative knowledge fostering the enlargement of scientific horizons.

Being a higher educational institution with prominent faculty continuously providing outstanding education we do comprehend, such academic forums are to be embedded within the continuing educational spectrum. Fending for the nation could have high-quality medical care we are enrolled in training specialists surmounting the challenges of today's world. Towards this end, such congresses are organised, being constituent components and ground basis of the professional growth medical professional pursue. Within the Congress live discussions with regard to perilous diseases will be held outlining vital issues. Latest achievements in the field of biomedical sciences and other medical related fields will be highlighted.

We cordially welcome and deeply hope to meet you in Yerevan for celebrating the 90th anniversary of Yerevan State Medical University establishment.

Sincerely,
Professor Gohar P. Kyalyan
Rector of Yerevan State Medical University

SHIFTS IN CONTENTS OF TNF-α, IL-2 AND PROLACTIN IN BLOOD SERUM AND ORGANS OF IMMUNOGENESIS UNDER CONDITIONS OF LONG-TERM HYPOKINESIA

Abgaryan K.[1], Zalinyan S.[1], Poghosyan G.[1], Avagyan S.[2], Shekoyan V.[1]

[1] Department of Microbiology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

[2] Scientific-Research Center, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

Phone: 093-322366

Changes in TNF-α, IL-2 and prolactin content in blood serum and organs of immunogenesis under conditions of three-day hypokinesia revealed by our previous investigations, as well as those on transformation of their correlation dependence, definitely prove development of disorders of various parts of the immunoneuroendocrine system in early hypokinesia conditions. Results of those investigations in association with available data showed that development of significant morphofuncional and metabolic changes in lymphoid organs have become the main reason to study TNF-α, IL-2 and prolactin contents in blood serum, thymus, spleen and lymph nodes under conditions of 30-day hypokinesia.

Investigations were carried out in 30 non-linear male rats (150-170 *g*). The model of experimental hypokinesia was obtained placing the animal into special individual cages, limiting their mobility in all directions and maintaining food and water access. The next day after finishing hypokinesia the animals were immunized by intraperitoneal injection of 8% suspension of sheep erythrocytes. Animals were divided into two groups: Group I: animals exposed to 30-day hypokinesia, Group II: animals exposed to 30-day hypokinesia with subsequent immunization. The content of cytokines (TNF-α, IL-2) and prolactin in blood serum and supernatants obtained from the thymus, spleen, and lymph nodes was revealed by immune-enzyme assay (ELISA). Statistical processing of the revealed data was done with the help of computer programs STATISTICA 6. and GraphPad Prism 4.

Investigations showed that TNF-α content increases in blood serum of 30-day hypokinesia exposed animals on day 5 of immunization. It was simultaneously found out that in spleen and lymph nodes TNF-α decreases in the same group of animals. In thymus we saw only increasing tendency of cytokine, but this change was not statistically reliable.

It was revealed that at a 30-day hypokinesia the level of IL-2 grows in blood serum, in the spleen, and especially in the thymus and the lymph nodes.

Data obtained is rather interesting, because it is known that T and B-subpopulations of lymphocytes and NK-cells can be as the source of IL-2 synthesis. Similar changes were revealed in spleen of experimental group animals. It was shown that in contrast to blood serum and spleen, the content of IL-2 in lymph nodes of Group II was significantly higher than in animals of Group I.

Studies on IL-2 content in thymus showed lower level of cytokine in animals of Group II in contrast to that in Group I.

The results of experiments showed 4-6 fold increase of prolactin in biosubstrates on day 30 of hypoki-

nesia. It was revealed that in Group II of experimental animals prolactin level in blood serum is higher than in animals of Group I, while in the thymus, spleen and lymph nodes its level decreased. Literature data show development of hypoplastic processes in central and peripheral organs of immunogenesis on day 30 of hypokinesia.

It is essential that along with dystrophy and atrophy centers the hyperplastic processes are obviously visible, which do prove development of adaptive reactions even in such late period of hypokinesia. Accidental involution of the thymus and hypoplastic processes in peripheral organs of immunogenesis are connected / associated with high content of glucocorticoids.

The shifts of TNF-α and IL-2 content in organs of immunogenesis at the late stages of hypokinesia can be caused besides prolactin, by high concentration of glucocorticoids, particularly by cortisol, in blood serum of experimental animals, which are known to possess stimulating action on T-killer population of thymocytes.

CEREBROVASCULAR EFFECTS OF NOOPEPT UNDER THE CONDITIONS OF CEREBRAL BLOOD FLOW ISCHEMIC DAMAGES

Afrikyan Sh., Balasanyan M.

Department of Pharmacy, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: shushafrikyan@yahoo.com; **Phone:** (091) 307702

Recent studies of modern neuroprotective drugs testify that endogenous neuropeptides containing pyrrolidine-carbonic amino acids (proline, pyroglutamic acid) and glycine display significant neuroprotective and nootropic activity. In view of this, one of the most promising ways of searching compounds with such activity is to design biologically active short (bi- or three-) peptides, which include mentioned amino acid residues in their structure or to imitate their biologically active conformation. Creation of these peptides is reasonable due to significant activity of the given amino acids and their high specific bioaccessibility for brain tissue as well.

Among the mentioned compounds, Noopept (N-phenylacetyl-L-prolylglycine ethyl ether), a new drug on the basis of short peptides, which were developed at the State Research Institute of Pharmacology (Russian Academy of Medical Sciences), possesses marked antiamnesic, anxiolytic and mnemotropic activity and is used as the nootropic agent.

As it is known, some anxiolytics of the new generation (for example, Afobazole, Mexidole) display neuroprotective activity; therefore, it was interesting to study the influence of Noopept on microcirculation of the ischemic brain.

The aim of this study is to investigate the influence of Noopept on local cerebral blood flow (CBF) in the model of global ischemia.

The experiments were carried out in adult male inbred albino rats weighing 180-220 *g*. The animals were housed under standard conditions and deprived of food the night before experiment, with free access to water. Operations were performed under Nembutal anesthesia (50 *mg/kg. i.p.*). Ischemia was caused by the right carotid artery ligation. Local CBF registration was carried out by laser Doppler flow meter "Transonic systems. Inc." model BLF-21 (USA). Flow meter needled sensor was fixed on rat's cortex perietal region (on ligation side), in a small 1.5-3 *mm* diameter hole. Local CBF registration was performed both at normal and ischemic conditions and on the background of Noopept injection, as well. Noopept has been injected at the dose of 1 *mg/kg* (*i.p.*).

Data obtained have shown that right carotid artery ligation decreased local CBF about 19.7±4.9%, in comparison with the initial level. Local CBF increased 15 *min* after 1 *mg/kg* Noopept injection (*i.p.*), and reached its maximal value (15.7±3.24%) at 25 *min* of experiment. It is interesting, that local CBF remained higher than its initial value (after ligation) on the background of drug influence, until 60 *min* of experiment. Some correlation between the rate of local CBF falling and the cerebrovascular effects of Noopept were also registered.

These data demonstrate that Noopept increases local CBF under the conditions of ischemia, which indicates that one of the mechanisms of its neuroprotective properties is ability to improve blood supply of ischemic brain tissue

MOLECULAR CLONING OF HOMOLOGOUS AND HETEROLOGOUS ARGB GENES OF L-ARGININE BIOSYNTHESIS IN CORYNEFORM BACTERIA

Aganyants H.[1], Asmyan A.[1], Koloyan H.[2]

[1] Biomedical Department, Russian-Armenian (Slavonic) University, Yerevan, Armenia [2] "Scientific-Research Institute of Biotechnology" CJSC, Yerevan, Armenia

E-mail: hovrau@mail.ru

Molecular cloning of homologous argB *Corynebacterium glutamicum* gene as well as of heterologous argB *Bacillus stearothermophilus* gene on the shuttle expression vector pEC-XK99E has been performed. The constructed new recombinant plasmids in cells of the strain-producer *Brevibacterium flavum* HK-19A obtained by us ensures higher level of L-arginine synthesis.

In recent years, there has been an increased demand for amino acid arginine, which is widely used in pharmacology (it is component of many therapeutic drugs and antiviral agents), medicine, animal husbandry, and food industry.

The industrial production of L-arginine mainly based on microbiological processes of production and creation of high active strain-producers of arginine seems to be of importance today. It is advisable to

design these strains on the basis of coryneform bacteria, as they proved to be the most effective ones in production of amino acids. To improve coryneform strain- producers, recombinant DNA technology is widely used.

It is known that the key enzyme for biosynthesis of arginine in coryneform bacteria is N-acetylglutamatekinase encoded by argB gene, which is inhibited by arginine. In thermophilic bacterium *B. stearothermophilus* the inhibition of this enzyme by the end product, *arginine*, was not revealed.

The purpose of this work is molecular cloning of homologous and heterologous argB genes into coryneform bacteria to obtain high-active strain-producers of L-arginine.

The following methods were used in this study: isolation of genomic and plasmid DNA, amplification of genes with polymerase chain reaction (PCR), restriction and ligation, transformation, electroporation, fermentation, determination of concentration of the synthesized L-arginine by colorimetric method.

Homologous and heterologous argB genes were amplified by PCR. Chromosomal DNA of *C. glutamicum* ATCC 13032 and *B. stearothermophilus* NCIB822 strains served as matrix for the synthesis of corresponding argB genes. On the basis of the shuttle expression vector pEC-XK99E recombinant plasmids bearing genes of the afore-mentioned strains were constructed. By transformation of the constructed recombinant plasmids into *Escherichia coli* mutant strain, it was determined that they contained argB genes of *C. glutamicum* and *B. stearothermophilus*. The *Br. flavum* HK-19 – arginine-synthesizing recipient strain obtained by us through multi-stage selection was transformed by these recombinant plasmids via electroporation method. Recombinant plasmids were transformed by electroporation method (through a multi-stage selection) to arginine producing strain *Br. flavum* HK-19. The obtained transformants were tested for arginine-producing ability. It was shown that the recombinant strains have a higher level of biosynthetic activity as compared with recipient strains.

However, the highest arginine-producing ability was shown by recombinant strains bearing the heterological argB gene of arginine biosynthesis. Thus, the molecular cloning of homologous and heterologous argB genes of arginine biosynthesis was performed.

The constructed new recombinant plasmids in cells of the strain-producer *Br. flavum* HK-19A obtained by us ensure higher level of L-arginine synthesis.

The employed principle of attraction of heterologous genes encoding key enzymes of biosynthesis for overproduction of amino acids is promising in constructing producers of other amino acids.

NEW METHOD OF RESEARCH ON SCLERAL FLEXIBILITY

Aghayan L., Gevorgyan T., Bazinyan L.

Ophthalmologic Clinic, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: lilitaghayan@mail.ru; **Phone:** (+374 93) 910 990

Variation of intraocular pressure (IOP) is an important factor in clinical judgment of eye pathologies where the evaluation of scleral flexibility is not considered.

The purpose of present research is a comparative evaluation of scleral quantitative description with the use of traditional methods (Friedenwald J.S., 1937) and those modified by us.

Flexibility coefficient of sclera was identified with the following methods: Friedenwald's method using automatic tonographer TNTs-100 and our modified methods with the use of electronic transpalpebral automatic tonometer IGD-02 (identification of intraocular pressure by tonometer) and automatic tonometer TGDts-01 (identification of authentic intraocular pressure).

Fifty patients aged 10-60 years developing myopia (32 patients), hyperopia (10 patients), mixed astigmatism (3 patients), and open-angled primary glaucoma (5 patients) were enrolled in the research.

The examination was made twice with the interval of 20 minutes.

As a result of research by a traditional method the acquired coefficient of scleral flexibility was not modified (0.02 – 0.3) thus signifying that it remained on a level higher than the norm. At the same time, scleral flexibility coefficient identified with transpalpebral electronic tonometer (our modified method) had different values considering different nosologic groups.

Relatively low scleral flexibility increased in parallel to the increase of myopia degree in the group of patients developing myopia and low degree of myopia. The case is more remarkable in patients developing glaucoma. Values of scleral flexibility coefficient had been lower than the norm if identified with a traditional method, while coefficients acquired as a result of applying our modified method of research had precisely defined low values. In patients with refractogenetic transfer from hyperopia to myopia, the decrease of scleral flexibility was detected.

The proposed and approbated modified method of evaluating scleral flexibility coefficient is more informative from the point of clinical judgment considering the state of exterior eye membrane of patients developing various eye pathologies.

THE EFFECT OF PROTEIN KINASE *C* ACTIVATION AND INHIBITION ON ANALGESIC ACTIVITY OF OPIOIDS DURING DIABETIC NEUROPATHY

Alaverdyan H., Vardanyan G., Gevorgyan N.
Department of Biochemistry, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia
E-mail: fmharut@yahoo.com; **Phone:** +(374)94-130030

Currently, the factors recognized in the pathogenesis of diabetic neuropathy are metabolic changes invoking a number of pathomechanisms, such as the oxidative stress, aldose reductase hyperactivity, protein kinase C (PKC) activation, advanced glycosylation, etc.,

There is no consensus in concern of a pathomechanism responsible for irreversible changes in nociceptive system. Another challenging problem is the effective treatment of pain syndrome during diabetic neuropathy, because there is no effective treatment for diabetic peripheral neuropathy and standard pain-reducing agents have reduced the efficiency. For example, during diabetic neuropathy the analgetic activity of opioids is weakened.

The possible role of protein kinase C in development of tolerance to opioids during diabetic neuropathy was investigated. The changes in analgesic activity of opioids during diabetes were studied after inhibition and activation of protein kinase C.

All experiments were conducted in non-linear male albino rats aged 3-5 months. Diabetes was induced by single intraperitoneal injection of streptozotocin at dosage of 55 *mg/kg*. Pain intensity was assessed using formalin test by means of semiautomatic ranking of pain behavior. It was preceded by subcutaneous intraplantar injection of formalin (4% 50 *mcL*) into the hind paw.

The calculations were made by registration of rating scores for each second and calculation sum of 180 seconds intervals. The resulted plot had a 2-peak graph structure, the first peak was describing the acute or phasic pain (first phase), the second peak lasting more than 30 minutes referred to tonic or chronic pain (second phase).

All experimental rats, both healthy and diabetic, were grouped into the following series:

1. rats with sham preinjection, preliminary injection
2. rats with preinjection of phorbol myristate-acetate ester (PMA, activator of PKC),
3. rats with preinjection of DAMGO (opioid agonist),
4. rats with preinjection of PMA and DAMGO,
5. rats treated by chelerythrine hydrochloride (inhibitor of PKC),
6. rats treated by chelerythrine hydrochloride and preinjected with DAMGO.

Duration of the first phase was increased during diabetes compared with the norm, but as an effect of DAMGO local administration the first phase was shortened among healthy (66%) and diabetic (50%)

rats. Among the healthy animals, the PMA preinjection had the reverse effect – the first phase duration was elongated from 9 minutes until 15 minutes, but not among diabetic subjects. Interestingly, the combination of DAMGO with phorbol ester resulted in reduced analgetic activity among diabetic rats, but preserved the analgetic activity among healthy rats. The second phase of formalin test was a slightly shortened during diabetes (36 minutes for healthy, 30 minutes for diabetic rats). DAMGO reduced duration of the second phase among healthy rats (17% reduction), but the second phase of diabetic rats was unchanged. Phorbol ester increased the second phase duration for 50% in healthy group and such an increase was not reduced in case of combination of PMA with DAMGO. In diabetic group, the second phase was again observed in both cases. Chelerythrine hydrochloride injection reduced a little the first phase, but the second phase and a pause were unaffected in diabetic rats. On the other hand, chelerythrine hydrochloride restored the analgesic activity of DAMGO.

According to revealed data, the activated protein kinase C in an organism of healthy animals might cause sensitization to noxious stimuli and tolerance to opioids, but these effects are somehow reduced in diabetic animals. One of possible explanations for the latter is an overactivated status of protein kinase C during diabetes. Although opioids have reduced analgetic activity in case of diabetes, the administration of protein kinase C inhibitor may restore analgesic activity of opioids.

Thus, during diabetic neuropathy the analgetic activity of opioids can be increased by simultaneous administration of protein kinase C inhibitors.

Our results demonstrated the probable role of protein kinase C in development of opioid tolerance during diabetic neuropathy. The important clinical implication of such a conclusion might be co-administration of opioids with protein kinase C inhibitors aimed to increase the analgesic activity of opioids during diabetic neuropathy.

THE PECULIARITIES OF *ESCHERICHIA COLI* AND *MYCOPLASMA ARTHRITIDIS* PERSISTENCE IN INTERNAL ORGANS OF EXPERIMENTAL ANIMALS EXPOSED TO X-RAY RADIATION

Alekyan N.

Scientific-Research Center Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: alek_nar@mail.ru; **Phone:** 077 30 80 23

Many aspects related to impacts of X-ray different doses towards biological objects are studied in details at present. However, the role of resident conditionally pathogenic microorganisms in alterative reactions in internal organs of mammals exposed to X-ray radiation is unsettled until nowadays. The expediency to perform relevant studies is conditioned by the following circumstance: under rather prolonged persistence of resident Gram-negative microorganisms in new econiches of the host dystrophic

and immunopathological reactions are triggered *in situ* and leave its imprint on the entire course of pathology processes.

The aim of research was to reveal the peculiarities of *Escherichia coli* (*E. coli*) and *Mycoplasma arthritidis* persistence in internal organs of experimental animals exposed to X-ray radiation.

Experimental animals (50 white male rats) weighting 150-180 *g* were exposed to single X-ray irradiation at 350 R during 3 *min*. Animals were sacrificed on days 3, 7, 20, after irradiation. The samples of internal organs were sectioned by cryostat and subjected to immunomorphological analysis to reveal endotoxin of *E. coli* and *Mycoplasma arthritidis*.

Due to immunomorphological analysis at the certain stage of the course of pathology process since day 20 of the experiment structural changes in internal organs (liver, pancreas, kidneys, lungs) were rather distinctly revealed, thus signifying to emergence of processes of bacterial translocation in experimental animals. In particular, endotoxin of *E. coli* in liver was found on the surface of single hepatocytes located immediately around the central veins in hepatic lobules, as well as on the surface of epithelium of proximal and distal convoluted tubules of nephron, acinar cells of pancreas, epitheliocytes of bronchopulmonary system, endotheliocytes. In immunofluorescent reaction for *Micoplasma arthritidis,* the persistence of abovementioned m*icoplasmas* was revealed exceptionally in lungs and pancreas. However, though under *E. coli* persistence endotoxin was predominantly characterized by extracellular localization, we managed to record micoplasmic antigen both intracellularly: in cytoplasm of epithelial cells of bronchioles and alveoles and extra- and intrasectoral system of the pancreas.

Peculiarity of persistence was studied in organs of immunity as well.

Thus, the new findings allow us to suppose/envisage the direct role of above-mentioned microorganisms in dystrophic processes induction, which were described in internal organs of laboratory animals exposed to X-ray radiation.

POTENTIAL THERAPY FOR ATHEROSCLEROSIS

Ali A., Grinevich E., Mansharipova A.

Coronary Heart Disease Department, Scientific Research Institute of Cardiology and Internal Diseases, Almaty, Kazakhstan

E-mail: grinkath@mail.ru

Endotheliotoxic hyperlipidemia provides impaired eNOS-derived NO production excessive free radicals, cytokines and adhesive molecules generation, vascular lipid accumulation, atherosclerosis development and progression. NO replacement therapy by NO donors could restore NO deficits. High density lipoproteins have anti atherogenic properties and are the carriers of excessive cellular cholesterol through the reverse cholesterol transport pathway. Plasma lipoproteins enrichment by anionic phos-

phatidylinositol (PI) increases the negative surface potential of all lipoproteins and stimulates reverse cholesterol transport pathway by high density lipoproteins. Moreover, incorporation of PI in lipoproteins stimulates a rapid flux and clearance of cholesterol from plasma compartment as a result of increased cholesterol uptake.

The investigation of morphological changes in atherosclerotic lesions of aorta after therapy with experimental combination of NO donor and PI was the aim of this study.

Thirty male *Chinchilla* rabbits (age 6 months, average weight 3,500 g) were fed with 2% cholesterol-enriched diet for 6 months. Five rabbits with regular diet served as control. After 6 months of feeding, 30 rabbits were subdivided into 3 groups of 10 animals each with continued feeding of cholesterol-enriched diet. Particularly, the groups were as follows:

- Group 1: without treatment.
- Group 2: treated with conventional oral isosorbide dinitrate (ISDN) twice a day.
- Group 3: treated with transdermal isosorbide dinitrate + phosphotidilinozitol (ISDN +PI) twice a day.

Drug doses were calculated according to interspecies coefficient of doses recalculation. Animals were sacrificed after 20 days of treatment. After termination, the abdominal aorta was fixed for general morphology and Oil Red O staining according to generally accepted techniques.

Morphological investigations of aorta showed:

- Group 1: numerous vacuoles with neutral lipids and foam cells under endothelium, proliferation of solitary smooth muscle cells (SMCs). Some SMCs contain small lipid vacuoles;
- Group 2: moderate deposition of lipid vacuoles and foam cells in subendothelial space, absence of proliferating SMCs;
- Group 3: expressed reduction of vacuoles with neutral lipids and foam cells;
- Control group: absence of atherosclerosis signs.

The obtained data provide clear evidence that treatment with ISDN+PI could provide expressed regression of atherosclerotic vessel damages and there is no doubt that ISDN+PI have therapeutic effect as well as ISDN. ISDN+PI provide antiatherogenic properties of NO donor and PI probably due to reduction of cytokines production, decrease in adhesion molecules expression, inhibition of monocyte-endothelium adhesion, increase of cholesterol efflux from peripheral tissues and clearance of cholesterol from plasma via hepatic uptake. This combined compound could be a potential therapeutic agent for the treatment of atherosclerosis.

NEUROPROTECTIVE AND ANXIOLYTIC EFFECTS OF AFOBAZOLE UNDER THE CONDITIONS OF LOCAL ISCHEMIA

Alikhanyan K., Balasanyan M., Mkhitaryan A.
Department of Pharmacy, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia
E-mail: achristina2003@yahoo.com; **Phone:** 093598760

Stroke is the most common life-threatening neurological disease and ranks as the third leading cause of death and first leading cause of serious long-term disability in the economically developed countries. Investigations with neuroprotective drugs have shown great promise in preclinical testing but disappointment in clinical trials. Several pitfalls have arisen in trying to extrapolate from animals to humans in the investigation of neuroprotective therapy. Unlike the animal model, usually involving middle cerebral artery occlusion in young, healthy animals, stroke patients typically have a multitude of associated variables. That is why one of the important goals of the experimental science is to develop new local ischemia models more precisely reflecting the clinical picture of stroke, when the acute ischemic disorders develop on the background of chronic ischemization of cerebral tissue.

The investigation was aimed to create new model of stroke with local ischemia performed after long-term restriction of movement activity – hypokinesia (HK), since it is known that HK causes chronic ischemization of cerebral tissue, and to evaluate anxiolytic and neuroprotective effects of Afobazole after local ischemia.

White inbred male rats were used. As a model of HK, all experimental animals were kept individually in narrow cages for 15 days. The anxiety was evaluated by elevated plus-maze (EPM). The acute local cerebral ischemia was performed by the ligation of middle cerebral artery (MCA). Afobazole (1 *mg/kg*, i.p.) was administered during 12 days after ligation of MCA twice a day.

Control animals did not receive Afobazole after ligation of MCA.

Coronal brain sections (4 *μm* thick) were stained with Luxol Fast Blue and photographed to obtain an independent measure of the ischemic injury. The corrected infarction volume was calculated using a formula given by T. Lin et al. (1993).

Data obtained showed that ligation of MCA after 15-day HK causes anxiogenic-like effects in rats. They displayed less time spent on [F (1.6)=0.41; p<0.5) and percentage of entries into the open arms [F (1.6)=0.03; p>0.5) of EPM. The morphological investigations of rat brains in control group assessed that the absolute infarct size was 100 ± 5 mm^3.

The psychopharmacological study of Afobazole revealed that if administered *i/p* during 12 days after local ischemia this neuroprotective drug displayed a significant anxiolytic-like effect. It affected the following behavioral measures: increases were observed for the percentage of open arm entries [F(1.6)=0.25; *p*>0.1), the time spent in the open arms [F(1.6)=1.642; *p*<0.5). Meanwhile, the infarct size in experimental group of rats administered Afobazole during 12 days after MCA was smaller, 60 ± 7 mm^3.

The new model of local ischemia combined with HK increases the relevance of laboratory studies,

and laboratory findings may provide cues regarding the clinical use of Afobazole. Moreover, the results obtained suggest that Afobazole evidently displays both anxiolytic-like and neuroprotective effect in animals with anxiety caused by the new model of local ischemia.

PHARMOCOGNOSTIC RESEARCH ON MULLEINS (*VERBASCUM L.*) OF ARMENIAN FLORA

Altunyan A., Chichoyan N.

Department of Pharmacognosy, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: pharmacognosy@ysmu.am; altunyan@yahoo.com

The Armenian flora is rich in several sources of raw materials, and their study and introduction to pharmacy is very important. From the phytochemical and pharmacological points of view, plants that are used in traditional medicine as well as in folk medicine are recently observable. Mulleins that grow at the territory of Armenia practically were not used in traditional medicine, though according to scientific information they are perspective medicinal plants.

The pharmacological study of wild growing mulleins of Armenian flora (genus -*Verbascum*, family- *Scrofulariaceae*) was performed for their standardization and creation of the appropriate normative documentation.

Wild growing mulleins collected from different areas of Armenia (Dilijan, Buzhakan, Sevan) were used to study. The flowers of mullein were collected as crude drugs (State Pharmacopoeia (SP) VIII USSR; WHO GACP 2003).

For microscopic study the microscopic preparation was made from the petals of mullein flowers according to USSR SP XI. The methods of cartography and route trip were used for resource analysis and for the definition of different types of brushwood.

As a result of analysis on scientific information, the mulleins that exist in Armenian flora during 23 years are out of view of scientists. Scientifically founded information was summarized in A.L. Tartajyan's work "Armenian Flora" (1987), where 31 species of genus *Verbascum L.* and 7 hybrid types were represented. According to USSR State Pharmacopoeia, the flowers of *Verbascum thapsiformae L.* and *Verbascum phlomoides Schrad.* are allowed for the pharmaceutical use. The method of cartography was applied for further pharmacognostic study of mullein flowers.

As a result of external examination of the collected raw material the flowers of black mullein (*Verbascum nigrum*), which have 5 dark violet stamens were distinguished. They are poisonous, according to USSR SP, and classified as forbidden mixture, while for the other collected species of actynomorph flowers, 2 naked and long stamens, 3 white short and puberulent stamens are typical. The microscopic analysis of the allowed species mentioned allowed to determine typical anatomic properties and the

selection was identified according to them.

In the microscopic preparation of mullein petals the following was found out: star shaped multicellular hair with multicellular legs and with monocellular beams radically moving out from the legs. These diagnostic properties, which were approved by the electronic microscope, are identical to the petals of *Verbascum thapsiformae* and *Verbascum phlomoides*.

Results of the microscopic analysis give a base to suppose that the mulleins existing in Armenian flora could be partially merchandised for preliminary standardization.

The cartography of wild growing mulleins of some regions of Armenia and definition of the species *Verbascum thapsifornae* and *Verbascum phlomoides* enables their further pharmacognostic analysis.

INTENSITY OF FREE RADICAL OXIDATION OF LIPIDS AND PROTEINS AND ACTIVITY OF OXYDOREDUCTASE SYSTEMS IN HEART MITOCHONDRIA OF OLD RATS UNDER EFFECT OF PRECURSORS AND MODULATOR OF UBIQUINONE BIOSYNTHESIS

Andrieieva G.[1], Kuchmenko O.[1,2], Petukhov D.[2]

[1] Department of Biology, National University of Kyiv-Mohyla Academy, Kyiv, Ukraine

[2] Department of Coenzymes Biochemistry, Palladin Institute of Biochemistry of National Academy of Sciences of Ukraine, Kyiv, Ukraine

E-mail: parpa@list.ru; **Phone:** (+38044)2347178

Ageing is a physiological process related to the morphological and functional changes in cellular structures, which leads eventually to progressive imbalance of the regulation systems in an organism, including hormonal, neuroendocrine, and homeostatic mechanisms. Nowadays there are a few theories of the organism ageing. One of theories is the free radical theory of ageing, which assumes that ageing is the result of damage to tissue and organs due to effect of free radicals. It results in changes of pro-and antioxidant balance that causes the oxidative stress.

The investigation was aimed to study the effect of a complex of precursors and modulator of ubiquinone (CoQ) biosynthesis (α-tocopherol acetate, 4-hydroxybenzoic acid, and methionine) on activity of oxydoreductase systems and intensity of free radical oxidation of lipids and proteins in heart mitochondria of old rats.

Adult male white rats aged 6-7 months and old male white rats aged 24 months were *per os* administered complexes of precursors and modulator of CoQ biosynthesis daily for 10 days. Then tissues of heart were homogenized and the mitochondrial fraction isolated. In heart mitochondria CoQ and vitamin E content was separated by thin-layer chromatography and determined spectrophotometrically.

NADH-CoQ-oxidoreductase and succinate-CoQ-oxidoreductase activities were determined using a spectrophotometric method. To investigate the correlation between the oxidative stress and ageing, we determined the levels of lipid peroxidation expressed as thiobarbituric acid reactive substances (TBARS) and conjugated dienes using spectrophotometric assays. Products of free radical oxidation of proteins, carbonyl products, were measured using the spectrophotometric method.

Changes were observed in heart mitochondria. NADH-CoQ-oxidoreductase and succinate-CoQ-oxidoreductase activities were increased in control old animals in comparison to control adults. The administration of the abovementioned complex led to decrease in this parameter to the level of adults. The same tendency was observed for the levels of conjugated dienes, TBARS and products of protein oxidation. Administration of the complexes of precursors and modulator of Q biosynthesis led to an increase in CoQ and vitamin E content in old animals in comparison to old control.

Administration of a complex of biologically active substances leads to correction of age-related changes in coenzyme CoQ content, functioning of mitochondrial electron-transport chain enzyme systems, intensiveness of lipids and proteins free-radical peroxidation in heart mitochondria of old rats.

INFLUENCE OF TOPAZ AND CONFIDOR ON CONTENT OF FREE AND BONDED ASCORBIC ACID IN APRICOT LEAVES AND FRUITS

Antonyan M., Karapetyan T., Mirzoyan V., Hanisyan R.

Department of Analytical Chemistry, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: analyticchem@ysmu.am

Apricot is one of the fruits of nutritional, medical, and industrial importance, which belongs to the fruits of Armenian areal and is well accepted and widely used by local people (fresh, dried and canned). It contains several valuable phenolics, flavonoids, tannins, microelements, vitamins (especially β-carotene, vitamins C, K, niacin, thiamin) and other substances with antioxidant activity.

Synthesis of these compounds in apricot can be modulated by application of pesticides, particularly fungicide Topaz® (Syngenta Co.) and insecticide Confidor® (Bayer Co.). However, it is known that these compounds are toxic, can absorb inside the protected plants and fruits with the possibility of long time preservation. The effects on ecosystem functioning and human health are complex and cannot be predicted with great certainty. The effect of mentioned chemicals on the biochemical patterns of apricot is not studied at all, though, agrochemicals, as biologically active substances, may have substantial influence on the processes of plant metabolism.

Ascorbic acid (AA) is one of the most important antioxidants of the flora, but actually, its functions in plants life are unclear.

Present work is aimed to study the effect of insecticide Confidor® and fungicide Topaz® (separately and in mixture, as it is, actually, well accepted) on the content of AA in apricot leaves and fruits.

Experiments were carried out in orchards of Kanakeravan using 8-year apricot trees of the "Shalakh" cultivar, which were treated by spraying the solutions of Confidor®, Topaz® and their mixture, twice in each 20 days, after the end of blossom, in accordance with the norms and standards of agricultural practice. Trees sprayed by water were used as blank experiments. Sampling of the leaves was done 2 hours after spraying and then at the 7th, 14th and 21st days. Fruits were collected after full maturation. The content of AA was determined by the well-known method of Moore. Total Antioxidant Activity (TAA) was evaluated by voltamperometry. AA solution (1%) was used as standard (1.15 *mmol/L·min*).

Experiments showed that AA content in apricot leaves is changing during the vegetation and can increase or decrease depending on the pesticide applied.

The sufficient increase of AA content (both free and bonded) during vegetation has been registered. The maximum concentration (1-4 *mg* %) of free AA was revealed at the 7th day of pesticide application with the further decrease, which is accompanied with bonded AA concentration increase (3.5-18.7 *mg* % at the 21st day).

It is very interesting that the type of pesticide used have ambiguous influence. Confidor® shows stimulation of AA synthesis up to 1.1 times and Topaz®, vice versa, inhibition up to 17% in comparison with blank.

Maximum antioxidant activity was revealed by the use of Confidor®+Topaz® mixture: 0.98 *mmol/L· min* in water extracts of fruits.

The variations of AA content, as well as apricot fruits antioxidant activity are indicating that redox processes are activating in fruits as a response to the toxic exposure of the pesticides applied.

SYSTEMIC INFLAMMATORY RESPONSE IN HUMAN ISCHEMIC STROKE

Arakelyan A., Boyajyan A., Hovsepyan T., Hambardzumyan M., Arakelova E.

Laboratory of Macromolecular Complexes, Institute of Molecular Biology, National Academy of Sciences of Armenia, Yerevan, Armenia

E-mail: aarakelyan@sci.am; **Phone:** +37410 282622;, Fax: +37410 282061

Stroke is among the most common diseases of advanced age becoming a steadily increasing financial healthcare problem in the industrialized world with the increasing longevity and aging of the population. No effective therapeutics are available till present. During the last years, promising findings suggest that systemic inflammation and neuro-inflammation play important role in the post-ischemic brain tissue damage and disease progression. The Laboratory of Macromolecular Complexes for decade is focused

on the study of inflammatory processes occurring at the systemic levels upon development of ischemic stroke (IS). Here we summarize the results of our studies and provide evidence on the involvement of systemic inflammatory response in pathogenesis of ischemic stroke.

The research was aimed to evaluate the levels of inflammatory markers: C-reactive protein (CRP), complement activators (circulating immune complexes (CICs) and cryoglobulins), the functional activity of classical and alternative complement activation pathways, their regulators and individual components (factor B, factor H and its variants, C1q, C3, C4, terminal complement complex), cytokines and chemokines in blood of patients with ischemic strokes.

Overall 300 patients with ischemic stroke have been studied during 1997-2010. Diagnosis of ischemic stroke was based on clinical history and neurological examination and was confirmed by brain computer tomography (CT) imaging and basal laboratory tests. The same number of physically and mentally age and sex-matched subjects served as a control group.

CICs and cryoglobulin concentrations were measured by spectrophotometric and Lowry assays, respectively. Cryoblogulin characterization and the measurement of C1q were performed using immunoblotting procedure. Levels factor B, factor H and its variants, terminal complement complex (TCC), cytokines (IL-1β, IL-6, TNF-α) and chemokines (GRO-α, MCP-1) were measured by ELISA. CRP, C3 and C4 levels were measured by nephelometric assays.

The result of our studies showed that the levels of systemic inflammatory markers are increased in IS patients compared to healthy controls. Significantly elevated CICs levels have been observed within 24 hours and at days 7 and 30 after IS onset are associated with worsening of neurological status and 30-day fatality. For the first time we demonstrated the increased levels of type III cryoglobulins in the blood of IS patients. Classical (CCHA) and alternative pathway hemolytic activities (ACHA) of complement were determined in serum of patients with acute IS on days 1, 3, 5 and 7 after onset of stroke. On day 1 no significant changes were found in CCHA in patients compared to healthy subjects, but on day 3 CCHA significantly decreased. On day 5 CCHA increased and returned to normal on day 7. For ACHA no significant difference, compared to healthy subjects, was detected on days 1 and 3, whereas on days 5 and 7 ACHA significantly increased with maximum level on day 7. There was a positive correlation between the CCHA and ACHA on days 1, 3, 5 and 7 after the onset of stroke. The levels of CRP, factor B, factor H-402H variant, C1q, C3 and C4, TCC as well as IL-1β, IL-6, TNF-α, GRO-α, MCP-1 were increased at the first day after stroke onset.

Our studies clearly indicate that in addition to development of local inflammatory processes in brain, the stroke evokes an immune response at the systemic level. The elevated levels of inflammatory molecules in periphery can be a consequence of blood-brain barrier breakdown and may permit specific immune response to the central nervous system. Moreover, severe inflammatory response may lead to development of Systemic Inflammatory Response Syndrome that worsens the prognosis of the disease outcome. Thus, the development of novel therapies acting via anti-inflammatory mechanisms might become a promising opportunity for the increase of the quality and effectiveness of stroke treatment.

APPLICATION OF BACTERIAL L-AMINOACYLASE FOR OBTAINING D- AND L-METHIONINE

Asmyan A.[1], Aganyants H.[1], Mkhitaryan A.[2]

[1] Biomedical Department, Russian-Armenian (Slavonic) University, Yerevan, Armenia [2] "Scientific-Research Institute of Biotechnology" CJSC, Yerevan, Armenia;

E-mail: *asmyana87@mail.ru*

Strain-producers of biologically active compounds including enzymes have found wide application in up-to-date biotechnology. Aminoacylases were found out and characterized for set of organisms. Distinctions of substrate specificity among bacterial and yeast aminoacylases are revealed and possibility of their application for obtaining different protein L-amino acids is shown. Aminoacylase genes of various organisms are cloned and studied in detail. Recently enzymes of thermophile bacteria were actively studied. In *E. coli* the aminoacylase gene of thermophile bacteria, *Bacillus stearothermophilus*, was cloned. Genes coding D-aminoacylases and enzymes themselves were also intensively studied. To study the mechanism of aminoacylase action, point mutagenesis, x-ray diffraction analysis and molecular modeling are used.

The aim of the study was working out a method of simultaneous obtaining D- and L-methionine from racemic methionine with application of recombinant L-aminoacylase of *E. coli* LGE 36 strain–producer.

The activity of enzyme was measured at temperature of 37°C with a modified method of W. Gade and J. Brown (1981). The concentration of protein was determined with a method of Grows and Davis (Peterson G., 1983). For obtaining a rough aminoacylase extract, the cells were subjected to ultrasonic disruption. The methods of acylation of racemic amino acids, enzymatic hydrolysis of racemic N-acetyl amino acid, methods of chemical hydrolysis of N-acetyl-D-methionine, sorption and desorption of L- and D-amino acids were also used. The optical purity of obtained crystals of D- and L-methionine was determined polarometrically.

The basic technological scheme for obtaining D- and L-methionine from N-acetyl-DL-methionine with application of bacterial L-aminoacylase of strain-producer *E. coli* LGE 36, containing recombinant plasmid with argE gene coding the synthesis of aminoacylase was developed.

The obtained results testify that the effective method for simultaneous obtaining D- and L-methionine from racemic methionine by means of L-aminoacylase was offered. Enzyme activity of 4000-5000 *u/mg* was achieved.

The proposed method allows obtaining D- and L-methionine with more than 80 % yield calculated from their content in racemate. The purity of obtained D- and L-methionine preparations was above 98 %. The specific optical rotation of obtained crystals of D- and L-methionine corresponds to preparations produced by Sigma-Aldridge.

CHANGES OF INDICATOR OF PATHOLOGICAL DAMAGE AMONG STUDENTS DURING EDUCATION

Avagyan K.[1], Khachikyan N.[2]

[1] Forensic Medicine Department, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

[2] Department of Hygiene and Ecology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: hygec@ mail.ru

It is known that health is one of the most important values for humans during their life-time. Formation of health starts at childhood, completes before mature age and determines health, working and defense capacity of nation in the future. A growing organism is the most sensitive to the impact of unfavorable environmental conditions leading to disorders of growth and development. Risk factors of different diseases influence the body at school and higher educational establishments and determine pathology among adults in the future. The problem of health status preservation in students is the focus of researchers' attention during the last years due to connection with unfavorable changes in students' health status. Different studies revealed the increase of morbidity prevalence and infringements of health status of students during education.

The aim of research was investigation of changes in health status of students during education at higher educational establishments.

The study was done among students of 7 higher educational establishments of Yerevan. Medical examinations were conducted among 2174 students (from the first and graduating courses) to give complex evaluation of health status of students. The indicator of pathological damage (prevalence of functional disorders and chronic diseases) was calculated basing on analysis of data on medical examinations.

Study results have shown that during education indicators of pathological damage increase among students at all investigated higher educational establishments.

The indicator of pathological damage among students increases at all universities from 1094.3 (among the first year students) up to 1381.2 (among students of graduating corses) during education. Moreover, this change is mainly determined by an increase of the number of chronic diseases. Thus,,the number of functional disorders increases by 1.2 times during education (from 822.1 among students of the first course up to 969.1 among students of graduating courses), while the number of chronic diseases increases by 1.5 times (from 272.2 among students of the first course up to 412.1 among students of graduating courses) over the same period of time.

Study results indicate to infringements of health status of students during education, which shows necessity of periodical medical examinations, as well as the awareness raising in students concerning healthy lifestyle to preserve students' health during education at higher educational establishments.

THE USAGE OF BOVINE PERICARDIUM FOR THE PLASTY OF THE EXTRAHEPATIC BILE DUCTS

Avagyan T.[1], Varzhapetyan A.[2], Barseghyan H.[2]

[1] Department of Morphology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia,

[2] Department of Surgery No.3, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

Phone: 098426070

Cases, which require replacement of the damaged organ by an intact one, are often in surgical practice. Congenital defects of development, iatrogenic injuries, and bile duct strictures require the restoration of normal bile passage to digestive tract. The surgeon is always faced with the choice of optimal surgical intervention in an effort to minimize its negative effects. In recent years the bile passage to the intestine lumen is provided by the reconstructive operations: the bile ducts are anastamosed with the loop of jejunum. As a result, the functions of papilla of Vater and part of the small intestine remain out of the digestive process.

In our study the bovine pericardium was used for the plasty of extrahepatic bile ducts, which has been pointed out as a good transplant in the cardiovascular surgery. This graft stimulates the process of regeneration by its low reactivity and high biocompatibility.

Thirty-five female rabbits (2.5-3.5 *kg*) were operated under general Nembutal anesthesia through the right subcostal laparotomic incision. The window defect of the gallbladder fundus was closed by the bovine pericardium patch. Rabbit gall bladder was studied by histological and histochemical methods. Accordingly the experimental animals were divided into 4 groups, by 10 in each. The histological investigation of gallbladder and graft was performed in the following terms: an early period (7 days), intermediate period (3 months) and late period (6 months). Ten experimental rabbits, which were not operated made the 4-th (control) group. The pieces from different parts of the gallbladder were fixed by 10% formalin solution paraffinized and sectioned. The paraffin sections (4 *μkm* in thickness) were stained with hematoxylin-eozin to reveal the general structure and with picrofuxin by Van-Gieson and Picro-Mallory to compare the differentiation of gall bladder connective and muscle tissues in different terms of experiment.

We investigated mucous membrane, muscle and serous tunics of the gall bladder wall.

In the early group (7 days), the bare mucous membrane was found out of the implant margins, which indicates to the epithelial layer necrosis and desquamation. The characteristic folding of the mucous membrane was smoothed out, the stroma was edematous, and single neutrophiles, lymphocytes and plasmocytes were detected in the perivascular space. The myocytes fragmentation was revealed in the muscle layer, due to edema and microcirculatory disorders. The ingrowths of young granulation tissue were revealed by the margins of the xenopericardium. New granulation tissue had numerous thin walled blood vessels and few differentiated cells. This feature points to the tendency of transplant to "vitalization".

In intermediate period in those parts of gall bladder wall, which were out of the operating area, the folding of the mucous membrane was preserved. The mucous membrane was covered with cylindrical epithelium, the integrity and polarity of which was preserved too. The proper tunic of mucous membrane located under the epithelium and presented by the loose connective tissue, was clearly visible. Here, in contrast to early period, edema was no longer detected. In the muscle layer, we did not reveal the edema and blood circulation disorders such as stasis, hemorrhage, and formation of thrombus. In contrast to that in the sections stained with Van-Gieson method, the single myocytes with dystrophic features (poorly contoured cell membrane and lightly stained chromatin substance) were noticed and might be explained by blood and lymph circulation disorders at the early period, which caused the myocytes dystrophic changes. The adventitial membrane of the gallbladder was deprived of blood and lymph circulation disorders and dystrophic changes of cells. In the second group, in contrast to the first one, we revealed the development of young connective tissue in the implant and around its margins, which can be explained by cells differentiation, fibers formation, and development of blood vessels. The number of collagen fibers and their bundles were markedly increased. This is directly due to the rapid increase of fibroblasts number. In the sections of gall bladder of control group animals stained by Van-Gieson, the fuxinophilia of the mature connective tissue and picrinophilia of the single recovered muscle fibers were detected.

In late group of animals (6 months after operation), the histological structure of whole gallbladder wall was similar to that of the control group. Actually, in the late period, even in condition of the reactive effect of the bile, the whole avital transplant was replaced by the connective tissue, that is to say became vital.

MODIFIED METHOD OF IMMUNE-ENZYME ASSAY (ELISA) FOR DETERMINATION OF HORMONES CONCENTRATIONS

Avagyan T.

Scientific-Research Center of the Yerevan State Medical University after M. Heratsi, Yerevan, Armenia
E-mail: admin@hay-link.net; **Phone:**099536665

For a great variety of diseases, the results of diagnosis depend largely on precision of determinations for hormones concentrations in blood serum and plasma, especially in case of extreme/ultimate values, for example, when indices of hormones concentration are on the borderline of norm and pathology.

The accuracy and precision in determination of extreme (utmost) parameters of hormones using the immune-enzyme assay on "StatFax® 3200" automated analyzer to a large extent depends on professional skills of the software operator. In immune-enzyme analyzers of the last generation upon calibration curves plotting the average curve is constructed according to obtained values of standard points. However, the programme package of the device does not consider the circumstance that upon prepa-

ration of samples the operator can commit mechanical inaccuracy, as a result of which the anticipated value of one (or certain) standard curves might deviate. Therefore, a certain site of the calibration curve also varies thus resulting in erroneous interpretation and treatment of obtained data.

In order to avoid similar errors at the Laboratory of immune-enzyme research of the Scientific-Research Center of YSMU the method was developed and applied for calculation of immune-enzyme assay outcomes based on the following software application.

Only the "Absorbance Mode" is used to obtain results. This choice is conditioned by the fact that in this case the device generates only values of real optical density. Due to capacity to connect the analyzer to the personal computer with the appropriate software (provided by the company) and perform automated data input, the results of calculation are transferred into "Advanced Grapher" programme application. Subsequently the programme generates the curve with values of standard points optical density on X-axis and the corresponding consentrations on Y-axis. In case of egregious errors, the amendments of a given point are possible.

Software application allows to choose the method for graph correlation and to calculate the appropriate mathematical formula for the calibration curve. If upon correlation it is impossible to present the calibration curve as one formula, the "Advanced Grapher" programme application allows to expand the initial curve into parts and then to device formulas for each part separately.

Having obtained the mathematical formula it would be possible to calculate the concentration of samples with the help of any mathematical editor/application (we propose Microsoft Excel or Oppenoffice.org Calc). Blank form was prepared for records of studies. In appropriate columns of the form using MS Excel, the formula generated by "Advanced Grapher" programme is introduced. As a result, upon input of optical density values the programme immediately generates the appropriate concentrations of analyzed samples.

COGNITIVE DISORDERS IN ARMENIA: TYPES OF DEMENTIA

Avetisyan A., Sargsyan R., Manvelyan H.

Department of Neurology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: DoctorAvetisyan@yahoo.com; Ruzikdoc@yahoo.com; Manvelian@yahoo.com

Dementia (Lat: *dementa*) is an acquired weak-mindedness, neurodegenerative disease or clinical syndrome, which includes three points: Cognitive impairment, Behavioural symptoms and Impairments of activities of daily living.

Alzheimer's disease (AD) is the most common form of dementia (50-60%) and the prevalence increases exponentially between 65 and 85, doubling every 5-year age group. Another frequent cause of dementia is pathology of brain vascules (vascular dementia: 10-20%). There are some diseases, which can cause

dementia: Alcoholism; Traumatic brain injuries; Brain tumors; Parkinson's disease and others.

Vascular dementia is a complex of clinical pathomorphological and clinicopathological syndromes, for which common are relationship of cerebrovascular and cognitive disorders. The most frequent and classical variant of vascular dementia is an atherosclerosis of brain vascules, because of which the cognitive function especially suffers.

The basic work-up should include patient and informant interview, physical and neurological examination, cognitive tests, evaluation of psychiatric symptoms and activities of daily living, a battery of laboratory tests, and CT (or MRI) of the brain. The most important instruments for differentiating between the causes of dementia are: Clinical interview; Neuropsychological assessments; Brain imaging; Routine blood tests and Neurological examination.

The research was aimed to unveil patients with Cognitive Impairment and reveal diagnostics efficacy of Dementia in Neurology clinics or patient counseling.

Within the framework of Yerevan State Medical University "AD & Memory disorders" ongoing program, 42 patients with dementia were detected and diagnosed (30 female/12 male), tested for mandatory examinations: neuropsychological testing, brain CT or MRI, blood tests for thyroid gland dysfunction or B12 deficiency as well.

We determined and investigated thoughtfully each case of cognitive impairment to unveil underlying problem and to find potentially curable situation. Distribution of different syndromological cases out of 42 patients was like following: Hypothyreoid conditions 10% (n=4), Alzheimer type 27% (n=12), Vascular type 24% (n=10), Mixed (Alz & Vasc) 24% (n=10), Parkinsonian syndrome 10% (n=4), Anemic or Vit B_{12} Deficiency 5% (n=2).

Our investigation proves the importance of screening, as 6 patients had completely reversible conditions (15%). Moreover, we are seeing almost equal numbers of patients with Alzheimer Dementia, Vascular Dementia and their mixed cases, which proves the high rate of vascular problems in Armenia, in line with our neighboring countries. About 10% makes Parkinsonian syndromes (Parkinson's disease, Movement disorders), half of them were primarily diagnosed during our screenings.

GIANT URINARY BLADDER DIVERTICULUM

Ayvazyan L.[1,2], Muradyan A.[1,2], Zohrabyan A.[1,2], Bablumyan A.[1,2], Avoyan A.[1,2], Grigoryan G.[1,2], Hajyan S.[1,2]

[1] Chair of Urology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

[2] Department of Urology, Republican Medical Center "ARMENIA", Yerevan, Armenia

E-mail: levon_ayvazyan@yahoo.com; **Phone:** (+37491) 60 9 006

Diverticulum of the urinary bladder is a congenital or acquired anomaly, at which one of the walls of the bladder is suckaly swolled. Diverticulum may be single and, more rarely, multiple. Most commonly, diverticulum is located on the posterior or lateral walls of the bladder. There are two types of bladder diverticula - true (when the wall of the diverticulum is composed of the same layers as the wall of the bladder) and false (when the wall of the diverticulum is composed of mucosa, which like a hernia is bulging through the muscle fibers of the bladder wall). Furthermore, one should distinguish between congenital (exists in the patient initially) and acquired diverticula (diverticulum is formed due to the substantial raise of intravesical pressure as a result of infravesical obstruction due to BPH, urethral stricture, bladder neck sclerosis, etc.).

A 69 years old patient ***A.G.*** was admitted in the department of urology of the Republic Medical Center «Armenia" on September 1, 2009.

Complaints: frequency, difficult urination with weak stream, feeling of incomplete emptying of the bladder, enlargement of the scrotum.

From history: the patient has experienced specified complaints for the past two years. Objective examination: asymmetry of the abdomen was determined visually. The smooth formations of 20x15 *cm* on the left and 10x 15 *cm* on the right were revealed upon palpation. Scrotum was increased in size, painless on palpation, and determined by right side hydrocele.

Laboratory test results: all data were in the normal range, except PSA: 4.7 *ng/ mL*, total urine: 12-14 white blood cells in the visual filed (leykotcyturia).

Ultrasound (01.09.2009.): The left kidney was increased in size, parenchyma sharply thinned, sometimes absent. From the lower extremity of left kidney till the suprapubic area, the formation of giant size was determined that mechanically resulted in hydronephrotic transformation of the left kidney. The right testis was 5.1x 3.1 *cm*; significant amount of liquid was revealed in the shells of testes.

Computed Tomography (01.09.2009.): The left kidney was hydronephrotically transformed with significantly thinned and sometimes absent parenchyma, pyelocaliceal system was noticeably enlarged. Left ureter was enlarged to 1.7 *cm*. Virtually the whole small pelvis and the left half of the abdominal cavity were occupied by formation of 20.0 x 16.0 cm in size, the density of which corresponded to the density of the bladder content. Bladder was on the right of above described formation and sharply enlarged; the upper contour of the bladder was 3.5 *cm* above the navel. The bladder was connected to the above-described formation through a narrow isthmus of 1.4 *cm* in diameter. Retrograde cystography indicated

an excretion of contrast material into the formation cavity through a narrow isthmus. The prostate was of 9.3x 4.4 *cm* in size and diffusely changed. Abdominal, pelvic, and retroperitoneal space lymph nodes were not enlarged.

Digital rectal examination of the prostate: on palpation prostate was of tightly-elastic consistency and increased in size, the median groove was flat, the upper pole of gland was reachable for palpation, bowel mucosa was sliding on the projection of prostate, while seminal vesicles were not palpable. On the basis of both objective and clinical examinations it can be interpreted that the giant diverticulum of urinary bladder was under consideration. The catheterization of the bladder was made and approximately 10 liters of urine was evacuated on the fractured basis. The patient was weighed before and after the catheterization, the difference in weight was 10 *kg*.

Repeated ultrasound (08.09.2009.): left kidney was deformed; parenchyma was preserved (1.5-1.8 *cm*), the cavities were enlarged, the outflow of urine was distorted by the type of hydronephrosis, the diverticulum was emptied. Clinical diagnosis: Benign prostate hyperplasia, a true diverticulum of the urinary bladder, chronic urinary retention, left-side uretherohydronephrosis, right-side hydrocele. Operation (09.09.2009.): The TUR (transurethral resection) of prostate and cervix of diverticulum, trockar epicystostomy, hydroceleectomy on right by Winckelmann were made to the patient under the spinal anesthesia. The postoperative period was uneventful. Urethral catheter was removed on the 7th day. The patient was discharged from the hospital on the day of recovery. Having studied the literature, we found out not so many cases with the description of giant urinary bladder diverticulum and in each case medical tactics, as well as in our case, was chosen individually, taking into account localization and complications caused by the diverticulum.

ANALYSIS OF *MACROVIPERA LEBETINA OBTUSA* SNAKE VENOM BY FPLC CHROMATOGRAPHY

Babayan B.[1], Blbulyan S.[1], Martirosyan A.[1], Voskanyan A.[2]

[1] Biomedical Faculty, Russian-Armenian (Slavonic) University, Yerevan, Armenia

[2] Laboratory of Physiologically Active Substances Purification, Certification and Standardization, Orbeli Institute of Physiology, National Academy of Sciences of Armenia, Yerevan, Armenia

E-mail: bbg.15.04@mail.ru

Investigation of physiologically active substances is very actual, due to their use in medicament and diagnostic means production and in different biological sciences. The relevance of the substances like animal toxins is inestimable for Armenia, because they can be used as the resource materials. Based on achievements of fundamental investigations these substances can bring economical profit to Armenia.

The aim of our study was to perform FPLC-chromatography and PAGE electrophoresis of *Macrovipera lebetina obtusa* snake venom.

The snake venom was isolated from *Macrovipera lebetina obtuse (*gyurza or gorzeh). Crystallic total snake venom was dissolved in distillated water to a final concentration of 30 *mg/mL*. Investigation of snake venom was carried out by FPLC-chromatography and PAGE electrophoresis. Chromatographic analysis was carried out by FPLC on Superose 12 column (Column - 1x15-20 *cm*; flow rate: 0.5 *mL/min/cm*^{2}, the sodium phosphate buffer was used as an eluent). Protein loading was 300 *mg* on column.

Polyacrylamide disc electrophoresis (PAGE) of snake venom was carried out in 7% dividing and 2.5% concentrating gels. Disk-electrophoresis was conducted about 3 *h* with electric field power of 4 *mA* per tube (250-300 *V*) at 4^{0}C in dark. Gels were stained for protein detection with mixture of 0.1% Coomassie Brilliant Blue 5D and 0.1% Amido black 10B. Gels were analyzed by FUJIFILM Image Gauge V4 program.

Data are expressed as means (standard deviation (SD) of at least triplicate determinations). Changes in variables were analyzed by a one-way ANOVA for multiple comparisons. Differences were considered significant at $p<0.05$.

According to FPLC chromatography results, there were obtained 5 fractions of venom with different molecular weights and proteolytic activities. The second fraction with average molecular weight 40-45 *kDa* has a significant caseinolytic activity excessing the enzymatic activity of *Macrovipera lebetina obtus* venom by 25-30%. Fractions received by chromatography have shown approximately 7 - 8 protein bands after PAGE electrophoresis.

The obtained results certify that the percentage of enzymes of *Macrovipera lebetina obtusa* venom with caseinolytic activity is high. Certain fractions of the venom may serve an instrument for directed destruction of microcircular blood vessels with the aim to localize injected medicinal preparations.

SURGICAL TREATMENT METHOD OF CONGENITAL MICROPHTHALMOS

Baghdasaryan E.[1]**, Galimova V.**[2]

[1] Ophthalmologic Clinic, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

[2] All-Russian Center of Eye and Plastic Surgery, Ufa, Russia

E-mail: baghdasaryany@mail.ru; **Phone:** (+374 91) 25 06 05

Congenital microphthalmos is one of the widespread malformations of eyeball. In children suffering from blindness its percentage is 3.2-11.2. Rehabilitation of children developing microphthlmos is the correction of cosmetic defects and prevention of asymmetric development of facial skeleton.

The purpose of research is surgical treatment of congenital microphthalmos with the use of biomaterial "Alloplant" promoting the oversizing of eyeball.

Forty-eight patients developing congenital microphthalmos were enrolled in the research. Patients were divided into two groups depending on the degree of eyeball hypoplasia. The operation of posterior bandage was performed in 22 patients of the first group (in this group of patients anteroposterior axis was 16-19 *mm*). The operation of posterior and anterior bandage was performed in 26 patients of the second group (in this group of patients anteroposterior axis was 8-16 *mm*).

Patients were examined prior to surgery and in 2-3 years postoperatively. The efficiency of operation was determined comparing the following parameters: anteroposterior and lateral axes of eyeball, depth of conjunctival fornix and the volume of eyeball according to the ellipsoid formula:

$v = 4/3 \times a \times b^2$.

In the first group of patients the average increase of anteroposterior axis of eyeball was 4 *mm* in 3 years postoperative (prior to surgery: 17.9 ± 3.2, in 2-3 years postoperative – 21.7 ± 1.7 *mm*). In the second group the average increase of anteroposterior axis of eyeball was 5 *mm* in 3 years postoperative (prior to surgery: 14.2 ± 3.3 and in 2-3 years postoperative – 19.2 ± 1.9 *mm*).

Operation of anterior and posterior bandages can be applied as a surgical treatment of congenital microphthalmos. Surgical procedure with biomaterial "Alloplant" is not accompanied by vicious cicatrix of conjuctival cavity. Surgical procedure improves the form of conjuctival cavity and depth of sac which is to be accompanied by prosthetics using light and thin sided prosthesis.

UTERINE CERVIX CANCER SCREENING EFFICACY ANALYSIS IN GEORGIA

Baliev D.

Public Health Department, Tbilisi State Medical University, Tbilisi, Georgia

Phone: +99599462312

In Georgia uterine cervix cancer is at the second place among oncological diseases in women's death rate structure, while 70% of the cases are diagnosed at late, incurable stages. The efficacy analysis concerning the relevant screening program was performed.

The research was aimed at prevention and changed health behavior of population.

Methods of expert analyses were applied.

According to the randomized inquiry of 400 women, 30% of them reported that they underwent Papanicolaou test at least once in their lives, while 42% have never heard about uterine cervix cancer screening. Twelve combinations of the screening were economically effective correlated with the longest-possible life years. The overall value of screening program varies from USD 2,950.00 to USD 4,100.00 per 1000 women.

The present day uterine cervix cancer screening program does not cover a great part of women in the society owing to the low awareness and educational level of women on this matter, the denial of the problem existence as such, the reasonless fear, and resources deficiency.

THE INFLUENCE OF VINPOCETINE, NIMODIPINE AND CINNARIZINE ON SENSITIVITY OF CEREBRAL VESSELS TO CARBON DIOXIDE IN HYPOKINETIC RATS

Baykov A.

Department of Pharmacology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: msejournal@yahoo.com; **Phone:** (37 41 0) 58 25 32/ 094 01 0615

Hypokinesia (HK) is believed to lead to significant pathological shifts in cerebral blood flow and metabolism; in particular, it causes cerebral hypoperfusion with the subsequent neuropsychological alterations. At the same time, HK is assessed as a serious risk factor in the development of cardiovascular diseases. The peculiarities of the action exerted by selective cerebrovasodilating drugs in HK conditions, as well as the possibilities of the pharmacological correction and prevention of HK-induced pathological shifts have both, theoretical and potentially practical significance.

The purpose of this study was to reveal the peculiarities of hypercapnic cerebrovasodilation under the influence of vinpocetine (V), nimodipine (N) and cinnarizine (C) during the experimental HK in rats.

The experiments were performed in 152 mongrel albino rats of both sexes weighing 190-210 *g*. Rats of the control group were kept in ordinary vivarium conditions. Experimental groups were kept in individual narrow Plexiglas cages for simulation of HK during 15, 30, 45, and 60 days. Experimental groups were divided randomly into four subgroups: 1) without treatment, 2) vinpocetine-treated (2 *mg/kg/day, i.p.),* 3) nimodipine-treated (0.5 *mg/kg/day, i.p.*) and 4) cinnarizine-treated (1 *mg/kg/day, i.p.).* The duration of treatment was 7 days up to the appropriate term of HK. The sensitivity of cerebral vessels to CO_2 was defined by registering the local cerebral blood flow (LCBF) using a laser-Doppler flowmeter (BLF 21, Transonic Systems Inc., USA) in a hypercapnic inhalation test. The reactivity of cerebral arterioles investigated by the laser-Doppler flowmeter was then tested by making the animals breathe an Air/CO_2 mixture (90%/10%). For the quantitative assessment of cerebrovascular reactivity, the coefficient of reactivity (CVR) has been used — quotient of ratio of LCBF value on the peak of inhalation load to its baseline value. The registration of LCBF, arterial blood pressure, heart rate and blood gas analysis were performed under general anesthesia with sodium pentobarbital (40 *mg/kg, i.p.*). The significance of differences between the corresponding mean values within and between groups was studied by Student's t-test with two-tailed distribution (paired-within group and with two-sample unequal variance-between groups) using Microsoft Excel 2000.

In the control group of rats, by the 30th *sec* of the hypercapnic test ICBF is found to be 149.8 $\pm$ 10.9% (to

its baseline value), on the peak of the inhalation load (on the 60th *sec)* — 199.6% ± 5.8%, and 3 *min* later after the stop of inhalation (on 240th *sec* of test) — 109.8 ± 8.0%. The coefficient of reactivity (CVR) is found to be 1.996 ± 0.058.

In vinpocetine-treated rats the authentic increase of CVR at the peak of inhalation load by the 30th day of HK (1.885 ± 0.090, p<0.05) in comparison to corresponding HK group without treatment (1.735 ± 0.089) was noted. In groups of hypokinetic rats with nimodipine-treatment, significant differences were not observed. On the other hand, although there were no changes of CVR on 15th and 30th day HK in groups with cinnarizine-treatment in comparison to the untreated HK groups, on the 45th and 60th days of HK opposite shifts were noted — an authentic decrease of CVR value on the peak of hypercapnic test (1.840 ± 0.077; $p<0.05$ against the index of corresponding untreated animals in 45 day HK group (1.990 ± 0.074); and 1.708 ± 0.079; $p<0.01$ against 1.940 ± 0.076 in 60 day HK).

We could note that the differences in the action of vinpocetine (increase of sensitivity to CO_2), on one hand, and blockers of calcium channels, on the other hand (no action or even decrease in case of cinnarizine in late periods of HK), have qualitative features. Perhaps, this difference is due to diverse mechanisms of action of the studied drugs: vinpocetine selectively blocks cGMP PDE; this amplifies the effect of endogenous NO, which is an important permissive factor mediating hypercapnic cerebrovasodilation. According to literature data, the degree of relaxation of isolated smooth muscles to vinpocetine diminishes after the treatment with indomethacin (inhibitor of COX-1); this shows the prostaglandin component of the vasorelaxing properties of vinpocetine — PGE_2 and prostacyclin are important permissive factors of hypercapnic cerebrovasodilation. Probably, the inhibition of reuptake of endogenous vasodilator, adenosine, by vinpocetine has a contribution role in the final response, whereas the calcium channel blocking property of vinpocetine is out of significance, because selective calcium channel blockers, nimodipine and cinnarizine, have the opposite influence on the studied process. Perhaps, nimodipine and cinnarizine decrease the activity of the calcium-dependent NO-synthase enzyme.

Thus, the results of this experimental study suggest that HK is a factor decreasing the sensitivity of cerebral vessels to CO_2, which is one of the signs of altered regulation of cerebral circulation in HK. At the same time, we showed that vinpocetine treatment can correct these shifts, but cinnarizine, and to a lesser extent nimodipine treatment, have opposite effect, especially in late periods of HK. Results could be useful for further clinical investigations in angioneurological patients.

INTERNETMANIA

Berishvili T.
Public Health Department, Tbilisi State Medical University, Tbilisi, Georgia
Phone: +995994623 12

Most of people more or less know one of the world's significant attainments of the 20th century – personal computer. The fact that computer is the positive event does not cause any doubt, but even the little period of relation with it showed us its negative sides.

The aim of research was prevention of risk factors in younger population.

The method of sociological investigation was applied.

The author tried to generalize world skills and branch it into Georgian reality taking part in research on internetmania among computer users in Georgia and carrying out a sociological research with the second course students of Public Health Faculty of TSMU.

In research 70 persons were enrolled. They filled in a Questionnaire, which included 30 questions approximately.

Preemptive questions are discussed in the theme and the conclusions are done on their basis.

The creation of World's global information web – INTERNET increased more negative influence of technical achievements on its users.

The results of research performed in different corners of the world and referring to the influence of computer on psychic of humans, were generalized with a special attention to "passion" with Internet and computer games in adults, as well as to the harmful influence on their psychic.

PH INFLUENCE ON THE SKELETAL MUSCLES AND MYOCARDIUM CONTRACTILE PROTEINS

Bogutska K.
Department of Biophysics, Taras Shevchenko Kiev National University, Kiev, Ukraine
E-mail: biophys@univ.kiev.ua

The interest in influence of pH of the medium on muscle contractile proteins is caused by the typical acidification of medium for the muscular fiber, which accompanies the process of contraction. The study of energy exchange in the myocardium is one of leading problems of biochemistry and cardiology for a period of recent decades. Development of pathology, first of all, can be connected with the disturbance of energy-formation and as a consequence, - function of the left ventricle of heart and its contractility. An increase in the H^+ concentration contributes also to the oppression of the calcium activation of the

actin-myosin interaction process and, in the final analysis leads to the reduction in the force of muscle contraction. It is also known that pH of medium is accompanied by the wide range of structural and functional changes in the appropriate molecules.

The ability of myosin and actomyosin to hydrolyse ATP was studied at varying hydrogen ion concentrations. The obtained results testify to the fact that the nature of a change of the Ca^{2+}-ATPase activity of the muscle myosin in the dependence on pH is identical for the skeletal and cardiac muscles. However, the maximums of their specific activity differ in the absolute values. The Ca^{2+}-ATPase activity of cardiac myosin was more at acidic pH than at alkaline pH, in contrast, the ATPase activity of skeletal myosin was more at alkaline pH than at acidic pH. Variation in the values of specific Ca^{2+}-ATPase activity most probably is caused by the qualitative and quantitative composition of light chains of the myosin of different types of muscles that can reflect differences in the charged groups of myosin molecules. Such special features of thin structural organisation and functional properties of the contractile proteins can specify the specific character of the normal functioning of muscles. The typical for the actomyosin optimum with the neutral values of pH was observed during a study of the pH-dependence of the Mg^{2+}-ATPase activity of the actomyosin of heart muscle under the conditions for low ionic strength and micro-molar concentrations of Ca^{2+}, i.e., the conditions close to the physiological ones. At the same time we revealed two additional small peaks of activity with the weak-alkaline and slightly acid values of pH that gives the possibility to assume the existence of certain strength reserve, which makes possible to support the ability of myocardium to function under the conditions for insignificant acidification or alkalisation of the intracellular medium of myocytes. However, the ATPase activity of the purified myosin preparation of heart muscle, measured under the optimum conditions for its manifestation, i.e., with high ionic force (I=0.5) and sufficiently high concentration of Ca^{2+} (5 *mM*), had two clearly expressed maximums: with pH of 6.0-6.5 and 9.0-9.5. These maximums briefly coincide with the same for the myosin of smooth and skeletal muscles. But the special feature of the myosin of heart muscle is the presence of small additional peak in the curve of the pH-dependence of its Ca^{2+}-ATPase activity with pH of 7.5-8.0, and also the less expressed maximum with pH of 9.0-9.5 in comparison with the myosin of skeletal muscles. The Ca^{2+}-ATPase activity of the myosin of heart and skeletal muscles practically coincided at pH of 6.0-6.5, and for the myosin of smooth muscles with the same conditions it is somewhat above. The Ca^{2+}-ATPase activity is above for myosin of skeletal muscles at pH of 9.0-9.5 in comparison with myosin of heart and smooth muscles, and the maximum of Ca^{2+}-ATPase activity of the myosin of myocardium occupies the intermediate position between the skeletal and smooth muscle myosin. It should be noted that the maximum of Ca^{2+}-ATPase activity with pH of 6.0 is sufficiently stable, whereas another maximum varies both on the position and in the intensity.

The obtained results testify to the fact that the nature of changes of the Ca^{2+}-ATPase activity of the myosin of myocardium in dependence on pH of medium is identical for the heart, skeletal and smooth muscles. However, the maximums of their specific activity differ in absolute values. This variation in the values of specific Ca^{2+}-ATPase activity is most probably caused by the qualitative (amino acid) and quantitative composition of light chains of the myosin of different types of muscles that can reflect the

differences in the charged groups of myosin molecules. It is known until today that light chains of the myosin of heart muscle are differed from their isoforms from the skeletal and smooth muscles. Such special features of thin structural organisation and functional properties of myosin molecules to some extent can specify the specific character of the normal functioning of the different types of muscles. The performed mathematical simulation, which describes the motion of ATP-hydrolyse biochemical reaction with a change of pH of medium in the muscle fiber, confirmed the experimental data given above.

The obtained results enlarge the existing ideas about the influence of pH of medium on the functioning of the contractile proteins of myocardium and skeletal muscles. They can be useful under the development of methods for correction of the possible muscular pathologies, which appear during the disturbance of the functional state of muscle in the conditions of changing the homeostasis in myocytes.

THE PROBLEM OF ENVIRONMENTAL POLLUTION WITH CHLORINATED PESTICIDES IN DEVELOPING COUNTRIES

Danielyan A.[1], Grigoryan K.[1], Minasyan S.[2]

[1] Department of Ecology and Nature Protection, Yerevan State University, Yerevan, Armenia

[2] Environmental Impact Monitoring Center, Yerevan, Armenia

E-mail: a.danielyan@hotmail.com, a.danielyan@ysu.am

Chlorinated pesticides belong to a range of the most dangerous pollutants related to high potential toxicity and properties to accumulate in all the trophic levels.

The influence of many chlorinated pesticides is obvious, and as a result, the use of these compounds was bounded in many countries of the world. These chlorinated pesticides are considered in the range of compounds restricted by the "Convention on Persistent Organic Pollutants", which is ratified by the Republic of Armenia as well. In spite of that, these compounds are being discovered in the environment, related to their high resistance and continuous use in any developing countries.

The continuous detection of DDT, its metabolites and isomers of HCH in the environment indicate to necessity to reveal the condition of pollution of the soil resources as a depot of land ecosystems, and of surface waters, as a transitional environment of water ecosystems. This is especially important in the certain residential-economical regions for having the complete toxicological idea, and for taking appropriate measures, when that is necessary, related to such very dangerous compounds for humans as DDT and HCH.

The Debed River watershed basin is located in the North of Armenia and one of the economical centers of the country. The spacious areas of land, propitious climate conditions, and rich water resources have favored to development of agriculture in this area. The development of agriculture was considerably preconditioned with the wide use of fertilizers and pesticides as well. Today agriculture is one of the

main fields of economy in the Debed River watershed basin too, as a result of which 51% of the land in this area belongs to the agricultural lands.

The objectives of the research were to reveal the condition of pollution with DDT and HCH in Debed River watershed basin of Armenia by their monitoring data.

The research was carried out in 2006-2008. The samples of waters of the rivers were taken monthly during the period spring - autumn, from the 12 sampling points of the Debed River and its main tributaries Pambak and Dzoraget. Analyses of DDT, its metabolites DDD and DDE, and HCH three isomers, α-HCH, β-HCH and Lindan (γ-HCH), in water samples were implemented by the gas-liquid chromatography method, according to the standard methodology. For data analysis and modeling different accepted methods were used, which allowed the reliable analysis of DDT and HCH monitoring data.

According to analytic data, the area of Debed River watershed basin is polluted with chlorinated compounds DDT, DDE, DDD, α-HCH, β-HCH and Lindane (γ-HCH) the concentrations of which in the samples usually exceed the Maximum Permissible Concentrations.

According to the research, DDT is not used in the watershed basin today. However, this very toxic compound and its metabolites (DDD, DDE) are revealed in the watershed basin until present, related to its application in the past and high persistence of this compound. Related to pollution with HCH, it is possible to mark that Debed River watershed basin is polluted with the isomers (α-HCH, β-HCH, γ-HCH – Lindane) of this compound due to past and present use of HCH and Lindane, which is banned in Armenia.

It is possible to suppose that the presence of abovementioned chlorinated pesticides in Debed River watershed basin is a consequent of their either past or present use. This state causes the anxiety, according to which we propose to implement preventive measures, taking into account both the international experience, including that of specialists in Armenia as well.

HEALTH CONDITION AND NUTRITION PROBLEM IN GEORGIA

Darsania T.

Public Health Department, Tbilisi State Medical University, Tbilisi, Georgia:

Phone: +99599462312

The main humane conception of public health claims that prevention is better than therapy. This idea can be put into practice completely exactly in case physician's care and, on the whole, government's concern will be aimed predominantly not to sickness, but to health. The main prerequisite of health preservation and its reinforcement is to correct recognition of daily processes, and their comprehensive administration. Any human being represents the unity of social, biological, physical, and mental thinking and feelings.

Good health and working capacity are the result of active adaptation, i.e. personal accommodation to environment.

We attempted to develop a new conception in health care services.

To achieve identified objectives and tasks, in this research were implemented the following methods: social researches, epidemiological method, method of actual nutrition appraisal, method of expert analysis. The special attention is paid to selective research to set representation. The indicated investigation was elaborated in Tbilisi to reveal violation in nourishment. For these purposes, three groups were chosen: people resettled from Abkhazia and now compactly residing in Tbilisi, temporarily unemployed people, receiving monthly cash benefit at the rate of 22 GEL – all belong to the 1st group. The second group consisted of people, whose income makes up to 300 GEL, and the third group was presented by people with monthly income over 1000 GEL. The age ranged from 20 to 65. To clearly recognize the violation in nutrition the Questionnaires-interrogatories were worked out. The first part of interrogatories comprised a journal, in which there were recorded all foodstuffs and dishes, taken by questioned people during a seven-day period. In the second part people were asked to indicate how often they took the basic food substances. The third part of Questionnaires included questions concerning psychological peculiarities of eating behavior. The obtained information was estimated in accordance to the method of actual nutrition appraisal. Each group was presented by one hundred people. Normative documents, state resolutions were evaluated using the method of expert analysis.

As a result of epidemiological investigations it was determined that imbalanced food intake, excess weight and obesity cause the diseases of cardiovascular and respiratory system, as well as diabetes, and oncological diseases. On the contrary, nourishment full of fruit and vegetables defends a person form some cancerous and cardiovascular illnesses. Child malnutrition leads to poor health. Imbalanced nutrition and excess weight of a pregnant woman have a negative effect on the fetus health. Imbalanced diet is rather spread within Georgia. Scientific data prove that balanced nourishment keeps up health condition. The obtained information shows that the majority of people either overeat (60%) or suffer from malnutrition (30%); there occurs the disturbance in consumption of the basic food substances, nutrients. Most of the interrogated people (60%) take meat and meat products fewer than once a week. A little number of people took fruit and vegetable uncooked.

The emotional, external, and limited types of eating behavior were discerned. The criterion was considered a state, which was accompanied with exceeding level of an appropriate alteration of eating behavior type, compared to the standard, based on data of questioner. Lack of exceeding eating behavior was appraised as rational. Rational type was observed among 59.5 % and alterations were shown by 40.5% of interrogated people. Among them: emotional alteration made 18.1%, external – 15.7%, limited – 6.2%.

Based on an example of the big city, Tbilisi, and on the ground of received data the goal of the research is to create a conception of Health Promotion. Accordingly, special Questionnaires-interrogatories were worked out about frequency of taken basic foodstuff to recognize psychological peculiarities of eating behavior. Each group consisted of 100 people aged from 20 to 65. Obtained information showed that

nutrition of interrogated people is neither balanced, nor adequate or satisfactory.

Nutrition and consequently public health depends not only on attending conditions (natural or social), but also is regulated by them. To manage public nutrition and to ensure prevention of elementary diseases is mandatory appropriate state policy. On the base of gained information purposeful references were elaborated to ameliorate eating behavior.

INFLUENCE OF MEXIDOL ON RECOVERY OF MEMORY AND BEHAVIOR OF RATS IN CONDITIONS OF HYPOKINESIA

Dheryan L., Balasanyan M., Panosyan N.

Department of Pharmacy, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: Dh.Lus2002@yahoo.com; **Phone:** 094252913

Assessment of consequences of restricted movement activity, hypokinesia (HK), is an important medical problem, as, without any doubts, it has a direct relation to the prevention and treatment of many diseases. Moreover, it enables us to complete the task of "pharmacology of the healthy man" in modern conditions of long-term restriction of the movement activity.

Investigations conducted at the Pharmacology Department of YSMU have shown that in conditions of HK experimental modeling by the 15-90 days restriction of movement activity there are pronounced impairments of blood circulation of the brain, biochemical disorders in thin functional mechanisms of the brain, pathologic histological changes in the brain tissues and its vessels, which are typical for the chronic ischemia of the brain. Besides the above-mentioned changes, in conditions of HK there also occur changes in behavioral reactions of animals, including development of anxiety, to which a great importance has been attributed recently.

The aim of the present study was determination of Mexidol exerted influence on the neurobehavioral changes and recovery of rats memory in conditions of restricted movement activity with different duration.

Experiments were performed in adult outbred albino rats weighing 180-200 *g*. HK was achieved by placing white male rats in the individual narrow Plexiglas cages during 15, 30 days according to the method of I.W.Federov (1982). The assessment of neurobehavioral parameters was performed with the help of cross maze test on the 15th and 30th days of HK under the influence of Mexidol. Memory has been assessed with active avoidance test on the 15th and 30th days of HK under the influence of Mexidol. The passive avoidance test was performed at nighttime using the step-down apparatus, which consisted of a box (30x20x30 *cm*) made of Plexiglas and stainless steel. An acrylic platform shelf (8.5x5 *cm*) was placed to one corner of the box.

Conducted investigations revealed that in conditions of 15- and 30-day HK there is an anxiety develop-

ment expressed by statistically proved decrease in the following parameters of cross maze test-percentage of the entry into the open branch (from 22.9±0.2 up to the 19.9±5.4 and 7.8±1.25, appropriately), time of the open branch (from 43.6±0.6 up to the 23.2±8.7 and 10.6±3.2, appropriately), centre time (from 18.0±0.1 up to the 12.3±1.9 and 9.7±0.83, appropriately), and movement activity (from 8.0±0.2 up to the 3.6±0.67 and 2.7±0.94, appropriately).

There are also impairments of memory recovery in the passive avoidance test. After intraperitoneal injections of 100 *mg/kg* Mexidol on the 15th and 30th days of HK anxiolytic action develops which is expressed by statistically proved increase in the following parameters of cross maze test-percentage of the entry into the open branch (up to the 21.53±0.2 and 56.14±0.6 appropriately), time of the open branch (up to the 62.8±0.2 and 177.4±0.1 appropriately), centre time (up to the 13.0±0.1 and 12.8±0.2, appropriately) and movement activity (up to the 6.0±0.4 and 5.4±0.2, appropriately). Besides that, the same dose of Mexidol statistically prevents development of amnesia in rats with conditions of 15- and 30-day HK (Ma=100).

Mexidol prevents a development of amnesia and anxiety induced by HK. Obtained results, including data concerning the absence of toxic action of the preparation, can have a very important clinical value for prevention and treatment of the above-mentioned disorders caused by the long-term restriction of movement activity.

ORGAN TOXICITY AND HYPOTENSIVE ACTIVITY OF NEW SYNTHESIZED WATER-SOLUBLE CATIONIC MESO-TETRA-PYRIDYL PORPHYRIN AND ITS METALLOCOMPLEXES

Dilbaryan K., Manukyan A.

Department of Pharmacology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

Phone: +374 77 42 31 86

There is an interest in synthesis and investigation of chemical and biological properties of porphyrins and metalloporphyrins, because these compounds have wide spectrum of biological activity and can be used in different fields of medicine. It should be noted that porphyrins can be used to treat cardiovascular diseases, but despite the wide-ranging scientific works in this field of medicine, there are many controversial data concerning the cardiovascular effects of porphyrins and their metallocomplexes.

The aim of this research was investigation on hepato- and cardiotoxicity in chronic experiments (rats), as well as species-dependent (rats and cats) hypotensive activity of new synthesized water-soluble cationic meso-tetra-pyridyl porphyrin (T4PyP) and its metallocomplexes (MnT4PyP, AgT4PyP, ZnT4PyP and CoT4PyP).

Blood pressure (BP) measurements were performed in acute experiments under nembutal anesthesia (40 *mg/kg*, intraperitoneal) in 40 cats of both sexes (1.5–2.5 *kg*) and 50 mongrel albino male rats (200–250 *g*). Registration of BP in chronic experiments was carried out in 50 awake rats (200 – 250 *g*). BP of cats was registered by microcatheter inserted into femoral artery using mercury monometer. Registration of BP in rats was carried out both in acute and chronic experiments with the help of tail cuff method. All the animals were randomly divided into 5 subgroups respectively to porphyrins.

In acute experiments the investigated compounds were injected intravenously (0.2 *mg/kg* and 0.4 *mg/kg*, cats and 0.2 -3 *mg/kg,* rats) and in chronic experiments - intraperitoneally (3 *mg/kg/day* during 6 days, rats). Cardio- and hepatotoxicity of the compounds (3 *mg/kg/day* during 6 days, *i.p.*, rats) was investigated in 72 mongrel albino male rats (180-220 *g*) by determination of activities of the following enzymes: aspartate transaminase (AST), alanine transaminase (ALT), creatine kinase (CK), lactate dehydrogenase (LDH).

In acute experiments all the investigated compounds exhibited a significant dose-dependent hypotensive activity in cats only. MnT4PyP (0.2 *mg/kg, i/v*) exhibited a maximum hypotensive activity (43.1±1.9%), and T4PyP manifested a minimal hypotensive effect (9.7±1.94%) at the same dose. At dose level of 0.4 *mg/kg* (*i/v*) MnT4PyP was also the most active compound (48.3±1.9%), and CoT4PyP manifested a minimal effect (15.3±2.7%). In chronic experiments (3 *mg/kg* daily, *i.p.*) only MnT4PyP demonstrated a hypotensive activity (20.7±1.77%) after 6-day administration. BP changes were not accompanied by any noticeable changes in the heart rate of cats and rats. None of the investigated compounds manifested any influence on the activities of AST, ALT, CK, LDH thus testifying to an absence of cardio- and hepatotoxicity in investigated compounds.

Thus, the mentioned compounds have no organotoxic activity and manifest dose-dependent (acute experiments, cats) and species-dependent hypotensive activity. MnT4PyP has a significant hypotensive activity in both acute (cats) and chronic (rats) experiments.

CYTOTOXIC EFFECTS EXERTED BY POLYARYLSULFONE DIALYSER MEMBRANES DEPEND ON DIFFERENT STERILIZATION PROCESSES

El Golli-Bennour E.[1], Kouidhi B.[1], Dey M.[1,2], Younes R.[1,2], Bouaziz C.[1], Zaied C.[1], Bacha H.[1], Achour A.[1,2]

[1] Laboratoiry of Research on Biologically Compatible Compounds, Faculty of Dentistry, Monastir, Tunisia

[2] Nephrology Service, Haemodialysis and Renal Transplantation, CHU, Sahloul, Sousse, Tunisia

E-mail: emnagol@yahoo.fr; Tel.: +216.98.61.56.37;

Polyarylsulfone group is one of the most important polymeric materials used in the biomedical field, due to its excellent properties, such as good thermal, chemical, and mechanical stability. There are three important polyarylsulfone polymers, all of which have excellent electrical properties: polysulfone (PSu), polyarylsulfone (PAS) and polyarylethersulfone (PAES). All these polymers have excellent creep, radiation and high temperature resistance.

In this study, we aimed to determine the effect of three sterilization processes (steam, ethylene oxide and gamma rays) on cytotoxicity of polyarylsulfone dialysis membranes.

Ten long-term dialysis patients and ten age-matched healthy controls were enrolled in our study. We analysed serum effect on cultured endothelial cell viability using MTT assay and lipid peroxidation assessed by serum malondialdehyde (MDA) formation at the beginning (T0), the middle (T2) and the end (T4) of haemodialysis (HD) session.

Our results clearly showed that steam-sterilized membranes improve endothelial cell viability when compared to ethylene oxide or gamma rays-sterilized ones. Moreover, there is an increased generation of MDA in patients' sera during HD session. The serum MDA concentration was about 3, 6 and 10 times higher, respectively, for steam, ethylene oxide and gamma rays sterilization procedures when compared to MDA amount in healthy subjects' sera.

Using steam instead of ethylene oxide or gamma rays for sterilization may improve the biocompatibility of polyarylsulfone membranes.

PREVENTION OF ADRENALINE-INDUCED PATHOMORPHOLOGICAL ALTERATIONS IN RAT LUNGS AND MYOCARDIUM BY MECHANICAL LUNG VENTILATION

Feschyan S., Semerjyan A., Mkhitaryan A., Krasnikov N.

Medical Biology Department, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: annasemerjian@hotmail.com; **Phone:** (37 41 0) 560 323

The emotional stress induces increased secretion of catecholamines releasing into blood that elevates oxygen consumption by myocardium and imbalance between myocardial contraction and oxygen supply. High doses of catecholamines exert cardiotoxic effect: progressive hypoxia and necrosis of cardiomyocytes.

However, pathogenesis of metabolic (adrenaline-induced) myocardial injury remains uncertain by now. Moreover, a number of pharmacological agents do not completely prevent myocardial infarction.

The aim of present research was to study adrenaline-induced pathomorphological alterations in the rat myocardium and lungs, as well as to prevent these changes by improving lung ventilation.

Experiments were performed in albino male rats:

- Control group 1: intact animals (n=6);
- Group 2: rats (n=6) injected 0.18% adrenaline hydrotartrate 0.5 *mL/kg* (intravenously);
- Group 3: rats (n=6) assisted to mechanical lung ventilation (MLV) prior to adrenaline injection (anesthesia by nembuthal).

Wet mass of lungs and heart was weighed. Morphology of lung and myocardium was studied by hematoxylin-eosin staining. Pulmonary and heart microcirculation was detected on freezing microtome sections by Ca-ATP histochemical method. Mean capillary diameter (D) was measured by ocular micrometer. Capillary density (CD) *per* mm^3 of myocardial tissue was assessed as total length (L) of capillaries. Capillary metabolic surface (CMS) and capillary bed capacity (CBC) were calculated.

NO synthase (NOS) activity was assessed by NADPH-diaphorase histochemical method.

Statistical analysis was realized by Student's *t*-test.

The lungs of adrenaline-injected rats weighed 3.7±0.4 *g vs.* 2.5±0.13 *g* in control (increased by 48%; $p<0.001$), and the heart weight increased by 15% (0.84±0.05 *g*; $p<0.05$) compared to control (2.5±0.05 *g*) due to edema. Lungs weighed 3.0±0.24 *g* in MLV-assisted group. Hematoxylin-eosin stained myocardial sections of Group 2 animals showed cardiomyocyte degeneration zones of necrosis, absence of linear orientation of myofibrilles, neutrophil infiltrations that were not observed after MLV exposition.

Ca-ATP staining method revealed capillary rupture sites in lungs, dilated alveolar acini along with destruction of interalveolar septa, deformation, and swelling of bronchiole walls in Group 2 animals. MLV-exposed rats exhibited no pathomorphologic changes in lungs, which were similar to intact animals.

Myocardial microcirculation parameters that were altered following the adrenaline injection returned almost to norm in MLV exposition conditions.

NOS activity was much decreased in lungs and myocardium of Group I rats, while in hyperventilated rats the NO synthase activity was restored.

Results obtained allow concluding that adrenaline-induced alterations of myocardium express in decrease of local myocardial blood supply, disorders of pulmonary microcirculation and reduction of NOS activity both in lungs and myocardium, which may lead to myocardial damage and lung injury that can be prevented by alveolar gas exchange improvement via MLV.

THE CONTENT OF ORGANIC MATTER IN RIVER WATER OF MEGHRIGET AND VOGHCHI RIVERS CATCHMENT BASINS

Gevorgyan G.[1], Danielyan A.[1], Grigoryan K.[1], Minasyan S.[2]

[1]Department of Ecology and Nature Protection, Yerevan State University, Yerevan Armenia

[2] Environmental Impact Monitoring Center, Yerevan Armenia

E-mail: a.danielyan@hotmail.com, a.danielyan@ysu.am

The oxidation is an index of organic matter content in waters, thus a quantity of oxygen that is used during the organic matter oxidation.

The organic matter composition in waters is formed by numerous factors. Among the major factors are synthesis and exchange biochemical processes, inflow from other water objects (surface and ground waters, atmospheric precipitations, agricultural, industrial, and municipal wastewaters).

Oxidation complies with regularities of seasonal variations. Their character is determined by the hydrological and hydrobiological regimes of rivers.

The variability of oxidation index is determinant of wastewaters' import regime into the reservoirs being under the active economic activity.

Catchment basins of Meghriget and Voghchi Rivers are located in the south of the Republic of Armenia. The area of the catchment basins includes Kapan and Meghri regions of the Syunik district. The industry and agriculture are developed at the catchment areas. Industrial and domestic wastewaters without sufficient cleaning flow directly to open water basins of the abovementioned catchment areas polluting them.

The objective of research is to explore the content of organic matter in catchment areas of Voghchi and Meghriget Rivers.

The research was performed in 2008-2009. As a determination index of organic matter's content in water samples values of the permanganate oxidation index measured by Kubel method were used,

Nonpolluted nature waters mainly contain small quantities of organic matter. Usually, high concentrations of organic mater content are observed in the waters polluted with industrial and agricultural wastewaters. In addition, the increase of oxidation index can be observed during the overflow of rivers, as a result of which large quantity of organic matter penetrates into the rivers from soil, however this regularity was not observed in rivers of catchment basins of Meghriget and Voghchi Rivers. According to the scale of water pollution, as for index of permanganate oxidation, water quality of explored rivers ranged from pure to dirty classes of waters. High values of permanganate oxidation index were noticed particularly after the big residential areas of the catchment basins, especially after Kajaran town.

Abovementioned discussions allow us to suppose that the high content of organic matter in the river waters is mainly conditioned by the industrial and municipal wastewaters, as well as by the agricultural water flows.

ACTIVITY OF OSMOTIC PRESSURE ON STABILITY OF BILAYER LIPID MEMBRANE

Gevorgyan H.[1], Potikyan G.[2]

[1] Chair of Molecular Physics, Physics Department Yerevan State University, Yerevan, Armenia

[2] Department of Medical and Biological Physics, Yerevan State Medical University, Yerevan, Armenia

E-mail: gevhaygo@mail.ru; **Phone:** +37477 377686

The problem of membrane stability is a central one in membranology. In view of exceeding complexity of cellular membrane, this problem is reasonable to study in a model: bilayer lipid membrane (BLM). It is known that frequently the electric field acts on membrane, likewise the osmotic pressure. Therefore, it is of interest to investigate the joint activity of the electric field and the osmotic pressure on the stability of BLM.

In the given work the joint activity of the osmotic pressure and transmembrane potential difference on the stability of BLM was experimentally investigated.

As the capacity parameter a mean lifetime of BLM was taken at the given magnitudes of electric field and the osmotic pressure.

The experiments were performed on BLM obtained from phosphatydilserine suspended earlier in nonane. BLM was formed by the method of Muller and co-authors on a hole with a 1 *mm* diameter in a polytetrafluorrethylene (PTFE) cell. On the both sides of the membrane a 0.1 *M* NaCl, pH=6.1 solution (10 *mL*) was positioned. All experiments ran at temperature of 20°C. Potential difference on BLM was provided by silver-chlorine electrodes, which were connected with ADS (NI USB-6008), measuring process controlled by computer. Potential difference was given from 150 *mV* to 300 *mV* in step of 50 *mV*. After whole blackening the osmotic pressure was created by addition of 0.1 *mL* NaCl solution in one

cell compartment, while in the second there was the same amount of solution with a certain content of saccharose, so that in the compartment the solution of saccharose was obtained at concentration of $6 \cdot 10^{-6}$ *mol/L*, which creates osmotic pressure of 15 *Pa* on BLM. The mean lifetime of BLM was defined by computer program.

At first the change of the mean lifetime of BLM with increasing of potential difference in absence of osmotic pressure was investigated. It was shown that the activity of electrostatic field brings decreasing of BLM mean lifetime. After the influence of potential difference on BLM mean lifetime was experimentally investigated at addition of osmotic pressure of 15 *Pa*. It was shown that a dependence curve of BLM mean lifetime with potential for the second case is lower than in the first case. Consequently, the comparison of theoretical curve with experiment points by least-squares method curve parameters A and B was determined, describing the dependence of the mean lifetime of BLM with potential.

$$\ln \bar{t}(\varphi) = A + \frac{B}{1 + \frac{C\varphi^2}{2\sigma}}$$

where $A = \ln((kT)^{3/2} / (4\pi n D\gamma(\sigma + C\varphi^2 / 2)^{1/2}))$, $\quad B = \pi\gamma^2 / (\sigma kT \cdot \lg e)$.

Where σ is surface tension of BLM ; γ - linear tension of edge pore of BLM, n - number of defects on membrane, D - coefficient diffusion of defects in radius space, , φ - potential difference on membrane , k - Boltzman constant, C - brought capacity, which is defined by correlation, $C = C_0 \left(\varepsilon_w / \varepsilon_m - 1\right)$, where $C_0 = \varepsilon_0 \varepsilon_m / h$ - specific electric capacity of membrane, ε_w - dielectric penetrability of water, ε_m - dielectric penetrability of BLM.

It turned out that for both cases the magnitudes of **A** parameter concur practically (A=3.64 and A=3.52), but the magnitudes of **B** parameter are different by value (B=19.49 and A=18.64).

The loss of stability of BLM at addition of osmotic pressure is conditioned by two factors: either changing linear tension of BLM pore, changing the numbers of defects on BLM. Upon analysis of theoretical formula and its confrontations with obtained dataon **A** and **B** show that decreasing BLM mean lifetime at addition of osmotic pressure can be connected with the changes of linear tension. It is indicated that the difference in osmotic pressure of BLM does not bring to increasing of an area.

THE BIOCHEMICAL MECHANISMS OF ACTION OF GUM FROM *ARMENIACA VULGARIS LAM*

Ghazanjyan M., Chichoyan N., Simonyan M.
Department of Pharmacognosy, Yerevan State Medical University, Yerevan, Armenia
E-mail: pharmacognosy@ysmu.am, mariamghazanjyan@yahoo.com; **Phone:** 091 5069 35

The apricot gum from *Armeniaca Vulgaris Lam.* possesses up to 1% Superoxide dismutase (SOD) mimeticl activity and after its 48 *h* incubation in aerobic conditions at 4°C has practically no effect on the optical-spectral properties and the level of antioxidative and prooxidative activity displayed by key metalloproteins *in vitro*. This gum elevates the level and activity of mentioned metalloproteins isolated from erythrocyte membranes and blood after 48-hour and 5-day incubation of the erythrocyte membranes and blood, correspondingly, in aerobic conditions *ex vivo*.

Simultaneously, the increase of the erythrocyte membranes (EM) stability decreases the releasing degree of cytochrome b_{558} from EM, as well as the increase of the NADPH-dependent O_2^- producing and metHb-reducing activities of cytochrome b_{558} from EM in homogeneous and heterogeneous phases take place.

The research was aimed at invention of molecular and biochemical mechanisms of the action displayed by apricot gums on metabolism of active forms of oxygen.

Cultivated apricot gums were used to study their molecular biochemical mechanisms. The aerobic incubation of 20 *mg/cc* gum was generated with cytochrome b_{558} fraction separated from spleen cell membranes under *in vitro* conditions. The SOD activity was determined by nitrotetrazolic blue (560 *nm*), considering the percentage content of formazane by superoxide radicals as a result of reduction of nitrotetrazolic blue.

The NADPH-dependant O_2^- producing activity of cytochrome was determined by counting the quantitative content of formazane formation stimulating protein.

To determine the methemoglobin reducing ability of a cytochrome the freshly prepared ferrihemoglobin was used and its reducing kinetics of optical absorbing intensity (565 *nm*) was determined in the conditions of the influence of the reaction mixture.

The results of analysis show that hemoprotein's NADPH-dependant O_2^- producing and methemoglobin reducing activities of apricot gum during the incubation time with cytochrome b_{558} fraction *in vitro* conditions practically do not change. However, in corresponding *ex vivo* conditions after the incubation time of the gum with spleen cell membranes, the hemoprotein's NADPH-dependant O_2^- producing and the methemoglobin reducing activities significantly increase, as well as cytochrome b_{558} fraction in homogenous medium and is directly dependant on the activity of cytochrome b_{558} separated from spleen cell membranes in heterogynous medium.

The corresponding mechanism of action of the gum is possibly connected to its superoxidedismutasing activity, decreasing the level of superoxides, where the level of hydroxyl radicals decreases as well

(especially in heterogenous medium), which appears as a denaturating factor for cytochrom b_{558}.

In conclusion, we can say that apricot gum can stimulate not only the immune system, but also the oxygen homeostasis.

THE PHARMACOKINETICS OF ARMENICUM PASTE TESTED IN RATS

Ghazaryan A., Topchyan H., Hovhannisyan A., Abrahamyan H., Muradyan R.

Department of Pharmaceutical Technology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-Mail: ani_ghazaryan88@yahoo.com; h_topchyan@yahoo.com

Preparation "Armenicum paste for external use" contains the complex of potassium iodide and iodine with dextrin and, according to the preclinical trials, accelerates the wound healing processes caused by burn.

The aim of this study is investigation on pharmacokinetics of iodide anion in rats after application of the "Armenicum paste for external use".

Preparation Armenicum: 100 *g* of the "Armenicum paste for external use" contain an active ingredient: solid concentrate of Armenicum preparation (22.5 *mg*); inactive ingredients: glycerol monostearate, ceteareth 12, ceteareth 20, cetostearyl alchohol, paraffin liquid and glycerine.

Eighty-four Wistar's rats (male, pubescent rats with body mass of 120-160 *g*) were used in the experiments. The animals were divided into three groups:

1. Intact group: 6 rats.
2. Control Group: 6 rats, which were exposed to burns, but were not treated with Armenicum paste.
3. Experimental group: 72 rats, which were exposed to burns and were treated with Armenicum paste.

Third-degree burn of 10-12 % of the body surface was inflicted to the' dorsal side of animals, exposing them to application of the hot copper plate with the area of 6 cm^2 on the skin.

The exposure duration was 10-15 *sec*, and the temperature under the skin reached 50-60°C. In 5-7 minutes after exposing the animal to burn, Armenicum paste was applied on the surface of the wound with 1 *mm* layer thickness, which completely covered the wound. Exposition of burns in animals and the following manipulations were done under ether anesthesia. After burns the rats were kept separately, without wound dressing. The rats of the intact group were neither exposed to burns, nor treated with Armenicum paste. Blood samples were taken after decapitation of the animals in 0; 0.25; 0.5; 1; 1.5; 2; 3; 4; 6; 8; 10; 14 and 24 hours after application of Armenicum paste. For each time-interval 6 rats were used.

Iodide anion was defined by the potentiometric method on the instrument 720A MTR using iodide-selective electrode "Ionplus iodide, W/B orion ionplus iodide electrode" (Thermo Fisher Scientific, USA).

The calculations of the pharmacokinetic parameters were implemented by pharmacokinetic program Kinetica 4.4.1 (Termo Electron Corporation, 2004).

Statistical analysis was performed by means of the computer program Statistic for Windows, version 6.0. All the statistical calculations were carried out for 95% confidence interval.

The results of the study showed that in 15 *min* after application of paste the concentration of iodide anion in blood plasma makes approximately 0.*3 μg/mL*. Subsequently it progressively rises to 13 *μg/mLl* and reaches to the first maximum in 3 hours after application of the paste. In the range of 3-4 hours, the level of iodide anion decreases to 7 *μg/ml*. Then the concentration of iodide anion rises again and the basic maximum of the concentration in blood plasma in 8 hours is 20 *μg/mL*.

Subsequently, the level of iodide anion exponentially reduces to 4 *μg/mL* in 24 hours.

Thereby the pharmacokinetics of the concentration of iodide anion in plasma is characterized by two peaks: in 3 and 8 hours after application of the paste, meanwhile the level of iodide anion in 8 hours is three times higher than in 3 hours.

It was established that the pharmacokinetics of iodide anion is characterized by rather slow absorption in circulatory blood system from the place of application of the paste. The maximal concentration of iodide anion in plasma reaches 20.68±7.00 *μg/ml* in 6-8 hours (mean value is 7.67±0.8 hours) after its application. The half-life of iodide anion is 10.21±1.931 hours, and mean retention time is 17.9± 3.1 hours. Calculations made according to the first-order pharmacokinetic equation allowed to establish that the absorption half-life ($t_{½abs.}$) is 4.71 hours. Therefore, the absorption in the circulatory blood system of iodide content in the paste may finish in 28-30 hours after its application to a wound ($7 \cdot t_{½abs.}$).

It can be concluded that in one day after application of Armenicum paste on the wound caused by burn, approximately 97% of iodine, as one of active components of the preparation, is absorbed in blood. The iodide anion is completely eliminated from the organism within 48-70 hours.

Long time preservation of iodine, a powerful anti-viral and bactericidal substance on the wound, can open a big prospect for use of Armenicum paste preparation in treatment of burns and infected wounds.

METHANOL EXTRACTS ANTIRADICAL ACTIVITIES IN SOME NUITRITIVE AND HERBAL PLANTS OF ARMENIAN FLORA

Ghochikyan A.[1], Mchitaryan S.[1], Topchyan H.[1], Ananikyan V.[2], Yeribekyan M.[2]

[1] Department of Pharmaceutical Technology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

[2] Scientific-Technological Centre of Organic and Pharmaceutical Chemistry, National Academy of Sciences, Yerevan Armenia

E-mail: anna_ghochikyan@mail.ru; **Phone:** (094) 35-00-35

Among the 24 plants (ethanol extracts and fresh fruits juices), which were examined during our previous studies antiradical activities were displayed by the following: *flores Sambucus nigra* – 92.5%, *fructus Sambucus nigra* – 85%, *folium Juglans regia* – 71.5%, unripe *fructus Juglans regia* – 86%, *fructus Ligustrum vulgaris* – 86%, *herba Achillea millefolium* – 49%. Some antiradical activities were also displayed in *rhizoma cum radicibus Inula helenium* – 17.5%. On the basis of the phytochemical extract analyses we can conclude that their antiradical activities depend first of all on the presence of highly-active phenolic combination (sambunigrin, anthocyans, flavonoids, juglon, tocopherol, tannins, sesquiterprnoid lactons), which displays neuroprotective influence according to literature.

The research includes determination of the antiradical activity depending on extract preparation technology, particularly depending on a solvent and herb substance crumbling degree.

The research was done in 15 herbal substances. The extract was obtained by maceration method, and 99.9% methanol was used as a solvent. The analyses for antiradical activity display were implemented by intensive method. The measurements were carried out three times. The content (% and *g*) of dry remainder was also determined in the investigated extracts with the help of "KERN MLB50-3" instrument (Germany).

Eleven extracts displayed antiradical activities for which quantity decision was made. The extracts differed from each other by the intensity display of antiradical activity.

Fructus Hippophae rhamnoides, total *fructus Cerasus vulgaris* and *fructus black Mulberry* did not show any antiradical activity in our research. *Rhizoma cum radicibus Inula helenium* shows a little antiradical activity.

In our research methanol extracts showed considerable antiradical activities compared with 70% methanol extracts and fresh fruit juice.

In our previous work ethanol preserved fresh black cherry, fresh cherry and cornel fruits did not display antiradical activity; at the same time their antiradical activity in methanol extracts is considerably high (72% and 73%, accordingly).

The leaves of black mulberry, black currant did not display antiradical activities in ethanol extracts, but in methanol extracts it is high: 81% and 72%, accordingly.

In two cases the antiradical activities are on the high level in *flores Sambucus nigra* extract (83% to 100%) and in *folium Juglans regia* extract (from 71.5% to 83%).

The crumbling degree of the material manifested essential influence on our researches. It is namely obvious in the example with total *fructus Cerasus vulgaris,* the extracts did not show antiradical activity, but the crumbled fruits of cherry showed significant antiradical activity (72%). The difference between antiradical activities is insignificant in the extracts of crumbled and total black cherry, which is connected with the thin peel, which does not make any difficulty for the extraction.

Methanol extracts show more significant antiradical activity compared with 70% methanol extracts and fresh fruit juice. Methanol is considered more optimal solvent for the research.

The crumbling degree of a herbal substance also has a great influence on antiradical activity.

SYNTHESIS OF NEUROPROTECTIVE DRUGS AND QUANTITATIVE ASSESSMENT OF THEIR ACTIVITY BY COMPUTED MODELING

Grigoryan A.[1], Mikaelyan A.[2], Karapetyan S.[1], Melikyan T.[1], Sargsyan V.[2], Nikoghosyan L.[2], Vardanyan A.[2,3], Hambarthumyan A.[3], Torosyan G.[3]

[1] Medical Chemistry Department, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

[2] State Engineering University of Armenia, Yerevan, Armenia

[3] "Scientific-Research Institute of Biotechnology" CJSC, Yerevan, Armenia

E-mail: anyuta742004@yahoo.com; **Phone:** 094 41 4490

Such cyclic homologues of glycine as l-aminocyclobutane-1-carboxylate (ACBC) were reported to exhibit activity at N-methyl-D-aspartate (2a5t-NMDA), which was characterized at a single channel level. Antagonists of the NMDA receptor are well recognized for anticonvulsant, antispastic, and neuroprotective properties. Moreover, according to recent data elaboration of convenient synthetic techniques for creating functionally substituted cyclobutane carboxylic acids is a very important task for synthesis of new rationally designed pharmaceutical agents with neuroprotective activities.

Generic methods for construction of highly substituted cyclobutane carboxylic acids were developed by transition metal catalyzing intramolecular cyclization reaction of alpha-halogen alkenoic acids and their derivatives via atom transfer radical cyclization (ATRC).

The driving force of this work is identifying new application fields of readily available intramolecular stereocontrolled cyclization reactions of the polyhalogenhexenoic acids derivatives into unique cyclobutane derivatives and their application for obtaining physiologically active compounds. It was known that trans-l-aminocyclobutane-l,3-dicarboxylic acid, which is the analogue of glutamic acid, is an extremely potent N-methyl-D-aspartate (NMDA) receptor agonist. *In vivo* studies of these dicarboxylic acid derivatives revealed highly expressed anticonvulsant activities.

Bioactivity investigation of the individual isomers, or isomers pairs yielded valuable knowledge about the site(s) of action and emphasize the three-dimensional structure-activity relationship. For prelimi-

nary estimation of physiological activity of newly synthesized compounds computed modeling is widely used now. AutoDock 4.0 is one of the most felicitous modeling programs, among the numerous ones. It is used by famous and highly rated pharmacological companies. Molecular modeling of the newly synthesized compound structure and their comparison with known analogous structures for activity estimation and preliminary docking studies of ligand with receptors models will give us valuable information about molecular mechanism of neuromodulating activities.

The AutoDock 4.0 testing of the following enantiomer cyclobutane derivatives gave: (1S,2S)-1-aminocyclobutane-1,2-dicarboxylic acid – ΔG = -18.58 *kcal/mol*, Ki = 24.09 fM; (1S,2R)-1-aminocyclobutane-1,2-dicarboxylic acid – ΔG = -10.56 *kcal/mol*, Ki = 18.19 nM; (1R,2S)-1-aminocyclobutane-1,2-dicarboxylic acid – ΔG = -10.57 *kcal/mol*, Ki = 18.01 nM; (1R,2R)-1-aminocyclobutane-1,2-dicarboxylic acid – ΔG = -29.39 *kcal/mol*, Ki = 284.71 yM; 1-amino-2,2-dimethyl-3-chloro-4-trichloromethylcyclobutanecarboxylic acid – ΔG = -26.97 *kcal/mol*, Ki = 16.90 zM; 1-amino-3-chloro-4,4-dimethylcyclobutane-1,2-dicarboxylic acid -ΔG = -1.05e+7 *kcal/mol*, Ki <0.10 zM.

Similarly, methods for synthesis of a series of practically important α-halogenecyclobutanecarboxylic acids highly substituted by functional groups were worked out. They have potential application for synthesis of α-aminocyclobutanecarboxylic acids, which can serve as a base for synthesis of new neuroprotective drugs. Moreover, this method allows preparation of a wide series of derivatives from readily available raw materials. The new quantitative parameters for estimation of neuromodular activity of newly synthesized compounds were also found out and they were compared with the known analogues of the compounds with described structure and property interaction of the suggested ligands with models of ion channels by Auto dock 4.0.

These results allow us to adjust the strategy of the synthesis of such kind compounds.

THE INVESTIGATION OF ANTI-INFLAMMATORY ACTIVITY OF SOME NOVEL SYNTHETIC A-NON-PROTEIN AMINO ACIDS

Grigoryan S., Saghyan A., Balasanyan M., Zhamharyan A.
Department of Pharmacy, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia
E-mail; sonagrigorian@mail.ru; **Phone:** 093582241

Creation of novel non-steroidal anti-inflammatory drugs (NSAID) continues to be one of the most important and contemporary problems of modern pharmacology, whereas despite the variety of medicines and wide application of this group (according to the statistical data, about 300 million people use NSAID annually), the majority of them is not free from serious side effects typical for this group. In the series of NSAID the investigation of the structure-activity relationship shows that the aminopropionic acid compounds with aryl and heteroaryl substituent possess cycloxygenase inhibiting activity and have

anti-inflammatory action as well. On the other hand, very interesting data concerning the high and wide specter of pharmacological activity of non-protein amino acids (including even the anticancer activity) was obtained. In view of this, the anti-inflammatory activity of derivatives of non-protein amino acids was investigated.

The aim of the present study was to investigate anti-inflammatory activity of α-methyl-L-phenylalanine, β-(N-benzyl amino)-L-alanine and L-5-propyl-4-allyl-1,2,4-triazol-3-yl-cystein and detect a relationship between the structure and anti-inflammatory action of the above mentioned amino acids.

The investigation was carried out in non-bred white rats weighing 170-200 *g* that were divided into 4 groups: the control group and 3 experimental groups. NaCl solution (0.9%) was injected intraperitoneally (*i.p*) to rats of the control group and solutions of the above mentioned compounds at a dose of 10 *mg/kg* were injected *i.p.* to the animals from the experimental groups. The anti-inflammatory activity was investigated in the experimental model of acute inflammation, according to which among experimental rats ear edema was induced by 0.03 *mL* xylene. The anti-inflammatory activity of the preparations was estimated by the rat ear mass difference (the right: inflamed and the left: intact) after 90 minutes of compound injections.

According to the obtained data, the mass of rat ear inflamed with xylene was increased by 127.6±24.57 *mg* compared with the non-treated ear. Intraperitoneal injection of α-methyl-L-phenylalanine at a 10 *mg/kg* dose results in reduction of the above mentioned sizes up to the 70.71±10.04 *mg* ($p<0.05$), which testifies the growth prevention of xylene induced ear edema in rats by 44.6% as compared with the control group. The anti-inflammatory activity of the compound was preserved in a case of displacing the aromalic ring with benzyl amino group. So, in case of β-(N-benzylamino)-L-alanine administration a difference between masses of inflamed and intact rat ears is 66.37±18.1 *mg* ($p<0.05$), which testifies that above mentioned compounds prevent development of the edema by 47.97% as compared with the control group. Thus, results of investigation on structure-activity relationship testifies that among the investigated compounds L-5-propyl-4-allyl-1,2,4-triazol-3-yl-cystein does not exert any activity in the model of acute inflamed rat ear.

Obtained results testify that a presence of aminopropionic acid residue in a molecule and benzene ring in the radical is very important for anti-inflammatory activity of the aminopropionic acid derivatives. Changing the aromatic ring by 1,2,4-triazole one ,as a substitution, leads to the sharp decrease of anti-inflammatory activity.

ELECTROPHYSICAL STUDY ON SENSITIVITY OF MICROBIAL CELLS TO SULFANAMIDE

Guliy O., Ignatov O.

Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Saratov, Russia

E-mail: guliy@ibppm.sgu.ru; guliy_olga@mail.ru

Study of the adaptation of microbes to sulfanamide action is an important problem that is of theoretical and applied significance. One can assume that changes in cell morphology and cell-wall disturbances in microorganisms should lead to changes in their electrophysical characteristics. These changes are reflected in alterations in the electro-optical (EO) characteristics of cell suspensions, which are recorded during experiments using electric-field cell orientation. On the basis of these alterations, one can draw tentative conclusions about the presence or absence of resistance to a given antibiotic in the cells under study.

The aim of this work was to study the effect of sulfanamide on the EO parameters of cell suspensions of *Escherichia coli* strains.

The strains *E. coli* B-878, K-12 and BL21 were used. The orientational spectra (OS) of the cells were measured with the ELUS EO analyser developed by the State Research Center for Applied Microbiology (Obolensk, Russia) at 670 *nm* (relative to vacuum), as described by O. Ignatov et al. (2002). A discrete set of frequencies of the orienting electric field (740, 1000, 1450, 2000, and 2800 *kHz*) was used. The cell suspension was standardized before analysis by dilution with distilled water (conductivity, 1×8 μS *mL-1*) to $4\times5\times10^8$ *cells Ll-1*.

We examined the effect of sulfanamide on the electrophysical characteristics of *E. coli* cells. Substantial changes in OS of suspensions of cells incubated with various sulfonamides concentration (0.04, 0.08, 0.3, 0.6 $\mu g/mL^{-1}$) took place only at the first five frequencies of the orienting electric field (740, 1000, 1450, 2000, and 2800 *kHz*). The change in the magnitude of the electro-optical signal occurred at 0.04 $\mu g/mL^{-1}$ of sulfonamides, and maximal change in the magnitude of the electro-optical signal occurred at 0.6 $\mu g/mL^{-1}$ of sulfonamides.

At studying the dynamics of influence sulfanamide (concentration of 0.6 *mg/mL*) on microbial cells during 5, 10, 20, 30 *min* it was shown, that there is a 31% decrease in EO signal after 5 *min* of influence, after 10 min of influence by 59 %, at the subsequent influence of sulfanamide the EO signal practically did not change.

Check experiments by standard seeding on the dense nutrient mediums were performed and the results confirmed data of the EO analysis.

Thus, it is shown that EO effect of microbial cells at sulfanamide action to a preparation depends on the concentration of a substance. Thus, the suspension-OS changes occurring under the effect of sulfanamide may be used as a test for resistance of studied cells to this sulfanamide. The possibility is suggested

of using electrophysical methods to study the mechanism of sulfanamide action on bacterial cells, with a view to check sulfanamide action of microorganisms. The obtained results can serve as a basis for the development of a rapid electrophysical test for the intra-species detection of sensitivity of microbial cells to sulfanamide.

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MOLECULAR DYNAMICS STUDY OF α-TUBULIN AND INDIBULIN INTERACTION

Hakobyan D.[1], Varzhabetyan L.[2], Nazaryan K.[1,2]

[1] Laboratory of Computer Modeling, Institute of Molecular Biology, National Academy of Sciences of Armenia, Yerevan, Armenia

[2] Department of Bioinformatics of Biomedical Faculty, Russian-Armenian (Slavonic) Yerevan, Armenia

Microtubules are known to be formed of spiral polymeric chains of α- and β-tubulin. They are the basic components of cell structure, which take part in a wide number of pivotal cellular functions. Drugs that are able to modulate the microtubule assembly either by inhibition of tubulin polymerization or by blocking microtubule breakdown are of great interest in anti-cancer therapy. The toxicity of these anti-mitotic agents is of the major concerns for drug developing organizations.

Our laboratory conducts experiment aimed at determining the binding details and estimation of binding free energy of indibulin (D-24851) and alpha-tubulin since the binding sites of this drug to tubulin are yet not well known, while indibulin seems to have certain therapeutic value in cancer treatment.

Methods of molecular dynamics and quantum mechanical (QM) calculations are used to build the indibulin approximate 3D structure and force field parameters for both all-hydrogen CHARMM22 and EEF1 implicit solvation models. Sixty different orientations were investigated (simulated with the help of implicit solvation MD techniques) for a period of 1.5 nanoseconds biological time with and without acetylated Lys40 at α-tubulin site.

EEF1 implicit solvation simulations of 30 different orientations of indibulin positioned around Lys40 as well as 30 orientations of the same indibulin positioned around acetyllysin40 were studied. The results show that effective interaction energy of indibulin with tubulin varies from 0 to -20 *kcal/mol*. Indibulin interaction energy with Lys40 laid in the range from -10 to -20 *kcal/mol*, while it was in the range from 0 to -8 *kcal/mol* when Lys40 was replaced with acetyllysine.

According to preliminary results 1.5 *ns* interaction simulations for indibulin with alpha-tubulin show higher interaction with Lys40 than with its acetylated derivative. This seems to agree with experimental

results where indibulin does not show inhibition of microtubule polymerization with acetylated alpha-tubulin.

The details of conformation of binding of indibulin to tubulin Lys40 site are subject to further implicit and explicit solvent simulations.

The results obtained will serve to better understand microtubule polymerization inhibition mechanism and will give a possibility of designing improved analogs.

LIPID REMODELING PROCESSES IN BLOOD LYMPHOCYTES AT CANCER

Hakobyan G.[1], Batikyan T.[1], Lazyan M.[1], Torgomyan T.[1], Galstyan H.[2], Alexanyan K.[2], Amirkhanyan E.[3], Tadevosyan Yu.[1]

[1] Institute of Molecular Biology, National Academy of Sciences, Yerevan, Armenia

[2] Centre of Oncology after V. Fanarjyan, Ministry of Health of the Republic of Armenia, Yerevan, Armenia

[3] Centre of Hematology, Ministry of Health of the Republic of Armenia, Yerevan, Armenia

E-mail: hakgohar@rambler.ru

Although evidence of alterations in lipids and fatty acids (FA) metabolism in diverse cell populations at cancer is well documented, the potential use of such changes, especially in blood lymphocytes, as index to the aggressive clinical behavior has not been addressed. Recent studies have shown the prevalence of regulatory T lymphocytes (T_{reg}) population in the blood and tumor microenvironment compared with healthy individuals. This fact supposed as a possible reason to promote immunosuppression accompanying neoplastic diseases. We hypothesized that investigation of different membrane-associated lipid modification mechanisms in the peripheral blood lymphocytes, which are responsible for aberrant lipid metabolism at malignancy, may be a successful strategy for cancer detection, state value of pathological process and discovery of novel targets and modes for the personalized treatment of cancer.

The current scientific investigation is intended at revealing the identity of changes in the processes going on the cellular and molecular levels which can be laid in the basis of the pathological responses of an organism at diverse forms of such system disease as cancer.

The aim of the present work was to conduct a comparative investigation of membrane phospholipids (PL) and neutral lipids (NL) FA content modification processes at norm and diverse forms of cancer, particularly, at acute lymphoblastic (ALL), acute myeloblastic (AML) leukemias, breast (BC) and ovarian (OC) cancers. It also follows the objective of studying the activities of certain lipid modification enzyme systems in the plasma membrane (PM) fraction isolated from lymphocytes of clinically healthy people and patients with cancer.

The intact lymphocytes of peripheral blood and PM fraction separated from them were used as objects

for the experiments. We applied some contemporary and sensitive radioisotopic methods using [^{14}C] labeled FAs and lipid substrates.

We revealed significant differences in the rapid (5 *sec*) acylation of primary targets of lymphocyte PM lipids at ALL, AML and BC patients compared to norm. The blood lymphocytes at acute leukemias are characterized by the activation of FA content modification processes in certain lipid fractions such as aminophospholipids, phosphatidylcholines, and 1,2-diacylglycerols. Noteworthy is also the discovery of abnormal high levels of arachidonyl-, oleoyl- and palmitoyl-lysophosphatidylcholines (LPC) at membrane-bound stage (5 *sec*) of lipid modifications in blood lymphocyte PM at ALL and AML. Some of the similar defects were observed also at BC and OC.

Judging from the aforementioned, we have performed a series of experiments to define the endogenous activities of certain lipid modification enzyme systems, such as phospholipases (PLase) A_1, A_2, lysophospholipase (LPLase) and acyltransferases (ATase) in lymphocytes at diverse forms of malignancy. It was shown that Ca^{2+}-dependent activities of PLases A_1, A_2 and C detected in the PM of lymphocytes at norm were significantly (PLase A_1) or completely (PLases A_2 and C) inhibited at all cancer types. It is also important to note that in lymphocyte membranes obtained from patients with ALL, AML and BC an abnormal high activity of LPLase was observed, which is distinctly individual for each patient, chemotherapy-sensitive and not detectable at norm. The studies revealed a high activity of Acyl-CoA : glycerophosphorylcholine-ATase in parallel to the unchanged activity of Acyl-CoA : LPC-ATase. The last facts indicate to the activation of synthetic ways of LPC generation at cancer.

In summary, data obtained display for the first time that:

1. There are regular disorders in the lymphocyte lipids modification processes, especially, in LPC formation/degradation and, consequently, in the enzyme activities, responsible for lipid-remodeling, leading to the alterations in content of diverse bioactive lipid metabolites in the PMs of indicated cells at ALL, AML, BC and OC.

2. The significant changes in the lymphocyte lipids modification processes and some enzyme activities revealed in blood lymphocyte PM are common characteristics for studied acute leukemias and BC.

The results of these investigations give a new, working knowledge of cancer pathology and open new perspectives for further studies in order to achieve common biomarker(s) for diagnosis and monitoring of malignancy treatment.

ORGANOPATHOLOGICAL CHANGES IN TANATOGENETICAL STRUCTURE OF FAMILIAL MEDITERRANEAN FEVER

Hambardzumian S.

Department of Pathological Anatomy, Yerevan State Medical University after M.Heratsi, Yerevan, Armenia

E-mail: dgh1996@gmail.com; **Phone:** 094354255

Familial Mediterranean fever (FMF) known as a wide-spread disease in Armenia and countries surrounding the Mediterranean basin. This is a recessively inherited periodic fever syndrome characterized by recurrent fever, peritonitis, pleuritis, pericarditis, arthritis; it is frequently complicated with amyloidosis. Renal envolvment is the main complication in FMF; it mostly presents with nephrotic syndrome and chronic renal failure.

The purpose of the present study was to characterize the morphological features and organopathological manifestations in FMF patients dead from complications and to evaluate tanatogenetical peculiarities of the disease.

We have investigated section material from 40 patients aged 15-45 years and dead from complications of FMF. In cadaver dissection the pathological changes in all organ-systems were evaluated macroscopically. Fragments from organs were taken for preparation of paraffin sections. For hystological investigation hematoxylin and eosin, kongo red, methyl violet staining methods were used. Renal involvment was detected in all cases. Morphohystological investigation revealed remarkable amyloid depositions in kidneys, spleen, gastrointestinal tract, adrenals, thyroid, and pancreas.

In kidneys amyloid depositions were observed in pyramids – along with vessels and collecting tubules, in glomerules – in mesangium, under the endothelium, in vascular walls, along with tubular basement membranes. In proximal and distal tubules hyaline-droplet and hydropic dystrophy, and in tubular lumines proteinous masses, hyaline cylinders, desquamated epithelial cells were detected. The cases with sclerosis and amyloid shrinkage of kidneys are prevailed in investigated material. In 4 cases non-amyloid renal disorders were observed. The latter were manifested by mesangioproliferative glomerulonephritis with proliferation of mesangial cells, and in 1 case with nephrothelial cells and podocytes proliferation with formation of crescent-shaped proliferate within capsular space.

Local and diffuse amyloidosis of the spleen was manifested microscopically, without clinical manifestations during the life. Amyloidosis of the vascular walls in mucosal and submucosal layers of gastrointestinal tract was determined.

In 3 cases we observed cardiac amyloidosis with cardiomegalia, thickening of intraventricular septa and left ventricular wall. Microscopically amyloid was distinguished in intramural vascular walls of myocardium, and in 1 case in the myocardium stroma as well. Localisation of amyloid depositions in adventitia of large arteries and in periadventitial tissue was characteristic. Amyloidosis was more expressed in arteriolar walls also with narrowing or completely closing of vascular lumina. Atrophic, dystrophic and necrotic changes with cardiosclerosis development in some cases were distinguished.in cardiomyocytes.

Cardiac amyloidosis resulting in heart failure and death in 1 case developed before renal failure and uremia.

Depositions of amyloid were obtained in several endocrine glands: adrenal, thyroid, and pancreas. In some cases amyloidosis of pancreatic islands was characteristic, somethimes completly replacing the islands with amyloid masses. Amyloidosis especially in cortical layer of adrenals, along with vessels was obtained, replacing the tissue and accompanied with atrophy of cells. Hystological investigation of thyroid showed amyloidosis of stroma with atrophy of follicles. Although amyloidosis of glandular stroma was sharply expressed, endocrine glandular insufficiency was manifested in rare cases only, usually clinically significant functional impairments were uncommon.

The most frequent causes of death of patients with FMF in cases investigated by us was kidneys amyloid shrinkage, or secondary shrinkage due to glomerulonephritis resulting in renal failure and uremic intoxication, intestinal bleeding, polyorganic insufficiency.

STUDY OF BURN PATIENTS IN THE SOUTHEAST OF IRAN

Hanafi-bojd H., Narouie B., Kaviani-far K., Shirkhoda M., Shikhzadeh A., Ghasemi-rad M., Khazaee A., Soltanpour N., Tajbakhsh-rigi M.

Clinical Research Development Center, Ali Ebne Abitaleb Hospital, Zahedan, Iran

Phone: 05413414103

Burn injury is one of the major causes of morbidity and mortality worldwide. In addition to the financial burden it inflicts on the health care system, it can cause psychological, social, and physical problems to the patient and family.

The aim of this study was to determine the epidemiological parameters in hospitalized burn patients.

In a cross-sectional study we evaluated data of 1073 hospitalized burn patients in Zahedan Khatam Al Anbia hospital from 2005 to 2008. Data was extracted from medical records and analyzed by SPSS software. The value of p less than 0.05 was considered significant.

Among 1073 patient with acute burn 55.4% were male and 44.6% were female. The mean age was 20.94 years and mean burn size was 49.11±29.65% total body surface area (TBSA). Total body surface area burned was significantly higher in those with self-burn (p=0.0001). In children younger than 15 years old scalds (hot liquid) were the most frequent case of burns (44%). There was a higher incidence of self-burning in women (p=0.0001) and the mean length of hospital stay was 6.32±5.27 days. The mortality rate in self-burn patient was 87.34 %; in total cases it was 41.47 %.

In the present study, most of the burns were registered in 16 to 20 years age group, and more often in male than in female subjects. Burn was more frequent in children and adults as a high-risk group with higher mortality and morbidity. This shows a need for burn prevention programs.

COMPARATIVE HISTOLOGICAL, HISTOCHEMICAL AND ULTRASTRUCTURAL CHANGES OF MYOCARDIUM AND VESSELS OF MICROCIRCULATORY BED DURING EXPERIMENTAL CRUSH SYNDROME

Hartenyan N.

Department of Morphology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: adelinatorgomyan@yahoo.com; **Phone:** 091460645

Crush syndrome is a pathological condition, which develops as a result of mechanical traumas sustained in case of a natural calamity, such as explosions, avalanches, earthquakes, etc., when extremities of injured remain under the tumbledowns for a long time. Decompression of squeezed down extremities initiates pathological processes in the organism. As a result, the increased production of catecholamines and glucocorticoids disturbs microvasculature in all organs, destroys cell membranes, and depresses the immune system. After decompression rhabdomyolysis occurs in a damaged region. There are revealed the plasmorragia, necrosis, myolis, swelling. Muscle injury leads to leakage of extracellular calcium ions into the intracellular space. The excess of calcium causes a pathologic interaction of actin and myosin, and activates cellular proteases with muscle destruction and fiber necrosis. With muscle injury, large quantities of potassium, phosphate, myoglobin, creatine kinase (CK), and urate leak into the circulation, and damage kidneys, liver, and heart leading to their insufficient function.

The aim of the investigation was to study the structural and functional changes of myocardium and vessels of microcirculatory bed during experimental crush syndrome and in the condition of trial correction with α-tocopherol.

The model utilized in the experiment has allowed us to have a non-serious form of crush syndrome. The experimental animals (rats) were exposed to squeeze over one pelvic extremity during 1 hour.

The experimental animals (rats) were exposed to squeeze over one pelvic extremity during 1 hour.

The results of the carried out investigations of myocardium and vessels in 1-3 days after the decompression and on the 7^{th} and 30^{th} days allow us to assert that at all these stages of developing crush syndrome from the first day till one month ultrastructural and microscopical pathology is displayed in exposed cells, *i.e.*:

a) histological (breeding ground of dystrophy and necrosis);

b) histochemical (fuchsinophilous distrophy, decrease of the quantity of glycogen and RNA);

c) histoenzymatic (decrease of lactate dehydrogenase and succinate dehydrogenase activity);

d) ultrastructural changes of apparatus, foremost, mitochondria and myofibrils.

These changes are distinguished with some general indications. They come early, then persist, and do not vanish during, at least, one month after decompression. Ultrastructural characteristics of the blood

capillary walls indicate to the high permeability of blood-tissue barrier.

At the same time, feebly marked and slow dynamics of intracellular regenerative processes attracted our attention. During crush syndrome an increase in the level of products of lipid peroxidation of cellular membranes is noticed, which can cause oxidation of different biological substrates and, so, damage the membrane proteins and lipids, inactivate enzymes and change the structures of micromolecules and the integrity of the cell with its organelles.

Hence, the support of normal level of the free-radial oxidation processes is an important condition for the cell functioning. Similar findings served as a basis to conduct a series of experiments in the early period of a syndrome by injecting to the animals α-tocopherol, which has antioxidant characteristics. The obtained morphological results indicate that α-tocopherol, to a certain degree, allows decreasing the severity of pathology processes development in the heart.

MORPHOLOGICAL CHANGES OF LOACH EMBRYO UNDER THE ACTION OF THE AMINO ACID DERIVATIVE OF NAPHTHOQUINONE

Heneha A., Mandzynets S., Bura M., Jaremkevych O., Lubenec V., Sanagurski D.
Department of Biophysics and Bioinformatics, Lviv National University of Ivan Franko, Lviv, Ukraine.
E-mail: anastasiyah2@gmail.com

Synthetic N-derivatives of 1,4-naphthoquinone are the prospects of practical use in medicine and pharmacology, as they are of low toxicity, active in the reactions with either peroxide or alkyl radicals, inhibit oxidation, and excess and low oxygen pressure, partly protect lipids during hypoxia and at the risk of heart attack. Natural quinones and their derivatives show their potential as inhibitors of lipid peroxidation under hypoxic conditions. Low toxic compounds having high antihypoxic, antiischemic and anticonvulsive effects may be used in the pharmaceutical industry to create new drugs.

The aim of this study was to determine the influence of potassium salts of amino acid derivatives of naphthoquinone (tyrosine) on the morphology of loach embryos at different concentrations: 7.1 *mg/mL* (10^{-3}M) and 0.071 *mg/mL* (10^{-5}M).

Studies were carried out in embryos of loach (*Misgurnus fossilis L.*) in the period from fertilization to 2 days of development. Ovulation was stimulated by the introduction of female chorionic gonadotropin (500 units). Hardroe was obtained within 36 *h* after stimulation and inseminated in Petri dishes for suspension of sperm by A. Neifakh method. Testes were obtained after decapitation and opening of the abdominal cavity of male species. In 5-10 minutes after fertilization, zygotes were washed and incubated in Goltfretera saline at a temperature of 20-22°C. Stages of development were visually monitored under a binocular microscope MBS-9. Control embryos were incubated in a Goltfretera solution for cold-blooded. Loach embryos were incubated in test solutions at concentrations of 7.1 *mg/mL* and 0.071 *mg/mL* from the moment of fertilization within two days.

Incubation of loach embryos in a medium with the addition of potassium salt of tyrosine at a concentration of 10.3 M (7.1 *mg/mL*) leads to a cessation of development at 3.5 hours after fertilization and embryo death a day after fertilization. In the first hours underdevelopment was observed: deformation of the first germ cells (2 blastomeres). Therefore, next blastomeres did not form.

At concentrations of 10^{-5} (0.071 *mg/mL*) in the presence of medium amino acid derivative of tyrosine at the fourth hour of incubation there was observed a clear slowdown in the development of embryos at the stage of 2 blastomeres. The impact of amino acid derivatives of 1,4-naphthoquinone tyrosine led to anomalous concentration of the cytoplasm, which can cause development of necrosis.

The investigations found that the effect of amino acid derivatives of naphthoquinone tyrosine through 3.5-4 hours delays the division of blastomeres, which, in turn, were deformed and lost their intercellular contacts with neighboring blastomeres. Further incubation of embryos in the presence of these substances led to their death on the stage of formation of somites in 27 hours after fertilization.

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HEALTH STATUS OF FOREIGN STUDENTS DURING EDUCATION AT HIGHER EDUCATIONAL ESTABLISHMENTS OF YEREVAN

Hovhannisyan M.

Department of Hygiene and Ecology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: hygec@ mail.ru

Health of young people is formed under exposure to some biological and social factors. Students are at the age, when biological and social processes of the body are not completely developed. Along with this body experiences intensive mental overload due to educational process. From this point of view, foreign students comprise a special group, which is first of all determined by new unusual conditions and environment, to which foreign students should adapt. Therefore, studies investigating health status of foreign students have special importance.

The study was conducted at Yerevan State Medical University after M. Heratsi, Yerevan State University, State University of Architecture and Construction and State Engineering University of Armenia. Health status and health complaints of 724 foreign students studying at 1-6 courses were investigated. Results of medical check-ups conducted among foreign students served as a basis for investigation. Health complaints of foreign students were investigated by a Questionnaire developed at our Department.

According to study results the majority, 47.7%, of students were from Iran, 39.5% from India and 10.8% from other countries. The results revealed that 46% of the first year students and 56% of students from graduating courses indicated worsening of health status after admission to university (χ^2=39.4;

$p<0.0001$). Moreover, among graduating courses percentage of students, who assess their health bad (from 1.5% to 5.2%) or fair (from 25.6% to 34.3%) statistically significantly increased. The majority of complains among the first year students are from neural (27%), gastro-intestinal (18%) and vision (17%) systems. The percentage of these complaints increased among graduating students. Complaints related to the neural system comprised 30%, to gastrointestinal and vision systems 22% and 18%, correspondingly ($\chi^2=100.7$; $p<0.0001$). The percentage of complaints relevant to cardiovascular system increased from 4.2% to 6% ($\chi^2=15$; $p<0.023$) as well.

The analysis of medical check-up results revealed that 7.2% of the first year students and 21% of graduating students had functional disorders of neural system ($\chi^2=115.3$; $p<0.0001$). During the period of education the percentage of students with myopia also increased from 20% up to 25%. The study revealed also that the percentage of students with gastrointestinal diseases increased during education from 0.5% to 5% ($\chi^2=136.7$; $p<0.0001$).

Cardiovascular disorders among Indian students (12%) were registered more frequently than among Iranian students (9%) ($\chi^2=121.5$; $p<0.0001$). Complaints related to vision and respiratory systems also significantly prevailed among Indian students. Results have show that 18% of Iranian students and 14% of Indians students have functional disorders of neural system ($\chi^2=26.7$; $p<0.001$). However, gastrointestinal disorders were more frequently observed among Indian students (4.4%) than among Iranian students (2%). Moreover, the percentage of Indian students with myopia is also high compared to Iranian students: 20% and 17%, correspondingly. Cardiovascular disorders were also significantly frequent among Indian than Iranian students: 36% and 23%, correspondingly.

Study results show that according to subjective and objective indicators health status of foreign students get worse during the period of education. It indicates the necessity of conducting periodical medical check-ups and appropriate measures among foreign students that will help to preserve foreign students' health during education at higher educational establishments.

THE EFFECTS OF HIGH LEVEL NOISE AND a_2-ADRENOBLOCKER ON THE FREE RADICAL OXIDATION INTENSITY OF WHITE RAT SERUM PROTEINS

Hunanyan L.[1], Sotskij O.[2], Khachatryan L.[2], Manukyan A.[1], Kocharyan K.[1], Shirinyan E.[3]

[1] Department of General and Bioorganic Chemistry, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

[2] Scientific-Research Center, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

[3] Institute of Fine Organic Chemistry, National Academy of Sciences of Armenia, Yerevan, Armenia

E-mail: prosc@ysmu.am

It is well known that the intensification of free radical oxidation (FRO) in cells is a universal mechanism of cell response to stress factors of different origin. It was shown that proteins, as well as lipids undergo modification as a result of free radical processes intensification and products of proteins oxidation serve as more informative markers of oxidative damage of cells compared with lipids.

The aim of this investigation was to study the free radical oxidation intensity of white rat serum proteins under conditions of acoustic stress and adrenoblockers administration, as well as the possible preventive effects of newly synthesized α_2-adrenoblocker Beditin possessing vasodilatatory, antihypoxic, antiagregation, antitoxic, antistressor, radioprotective properties that was synthesized at the Institute of Fine Organic Chemistry NAS RA by E.A. Shirinyan and colleagues and a well known α_2-adrenoblocker idazoxane on the intensity of FRO under condition of the acoustic stress.

Investigations were carried out in white male rats weighing 150-200 *g* kept on a diet in the vivarium. The animals were divided into 6 groups: rats of the 1st group were referred to as a control, rats of the 2nd , 4th and 6th groups underwent noise influence (91 *dB A*) with maximal energy in the region of the average and high frequency. The animals of the 3rd and 4th groups were injected 2.0 *mg/kg* Beditin 30 *min* before the experiment. The animals of the 5th and 6th groups were injected 2.0 mg/kg Idazoxane. So, the 4th and 6th groups underwent combined action. The animals were decapitated under slight ether anesthesia. Previously it was reported about two main types of products of protein oxidation, which can serve as markers of FRO intensification. To estimate carbonyl products of protein destruction (oxidation) we used the spectrophotometric method suggested by R. Levine et al. (1990). The method is based on the reaction of interaction of carbonyl products of protein oxidation and 2,4-dinitrophenylhydrazine (2,4-DNPH) with 2,4-dinitrophenylhydrazones formation. Fluorometric method was applied for detection of bityrosine, product of oxidation of aromatic amino acid tyrosine residues in protein chains, by its fluorescence. Protein concentration was determined by Lowry.

The carbonyl products of protein oxidation were registered at different wavelengths: the basic ketone dinitrophenylhydrazones were recorded at 430 *nm* and the neutral ones at 356 and 370 *nm*. Data obtained revealed an increase of carbonyl products of protein oxidation in serum proteins of all the studied groups compared to intact animals data. This effect was more expressed in the 2nd, 5th and 6th groups, in which the animals underwent noise action, idazoxane influence and their combined action

(80-90%), correspondingly. The most intrinsic was the increase of basic ketone dinitrophenylhydrazones content: more than 18 times higher compared to the neutral ones in all the groups. Being administered to the intact animals both adrenoblockers caused a verified oxidation of proteins according to the end products. Nevertheless, unlike idazoxane, administration of Beditin to the animals 30 *min* prior to noise exposure prevented the development of any noticeable changes in the studied parameterAccording to the second studied marker, it must be emphasized that there was no specificity in the changes of aromatic amino acids oxidation intensity in all the studied groups: in models of either free- or metal-catalyzed oxidation. Results obtained have proved our expectations on development of protein damage due to free oxidation intensification in acoustic stress conditions. In our opinion, the significant difference in the levels of basic ketone dinitrophenylhydrazones compared to the neutral ones is the result of activation of carbonyl groups glycolyzation.

Determination of carbonyl products of protein oxidation with dinitrophenylhydrazones formation is an adequate informative parameter for estimation of the level of protein damage under studied conditions. Administration of α_2-adrenoblocker Beditin to animals prior to the noise exposure prevents development of any significant changes in the dinitrophenylhydrazones content and reveals noticeable regulatory effect in acoustic stress condition

PATENT FORAMEN OVALE (PFO) AS A POSSIBLE RISK FACTOR FOR ISCHEMIC STROKE IN A YOUNG PATIENT: CASE REPORT

Kalayjyan V., Manvelyan H.

Department of Neurology, Yerevan State Medical University after M.Heratsi, Yerevan, Armenia

E-mail: vahan79@yahoo.com

Ischemic stroke is still one of the most frequent neurological disorders in the modern world and may cause patient's disability or even death. Recurring strokes are more life-threatening and have worst prognosis. Although stroke is more prevalent in higher age groups, its socioeconomic impact is greater when it affects young patients. In this population, the annual incidence of ischemic events is estimated between 6 and 26 per 100,000.

Patent foramen ovale (PFO) has been identified as a potential risk factor for stroke by several investigators, but the mechanisms of PFO-associated stroke remain unsettled.

In this case report a young patient is introduced who has survived an ischemic stroke with no well-known stroke risk factors. The aim of the report is to view the PFO as a possible risk factor for ischemic stroke in this patient.

The patient (general information – age: 36 y.o.; male; moderate smoker) was admitted in hospital 10 hours after clinical signs started. At home he felt himself bad and speech disorder, facial asymmetry and

right side weakness developed. No vascular events were observed in the past. The patient does not suffer from arterial hypertension and diabetes mellitus.

Status presens sub. et object.: The somatic status was without significant pathological changes.

The neurological status:- consciousness was soporous, meningeal signs absent. CN – pupils D=S, with intact fotoreaction; eyes spontaneous movements were present, without nistagmus and strabismus; corneal reflex was intact; central paresis of facial nerve was present in the right side; pharyngeal reflex was intact; tongue was along down middle line.

Muscle tone was D≥S. Deep hemiparesis was present in the right side. Tendinous reflexes were D≥S. Pathological Babinski sign was present in the right site. Sensory and coordination were not checked because of patient's status.

Laboratory tests: 1) routine blood and urine tests were without significant changes. 2) CRP: 192 *mg/L* (N: 12 *mg/L*), RF: abs., ASLO: 600 *u/mL* (N: <200 *u/mL*). 3) immunologic tests: ANA: 0.5 *U/mL* (N: <1.0 *U/mL*), Anti-ds DNA: 22.0 *U/mL* (N: <25.0), Anti-Cardiolipin (IgG): 7.7 *U/mL* (N: <10.0 *U/mL*), Anti-β2 Glicoprotein (IgG): 0.8 *U/mL* (N: <5.0), CIC: 0.9 *U/mL* (N: <2.0).

Investigations: 1) ECG – Sinus rhythm - 55 beats per *min*., w/o any pathological signs. 2) EchoCG (w/o contrast) – no evidence of heart significant organic disorders. Suspicion to presence of PFO. Hence the exact verification demands further investigation.

3) Brain CT (performed about 12 *h*. after clinical signs started): hypodense "fresh" ischemic nidus in the left frontotemporo-parietal area (about 7.5x4.9 *cm*). A significant brain edema around the ischemic nidus with the brain middle structures displacement from left to right on 3-4 *mm* occurred.

Brain CT-angiography: – Massive trombosis of LCMA observed. There were no evidence of brain arteries atherosclerotic stenosis.

Treatment: antiagregants – Plavix 75 *mg*, Cardiomagnyl 75 *mg*; anticoagulants – Fraxiparin 0.6 *mL*; infusions, antibiotics. During the treatment positive changes were seen, but the neurological deficit (motor aphasia, right deep hemiparesis) still persisted.

In fact, in this patient all well-known stroke risk factors were totally excluded. He is a relatively young, not severe smoker. He has no arterial hypertension, diabetes mellitus and cerebral arteries atherosclerosis. There is no evidence of atrial fibrillation (permanent form), endocarditis, and heart's cavities dilatation. The immunological tests excluded vasculitis. Hence we strongly recommended the patient to pass Holter-ECG for revealing paroxism of atrial fibrillation, and more detailed investigation for detecting PFO (transthoracal echocardiography with contrast, and/or transesophageal echocardiography), which may be a potential risk factor for ischemic stroke.

NUCLEAR PHENOTYPE MODIFICATIONS IN HUMAN CELL CULTURES AFTER ENCEPHALOMYOCARDITIS VIRUS INFECTION.

Karalyan Z.[1,2], Abroyan L.[1], Susanyan M.[2], Karalova E.[1]

[1] Laboratory of Cellular Biology, Institute of Molecular Biology, National Academy of Sciences, , Yerevan, Armenia

[2] Department of Medical Biology, Yerevan State Medical University after M. Heratsi,, Yerevan, Armenia

E-mail: zkaralyan@yahoo.com; **Phone:** 094202405

The encephalomyocarditis virus (EMCV) can produce a severe lytic infection described by fast development in susceptible cell morphological and biochemical changes with farther destruction. However, even in cellular lines susceptible to EMCV infection, after several cycles of replication a virus presents individual viable cells. Resistant to picornavirus infection cells are characterized by essential changes in morphology and metabolism.

EMCV (Columbia-SK strain) was used at multiplicity of infection 0.1 TCD_{50} per cell. A continuous rhabdomyosarcoma (RD) culture, human embryonic kidney (HEK) 293 cells, and HEp-2 a continuous transformed cell culture derived from a human laryngeal cancer. Cell viability was measured by trypan blue staining. A native interferon (IFN) in a dose of 100-1000 *U/mL* was used in experiments. Cytophotometry was done with routine Feulgen staining. DNA changes in chromatin texture in apoptotic cells were evaluated by image analysis of Feulgen-stained preparations.

Infected RD cells arrested cell growth for 12 hours post infection (*h p.i.*), involved cytopathic effect, and reduced the cell number to less than 10% of that oobserved in non-infected cells at 24 *h* p.i. The peak of viral titer was at 24 *h p.i.* (3.5 $\log_{10}$). This is a first report of EMCV adaptation to propagation in HEK cell line. Infected HEK 293 cells arrested cell growth for 12 *h. p.i.,* involved cytopathic effect, and reduced the cell number to 28% of that in non-infected cells at 24 *h p.i.* The peaks of viral titers were at 24-48 *h* p.i. (4.0 $\log_{10}$). Infected HEp-2 cells arrested cell growth for 12 *h p.i.,* involved cytopathic effect, and reduced the cell number to less than 5% of that in non-infected cells at 48 *h p.i.* The peaks of viral titers were at 24 *h p.i.* (7.5 $\log_{10}$) and then slowly decreased.

In all cell cultures the most part of cells with apoptotic changes were observed in significantly increased quantities beginning 12 *h. p.i* of EMCV. This growth continued up to 24 *h p.i.*

Intact RD cell line was hypotetraploid (average ploidy of the summarized passages was 3.9"c"). Here, under the action of EMCV the ploidy began to decrease by 12 *h* after infection and at 12 and 24 *h* the difference between infected and control cells became significant (2.6"c").

Intact HEK 293 cell line was hypertetraploid (average ploidy of the summarized passages was 5.4"c"±0.41). Here, under the action of the EMCV the ploidy began to decrease after infection and at 24*h* the difference between infected and control cells became significant (4.19"c").

Intact HEp-2 cell line was hypertetraploid (average ploidy of the summarized passages were 4.7"c"); under the action of EMCV the ploidy began to decrease after infection and at 24h the difference between infected and control cells became significant (3.4"c").

Under the EMCV influence not only a decrease in absolute parameters of DNA amount, but also changes structures of cells in ploidy classes were observed in all cultures. In a final stage of a virus infection an increase of euploid population was observed in all investigated cultures (control RD: 22%, EMCV: 40%; control HEK 293: 25%, EMCV: 48%; control HEp-2: 16%; EMCV - 24%). Besides the increase of euploid population, in RD and HEp-2 cultures diploid cells appeared under the action of a virus.

Under the EMCV infection in all cells, the number and total area of nucleoli was markedly reduced. Cells with four and more nucleoli per nucleus completely disappeared and there were few with three nucleoli. Most of the cells of the populations had a single or two nucleoli.

For differentiation of the IFN-mediated influence, the direct IFN action on cellular cultures was investigated.

IFN inhibited the proliferative activity of cells: it significantly reduced mitotic activity and raised percent of dead cells, there was a tendency to decrease the number of cells as well. However, at IFN-mediated influence there was no decrease in DNA amount, the euploid population increased insignificantly. Under IFN action, the average number of nucleoli on a nucleus was significantly reduced.

Similarity of the changes proceeding in all three cellular cultures gave possibility to assume that one of main reason of the described phenomenon can be selection of cells by the virus. Most likely, the selective EMCV action causes apoptosis of the multinucleolar cells containing aneuploid genome. The number of nucleolar-forming regions is genotypically determined, so differences among nucleolar indices can be a result of the selection and arising of new clones. Selection can occur, for example, due to increased expression of EMCV receptor, VCAM-1, on susceptible cells. However, we can also conclude that EMCV not only carries out selection of cells of all described cultures, but is capable to modify cells as well. Modification consists first of all in occurrence of diploid populations in RD and HEp-2 cultures absent in the control. The basic selection factor is apoptosis induced under the influence of a virus in susceptible cells. Modification of cells is produced by deblocking of G_2 cells and stimulation of their division. As a whole, the phenotype of similar cells can be characterized as less transformed in comparison with an intact cellular population.

PROLINE RICH PEPTIDE (PRP) AS A PROTECTOR OF NEURONS AND HEPATOCYTES ULTRASTRUCTURE AT CRUSH SYNDROME

Karapetyan G., Kukurtchyan N., Kevorkian G.
H. Buniatian Institute of Biochemistry, National. Academy of Sciences, Yerevan, Armenia
E-mail: sapootraa_a@yahoo.com

Crush syndrome (CS) is one of the most investigated subjects in nowaday science; however, many questions concerning this problem are still unknown.

The aim of this study was to find the effect of proline rich peptide (PRP) administration on neurons and hepatocytes ultrastructural changes at crush syndrome.

Liver and brain of 9 Wistar rats were used in this study. The animals were divided into 3 groups: 2-hour compression, 4-hour decompression and 4-hour decompression + PRP. CS was used by a compression of femoral soft tissues using a special press during 2 *h* by forces of 100 *kg/kg* body weight. At the end of experiments liver and brain specimens were taken. The material was treated by the standard method used in transmission electron microscopy and viewed under the electron microscope TESLA.

As revealed in our study at 2-hour compression brain and liver mitochondria (Mch) were presented in groups where the organelles closely contacted to each other, but sometimes the swollen organelles were met as well. The cristae were presented like tubules or vesicle type. Two hour compression increased the per cent of investigated double nuclei hepatocytes; however, at the same time we did not meet such changes in neurons. Rough endoplasmatic reticulum showed the tendency of fragmentation in hepatocytes and vesiculation in neurons. At 4-hour decompression swollen Mch with various size and shape were presented. The process of Mch inner membrane with practically completed reduction took place. The presence of giant Mch in liver and increase of the number in brain was typical. Four-hour decompression led to swelling of nuclei both in hepatocytes and in neurons. Rough endoplasmatic reticulum was in a process of fragmentation and vesiculation in both hepatocytes and neurons. At the injection of PRP such destructive changes of structural safeness of Mch were not presented. Mch were small in size and presented in groups with tubular or vesicle type of cristae. The presence of Mch fission process in brain was typical for this group. PRP administration led to formation of multi nucleolus neurons; therefore hepatocytes nuclei were close to norm. Rough endoplasmatic reticulum was close to norm in hepatocytes, and in neurons the tendency to decrease of vesiculation process was observed. At 2-hour compression the increasing of catecholamine level led to dilation of district circulation of the blood; as a result there took place the ultrastructural changes in both neurons and hepatocytes. In addition, at 4 hour of decompression the toxic peptides formed by ischemic muscle made the condition of organelles more critical. PRP administration into organism on the background of CS neutralized the toxic peptides effect and decreasesd the negative influence od catecholamine on hepatocytes and neurons ultrastructure.

Our investigation demonstrated that neurons are more subject to changes at 2 hour of compression than hepatocytes. Moreover, one-time PRP administration on the background of CS prevents the progress of ultrastructural changes typical in 4-hour decompression.

MONITORING OF POLYCHLORINATED BIPHENYLS IN ARMENIA

Khachatryan A., Bunyatyan Y.
Risk Assessment Division, Waste Research Center, Yerevan, Armenia
E-mail: khachart7@yahoo.com; **Phone:** (37 41 0) 55 47 32/ 094 04 9955

The extensive scientific research has shown that polychlorinated biphenyls (PCBs) are toxic, bioaccumulative and persistent compounds posing risks to human health and the environment. Moreover, PCBs are suspected human carcinogens. Any direct exposure to PCBs, such as inhalation and skin contact, could lead to serious headaches, drowsiness, and skin irritation. The most common signs of exposure to PCBs are chloracne and elevation of liver enzymes.

PCBs are also associated with immunological effects in animals and some developmental effects in humans. People exposed to PCBs for a long time have such problems as irritation of the nose and lungs. The increasing concern over health risks posed by PCBs and their undesirable environmental effects has resulted in PCBs banning for manufacture, processing, and distribution in commerce.

Despite the cessation to produce PCBs in numerous countries, these compounds continue to be environmental pollutants of high concern.

As the main source of PCBs in Armenia there were identified numerous enterprises (hydropower plants, thermal power plants, transformer stations of energy production sector) and equipment (capacitors, oil switches), as well as service providing sector with the use of PCB-containing technical oils.

The aim of research was to monitor residual amounts of PCBs in samples of water from rivers of Armenia in order to reveal the degree of PCB-related environmental pollution.

The study embraced surface water near 7 hydropower plants and 2 thermal power plants.

PCB determinations were done by means of Gas-chromatography analysis, including extraction, clean-up, concentration and analysis of samples.

In samples of water from Vorotan River in the vicinity of the hydropower plant PCB content averaged 1.49 *mcg/L*, while in water samples taken near Hrazdan hydropower plant it reached 1.82 mcg/L. Taking into account that in the water catchment area of Vorotan River no major enterprises are functioning, it was possible to suppose that determined PCB concentration (1.49 *mcg/L*) resulted from the activity of hydropower plants on Vorotan River. However, findings obtained at other rivers that in no way were related to hydropower plants or major enterprises demonstrated that the revealed PCB concentrations dependence on water basin characteristics was insignificant.

In samples of water taken from the part of Pambak River that flows along the agricultural regions the pollution by PCBs was at 0.83 *mcg/L*. On the other hand, in Arpa River that also flows along the agricultural regions rather high amounts of PCBs were determined: 1.57 *mcg/L*, i.e. concentrations similar to those of Vorotan River.

In samples of water from rivers, in the water basins of which there are major enterprises, in particular

mining entities, the concentration of PCBs did not differ significantly from others. Thus, in rivers Voghchi and Debed PCBs content made 1.33 *mcg/L* and 0.68 *mcg/L*, appropriately.

Monitoring studies allowed to reveal that all studied water reservoirs of the Republic of Armenia are at least insignificantly polluted by PCBs.

ENVIRONMENTAL REGULATION OF LANDFILLS FOR PREVENTION OF DIOXINS/FURANS RELEASES

Khachatryan Kh.[1], Aleksandryan A.[2]

[1] Environmental Law Department, Yerevan State University, Yerevan, Armenia

[2] Ministry of Nature Protection of the Republic of Armenia, Yerevan, Armenia

E-mail: khachartryan85@yahoo.com; **Phone:** 093286085

Open landfills or waste-dumps are considered be areas of visual (evident) pollution and, thus, presenting threat for the human and environmental health. Solutions, filtrates are released from the area of dumps polluting ground waters and soils of the surrounding lands.

Usually, wastes are composed of sweepings, plastics, glass, ceramics, clothing, leather, packaging of soft drinks, tin and aluminum cans, paper, cardboard, polymer composites of packing material of domestic appliances, aerosol containers, organic substances, such as food remains, safe wastes from shops, canteens, etc., debris of construction wastes, washing detergents, paints and dies, solvents, organic and inorganic chlorine-containing compounds (e.g. polyvinyl chloride). The content of waste depends on housekeeping (urban or rural type).

During a certain period of time the population of Armenia used to dispose domestic and sometimes non-toxic industrial waste to open-air dumps (iron boxes) in yards and/or streets. For elimination, the waste was exposed to open-air burning that had random character. Burnt wastes such as ash, noncombustible components of refuse were actually thrown to the surface. Therefore, in case of uncontrolled waste burning citizens were exposed to a potential threat of toxic exposure, smoke and oppressive smells.

In case of low-temperature open waste burning at dumps the general population of Armenia is exposed to a specific threat posed by releases of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDDs/PCDFs), because periodically occurring spontaneous waste ignition and smoldering bring forth environmental pollution by these hazardous substances also known as Dioxins/Furans.

PCDDs/PCDFs are unwanted by-products of many chemical industrial and combustion processes. The known toxicity and persistence in the environment of some congeners has emphasized the necessity (a) to identify processes, which are suspect to generate PCDD/PCDF, (b) to determine levels of PCDD/

PCDF in environmental compartments (e.g. in soil, sediments, and air), in products (e.g. in chemicals, paper, other consumer products, and food), in residues and emissions (e.g. municipal waste, fly ash, flue gases of incinerators, sewage sludge, etc.), (c) to understand transport and distribution of PCDD/PCDF, and d) to determine human exposure. In addition, technological measures were developed to minimize dioxin emissions and exposure.

The study was performed to assess PCDD/PCDF releases into air, water, soil and residues with the use of UNEP Toolkit proposed coefficients of toxic equivalent (TEQ).

Monitoring study revealed that open burning of wastes caused PCDD/PCDF releases of 0.3 *g* TEQ/year (to air) and 0.6 g TEQ/year (to residue and soil).

In Armenia there is no plant for domestic wastes processing. Therefore, such wastes cumulate at the dumps. Despite the fact that special areas are allotted for dumps in rural inhabited locality, the wastes cumulate in different places; very frequently it occurs along the banks of rivers. A definite part of wastes from the dumps is then spread in the vicinity of landfills and on the roads to the dumps.

During the last 3 years, the amounts of domestic wastes varied within the range of 1800-2200 thousand cubic meters, the main part of which made wastes generated in Yerevan.

Proper environmental regulation (norms and standards) is required for environmentally sound management of landfills in order to prevent open-air burning of hazardous wastes known as the main source of PCDDs/PCDFs in Armenia.

BROKEN RELATIONSHIP BETWEEN HOST AND MICROBES IN FAMILIAL MEDITERRANEAN FEVER

Khachatryan Z.[1], Ktsoyan Zh.[1], Manukyan G.[1], Sedrakyan A.[1], Arakelova K.[1], Hovhannisyan A.[1], Beloborodova N.[2], Aminov R.[3]

[1] Laboratory of Molecular Genetics, Institute of Molecular Biology, National Academy of Sciences, Yerevan, Armenia

[2] Research group of Academician Yu. Isakov, Bakulev Scientific Center of Cardiovascular Surgery, Russian Academy of Medical Sciences, Moscow, Russia

[3] Division of Gut Health, University of Aberdeen, Rowett Institute of Nutrition and Health, Aberdeen, United Kingdom

E-mail: zkhachatryan@mbi.sci.am; **Phone:** +37410-231499; **Fax:** +37410-282061

The structure and function of the commensal microbial communities is a result of the long-term host-microbe co-evolution. Selective pressure imposed by the host is believed to be a dominant shaper of diversity in the gut, resulting in a stable community. However, presently, we are witnessing a rapid increase in the number of diseases, pathology of which involves commensal microbiota, as well as aberrant host responses directed towards commensal microbiota. Thus, the question is: *what is the underlying nature of*

gut microbiota transformation and which properties make it pathogenic? Here, we investigated a disease of innate immunity, where this intricate molecular cross-talk between the human host and gut microbiota may be compromised. The disease is Familial Mediterranean fever (FMF): a recessively inherited autoinflammatory disorder caused by mutations in the *MEFV* gene, which encodes regulatory protein pyrin.

The present study aimed to test the hypothesis: if the structural composition of gut microbiota is affected and acquires pathogenic properties in the autoinflammatory condition such as FMF, possibly through the production of the unusual range of gut microbial products.

The FMF and control subjects enrolled in this study were genotyped for mutations in the *MEFV* gene. The gut bacterial diversity was determined by two independent techniques: (i) sequence analysis of 16S rDNA clone libraries (1,328 unambiguous sequences) and (ii) Fluorescent *in situ* hybridization analysis using 12 hybridization probes covering almost the entire human gut bacterial diversity. The composition of chemical compounds of microbial origin in systemic circulation was assessed using gas chromatography (GH) – mass spectrometry (MS) approach, while the metabolite profile of fecal extracts was examined by gas-liquid chromatography method.

These analyses demonstrated that the disease caused significant changes in microbial community structure and activity, characterized by major shifts in bacterial populations within the *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* phyla in different stages of FMF. More specifically, the gut microbiota in active disease was characterized by diminished bacterial diversity and significantly different distribution of main bacterial groups. In the asymptomatic remission period, bacterial diversity values were higher than in control; however the bacterial composition within the three major phylogenetic clades was significantly deviant from the norm. The detailed MS-based characterization of microbial chemical markers at the biochemical level revealed a cardinally biased profile of microbial patterns in systemic circulation of FMF patients. The highest concentrations of microbial markers were observed in FMF remission, whereas in the attack period the corresponding values were significantly reduced. Furthermore, the metabolic differences in fecal extracts of FMF patients were characterized by elevated levels of acetate, butyrate and propionate, as well as of minor short chain fatty acids compared with healthy state. Finally, the discriminant functional analyses based on these molecular and biochemical investigations of microbial system revealed distinct clustering patterns in the healthy and diseased states, as well as in different stages of the disease, suggesting a disease-specific microecological status in FMF.

The findings lead to the conclusion that the autoinflammatory disease state such as FMF caused by mutations in the *MEFV* gene results in a dysfunctional interaction between gut commensal microbiota and the host. Our findings conclusively establish the link between the disease state and the corresponding shifts in the gut microbiota by analyzing simultaneously the genetics of both sides in this host–gut microbiota relationship. We found, for the first time, that FMF affects bacterial diversity in the gut resulting in specific restructuring of its composition and metabolic imbalance both in remission and acute stages of the disease. Nevertheless, no particular species or microbial products were specifically linked to FMF, thus leading us to the conclusion that the restructured community as a whole contributes to the disease state.

CEREBROVASCULAR EFFECTS OF NUCLEON-CMP FORTE UNDER THE CONDITION OF GLOBAL ISCHEMIA

Khachaturyan M., Balasanyan M.

Department of Pharmacy, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

milena_s85@mail.ru, 093966721 (mob.)

Cerebrovascular diseases remain the most important case of human disability. Moreover, recently there is noticed a stable tendency to the rejuvenation of the patients with acute ischemic stroke. One of the important directions of neurological correction is the usage of endogenous compounds, which have been proved as essential agents for physiological regulation of CNS function. One of the mentioned compounds is nucleoside. Experimental researches have shown that in ischemic conditions the synthesis of nucleosides is markedly decreased. In view of this, the investigation of these compounds is of a great interest. Nucleon-CMP forte is one of the preparations, which consist of two biologically active endogenous pyrimidine nucleosides: cytidine-5-monophosphate and uridine-5-triphosphate. CMP/UTP nucleosides are used during the neurological disorders, since they contribute to the axon and its membrane regeneration. Nucleon-CMP forte increases the nerve fiber density and diameter and improves the conductibility at histological level. Data concerning its action on CNS is rather limited.

The aim of this study **was** to investigate the influence of Nucleon-CMP forte on local cerebral blood flow (CBF) in normal conditions and in a model of global ischemia.

The experiments were carried out in outbred, adult male, inbred albino rats weighting 270.0±25.0 *g*. The animals were housed under the standard conditions and were deprived of food at night before the experiment with free access to water. Experiments were realized under Nembutal anesthesia (50 *mg/kg, i.p.*). Global ischemia was caused by occlusion of common carotid artery (CCA). Local CBF registration was carried out by laser Doppler flow-meter "Transoic systems. Inc" model BLF-21 (USA). For data registration flow meter needled sensor was fixed on rats' cortex perietal region (on ligation size) in a small 1.5-3 *nm* diameter hole. Nucleon- CMP forte was injected at a dose of 10 *mg/kg* (*i.p*).

Our experiments showed that in normal condition there were no significant changes in CBF under injection of Nucleon-CMP forte. In case of CCA occlusion a decrease of CBF by 25±3.6% ($p<0.05$) was registered. Intraperitonial injection of CMP/UTP to rats with carotid artery ligation led to enhancement of brain tissue microcirculation more than 30% (32.6±4.8) after 60 minutes of drug administration achieving even control value of CBF.

It was obtained that the combined injection of two nucleotides cytidine-5-monophosphate and uridine-5-triphosphate as a Nucleon-CMP forte improves the blood supply of ischemic brain tissue. Date indicate that these compounds could be investigated as neuroprotective agents in case of brain ischemic disorders

THE COMPARATIVE ANALYSIS OF THE PHARMACOKINETICS OF MERONIDAZOLE IN SALIVA AND BLOOD

Khurshudyan K., Hovhannisyan A.
"Expertise Center of the Republic of Armenia" SNPO, BFDFA Laboratory,
Yerevan, Armenia
E-mail: kristina.khurshudyan@gmail.com; **Phone:** (091) 180678

The primary presuppositions for saliva use in the pharmacokinetic investigations data on the effect that drug concentrations in saliva and blood are the same, because of this matter depends the evidence of drug therapeutic and toxic actions.

The metronidazole is one of the drugs, which require the recommended use of saliva for its pharmacokinetic and bioequivalence studies. It is established that in the interval of 0.5-8 hours after administration of metronidazole tablets the ratio of its concentrations in saliva and blood of volunteers is practically equal. However, till now no optimal schedule for saliva sampling has been developed to allow using the saliva for investigations of bioequivalence of metronidazole.

The aim of this study was to develop an optimal pattern of saliva use for the pharmacokinetics and bioequivalence investigations of metronidazole.

In this study Klion® 250 *mg* tablets (Gedeon Rhichter pharmaceutical company, Hungary) that are registered in the Republic of Armenia were used. The approval for study of the pharmacokinetics of metronidazole was obtained from the Ethics Committee of the Scientific Center of Drug and Medical Technology Expertise (Republic of Armenia) and informed consent from three healthy human volunteers. The age of volunteers was 31, 29 and 19, and BMI accordingly were 19, 23.8 and 20 kg/m^2. Each of the volunteers was given by one tablet of 250 *mg* metronidazole to receive with 200 *mL* of water on an empty stomach in the morning. Saliva and blood samples were collected before ingestion (0 *h*) and in 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 24 *h* after administration of metronidazole tablets. The levels of metronidazole in each saliva and blood plasma sample were determined by gas chromatography method (Crystal 2000M, Chromatec, Russia).

Pharmacokinetic parameters of studied samples were calculated by Kinetica 4.4.1 (Termo Corporation, 2004) program.

The statistical analysis of the pharmacokinetic parameters was done using a Windows Statistic 6.0 software.

As a result of the pharmacokinetic investigations we established that the peak saliva and blood concentrations (C_{max}) achieved in 1 hour after administration of metronidazole tablets and accordingly made 13.3±0.9 and 13.2±0.7 *mkg/mL*. Immediately after hitting the maximum, the levels of metronidazole in saliva and plasma exponentially decrease and in 24 hours, almost the same concentration of metronidazole can be recorded in both biological fluids. The comparative analysis of concentrations variations of metronidazole in saliva and plasma of volunteers in the interval of 0.5-8 hours indicated that the

saliva/plasma concentration ratio of metronidazole was practically equal and constituted 0.95±0.02 that is close to the accepted literature data of 0.87-1.03. In the meantime, in 24 hours, the value of saliva/plasma ratio is definitely lower than in the interval of 0.5-8 hours and constitutes 0.82±0.04.

The pharmacokinetic parameters of metronidazole received after analysis of variations of its concentrations in saliva and plasma showed about the same values in the interval of 0.5-8 hours. However, after the analysis of variations of metronidazole concentrations in saliva and plasma in the interval of 0-24 hours, between the pharmacokinetic parameters, except for C_{max}, statistically reliable differences were observed. The most expressed differences were recorded for the value $AUC_{0-\infty}$, (p=0.081), which is the main pharmacokinetic parameter for bioequivalence investigations. At the same time after analysis of variations of metronidazole concentrations in saliva and plasma in the interval of 0-8 hours the pharmacokinetic parameters statistically did not differ.

Granting the fact on application of the interval 0-8 hours the value of interpolating area of pharmacokinetic curve was 6% for metronidazole and that satisfied the requirements of bioequivalence studies (>20%), one may conclude that usage of this interval is quite enough for getting reliable results in the pharmacokinetic and bioequivalence studies of metronidazole.

Though sampling of blood throughout 24 hours is more correct for the pharmacokinetic studies of metronidazole, however it cannot be accepted for sampling of saliva, since the metronidazole concentrations in saliva will not correctly reflect the level of metronidazole in the blood during 24 hours after its administration.

The optimal pattern for using the saliva for pharmacokinetics and bioequivalence investigations of metronidazole is the study of its concentrations variation in saliva in the interval of 0-8 hours, when the ratio of its level in saliva and blood practically remains unchanged.

THE RIGHT IN ECOLOGY

Malkhasian K., Usupashvili K., Sekania J., Mesxi B.

Department of Public Health, Tbilisi State Medical University Tbilisi, Georgia

Phone: +995994623 12

The problem of preservation of the environment is one of the most vital problem of modern mankind. Global and irreversible changes of the environment are obvious and indisputable today.

Realizing a reality of threat for the human life, all developed democratic states take measures on protection of the environment. The big role in the international cooperation of the states concerning preservation of the environment play the United Nations, and also the intergovernmental organisations, the international non-governmental organisations and other specialized establishments.

The study was aimed at evaluation of the environmental health.

Methods of expert analyses were used.

The important precondition of formation of the ecological right as independent branch of the right is presence of the ecological legislation. It includes numerous statutory acts which regulate use and protection of the environment.

The purpose of our work was to define a concept of sources of the ecological right, and the requirements to them, to result classification of sources by the various bases, and to consider the most important sources of the ecological right.

The specified questions were analyzed on the basis of the current legislation of Georgia, the special literature on the given problem, and the published practice.

Right sources in legal sense are understood as the special form of expression of rules of the behaviors, doing them obligatory. In other words, sources of the ecological right are the regulatory legal acts containing norms, regulating relations in sphere of interaction of a society and the nature.

Thus, one of essential problems of the State consists in caring of wildlife management and the most rational use of natural resources in interests of humans. Strict observance of laws in the field of preservation of the environment, as well as resolution of this problem is inseparably linked with public health care, and will affect well-being of the present and future generations.

PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL, ANTIFUNGAL ACTIVITIES OF *SATUREJA HORTENSIS* PLANT

Manjikyan A., Balasanyan M., Gabrielyan S., Melikyan L.

Department of Pharmacy, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: annapharm@web.am; **Phone:** 093905460 (mob.)

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. Therefore finding new anti-infective agents still remains one of the main important medical problems New sources, especially plant sources, are also being investigated. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which were found to have antimicrobial properties *in vitro*. The genus *Satureja hortensis* (savory) belonging to the family *Lamiaceae* (*labiatae*) is rich of phytochemical compounds and is recognized for its wide therapeutic values. Therefore, it was interesting to study *Satureja h.* plant of Armenian flora as a new source for antimicrobial agent.

The aim of research was to investigate the chemical content of Armenian flora *Satureja h.* plant and its antimicrobial activity against the several types of microorganisms.

The essential oil was released from the plant leaves obtained during the flowering period by Ginsburg's steam distillation method. Aqueous extract was obtained from plant leaves by percolation method. The analysis for essential oil chemical content was carried out by gas-liquid chromatography (GLC) method. Essential oil antimicrobial effects were studied in a number of conditionally pathogenic microorganisms. An experiment was carried out *in vitro* in the Petri plates using Mueller-Hinton media. Different types of microorganisms were tested on one plate for the purpose of preliminary qualitative comparable estimation of the antimicrobial effect.

Data obtained by GLC method revealed that the essential oil of *Satureja h*. contained the following chemical compounds: α-tujene (1.123%), α-pinene (0.761%), pseudopinene (0.471%), β-pinene (1.731%), phelandrene(0.392%), 2 carene (4.226%), cymol (3.741%), α-terpinene (carvacrol) (31.48%), terpineol (0.287%), thymol (41.742%), acetylthymol (0.176%), β-carriophylene (0.440%), bisabolene (0.677%). Thus, according to our data thymol and carvacrol were found to be the main components in essential oil of *Satureja h*. of Armenian flora. According to literature data, they display antimicrobial and antiseptic activity. That is why at the next step of our research the antimicrobial activity of *Satureja h.* essential oil was investigated. Preliminary results indicated a certain activity of the essential oil on *E. coli, S. aureus, Bacillus sp.,* and fungi of *Candida* type. *Ps. aeruginosa* appeared absolutely resistant to tested essential extract. It was revealed that the aqueous extract failed to show antimicrobial effect to any of mentioned microorganisms *in vitro*.

The obtained results concerning the antimicrobial activity of *Satureja hortensis* essential oil demonstrate that, obviously, the fat-soluble compounds are responsible for studied activity. These results indicate that *Satureja hortensis* essential oil could be a good source for creation of new agents with antimicrobial activity

INCREASED IMMUNE REACTIVITY TOWARDS BACTERIAL ANTIGENS IN FAMILIAL MEDITERRANEAN FEVER

Manukyan G.[1], Ghazaryan K.[1], Ktsoyan Zh.[1], Khachatryan Z.[1], Tatyan M.[1], Aminov R.[2]

[1] Group of Molecular and Cellular Immunology, Institute of Molecular Biology, National Academy of Sciences, Yerevan, Armenia

[2] University of Aberdeen, Rowett Institute of Nutrition and Health, Aberdeen, UK

E-mail: gaya.manukyan@yahoo.com; **Phone:** +3741023149

Familial Mediterranean fever (FMF) is a systemic autoinflammatory disorder, which is caused by recessively inherited mutations in *MEFV*, encoding the pyrin protein. FMF is characterized by recurrent attacks of fever and serositis at the affected sites such as peritoneum, pleura or synovium. Despite the evident progress in investigating FMF, the triggers of periodical attacks are still remaining unknown. Since the breakdown in recognition of commensal bacteria by the host may result in severe inflammatory disease,

investigation of the immune response against commensal bacteria in patients with FMF may be crucial for the better understanding of FMF pathogenesis and for new treatment strategies to be devised.

In our study, we investigated the immune reactivity to commensal bacteria/antigens in FMF in comparison with healthy subjects.

We used serological expression cloning (SEC) approach to identify bacterial antigens that could contribute to the pathogenesis of FMF, as well as the ELISA and Western blot to evaluate the bacteria-specific IgG and IgA titers directed towards the most frequently isolated commensal bacteria from the fecal samples of FMF patients. Taxonomic affiliation of isolated bacteria was confirmed by sequence analysis of 16S rRNA genes. SEC involved the screening of DNA expression libraries in lambda phage vector with defined sera.

The most frequently isolated bacteria were species of *Bacteroides*, followed by *Escherichia coli*, *Parabacteroides*, *Enteroccocus*, and *Lactobaccilus*. In FMF patients, we observed elevated bacteria-specific IgG titers primed against the harmless intestinal bacteria; the highest titers were against *E. coli* and the species belonging to the *Parabacteroides*, and *Bacteroides* genera. At the same time, the reactivity of systemic IgA antibodies against the epitopes of selected bacteria was not different between FMF patients and controls. Immunoblotting results confirmed ELISA data and demonstrated the presence of IgG antibodies directed against the multiple protein antigens of commensal bacteria. To identify the nature of these bacterial antigens we performed SEC. Six metagenomic lambda expression libraries from feces of four FMF patients and two healthy subjects were constructed and screened with the corresponding host sera. We identified recombinant proteins of bacterial origin generating increased systemic immune response in FMF. The majority of ORFs in these clones had the closest matches with proteins (hsp60, enzymes, bacterial surface proteins, ribosomal proteins, etc.) from the bacterial genera *Bacteroides, Parabacteroides* and *Escherichia*.

Thus, FMF is characterized by the increased systemic reactivity against the commensal gut microbiota, which is usually well tolerated by the normal immune system. Therefore, the next question to ask is, whether the exaggerated immune response towards commensal bacteria in FMF is due to the reduced gut epithelial integrity facilitating bacterial translocation or it is the result of heightened sensitivity to normal bacterial antigens.

PREVALENCE OF BURNOUT IN ARMENIAN PHYSICIANS

Margaryan A.
Department of Family Medicine, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia
E-mail: an_margaryan@yaoo.com; **Phone:** (010) 581794

Burnout is a psychological term for the experience of a long-term emotional and mental exhaustion and diminished interest. Job-related "burnout" was identified as an occupational hazard for various professionals involved in people-oriented services. It most frequently affects human service professionals, like educators, job nurses, and physicians, due to chronic emotional and interpersonal job related stressors.

The aim of this study is to determine the prevalence of burnout among the physicians of primary health care.

To evaluate the prevalence of burnout in Armenian physician, a survey was conducted among doctors of primary health care (policlinics) during the autumn of 2009, and a Questionnaire has been used as a tool. Random, non-stratified sample was selected. The Questionnaire embraced questions regarding the age, gender, marital status, years since qualification as a doctor, years in current workplace, earning, workplace conditions, (rural/urban setting), working conditions (working hours per day, patients per day, night shifts). The second part of Questionnaire involved the MBI (Valid Russian version), which was used to measure burnout. Each participant was explained the purpose of the research and the voluntary basis of participation, and his verbal consent was received. The study was approved by the local ethics committee.

Of the 240 Questionnaires distributed 32 were not returned and 24 had missing data and were not used (response rate 76.7%). Of the 184 available Questionnaires, 50 had to be excluded because respondents worked in the secondary health care system; those Questionnaires were supposed for further analyses. Of the respondents 14 physicians (10.4%) were males and 120 physicians (89.6%) were females. The 134 respondents had a mean age of 48.9 years (SD: 11.9 years). The analysis of the data obtained from the survey showed that the 36.6% of physicians had narrow specialization, 34.4% physicians were therapists, 18.7% were pediatricians, and 10.4% were general practitioners and worked in a rural environment. Most of the respondents (53.0%) had graduated 20 years prior to filling in the Questionnaire and 8.2% had graduated 4 years ago. The mean estimated that physicians worked 6 hours per day (SD: 2.6 hours) and saw 13 patients per day (SD: 8 patients).

The analysis of the survey showed that the 79.8% of respondents answered all 22 items of the MBI, 13 respondents had only 1 missing value, 4 respondents had 2 missing values, and 10 respondents from 3 to 12 missing values.

The mean score on the emotional exhaustion (EE) subscale was 24.3 (SD: 5.8), on the depersonalization (DP) subscale: 10.1 (SD: 3.3), on the personal accomplishment (PA) subscale: 33.7 (SD: 4.4). Our results are comparable with the data of research conducted among European family physicians.

The three dimensions of burnout were transformed into dummy categorical variables for high, aver-

age and low burnout in the dimensions of EE, DP and PA as recommended by Maslach, namely: EE: low burnout ≤13, average burnout 14-26, high burnout ≥27; DP: low burnout ≤ 5, average burnout 6-9, high burnout ≥10; PA: high burnout ≤ 33, average burnout 34-39, low burnout ≥ 40). In all, 32.8% of respondents scored high for EE, 49.3% for high DP and 48.5% for high PA.

The analysis of the BMI revealed that 16.4% of respondents scored high for burnout in all three dimensions. Only 19.4% of doctors did not score high for burnout in any dimension, whilst 21.7% score high for burnout in at least two dimensions.

Thus, analyzing the results of the survey carried out among physicians of primary health care, we can draw a conclusion that burnout seems to be a common problem in with high levels apparently affecting two-thirds of respondents in this study. Future research is needed to explore the problem in depth, develop models to describe the phenomenon and identify causative factors and effective intervention strategies.

ESTIMATION OF THE LEVEL OF ANXIETY AND SUB DEPRESSIONS AMONG FUTURE DOCTORS

Margaryan Ar.[1], Margaryan A.[2], Sydorchuk L.[3]

[1] Department of Family Medicine, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

[2] National Institute of Health, Yerevan, Armenia

[3] Department of Family Medicine, Bukovina State Medical University, Chernivtsi, Ukraine

E-mail: lsydorchuk@ukr.net; **Phone:** 093 50 1842

Nowadays the health care issue of young people, especially students, is one of actual and key problems of modern public health services because youth, being the basic reserve of highly skilled experts, defines a mental potential of a society, the future of a national economy, as well as reproduction and health of the future generation. It is well known that the basic requirement to higher educational institutions is preparation of experts qualified not only in the special field, but also experts with psycho-emotional and physical stability, being the important quality of an individual for adequate adaptation to conditions of further labor activity. The insufficient attention to the problem of students health can lead to essential deterioration of a condition of their health and ability to study, that will be later reflected both in a level of their competitiveness on a labor market, and in a success of their labor activity.

The aim of this research was to study some psychological parameters of future doctors' health, especially the level of anxiety and sub-depression condition during training.

The psychological health status of medical students was assessed using an anxiety scale (Ch. Spielberger, 1976) and depression scale (W. Zung, N. Durham., 1965). The study involved 824 medical students (323 boys and 501 girls). Middle age of students has made 17.1-22.3 years old. The examination was made

during the term, before an examination session.

The analysis of obtained data showed, that an average parameter of personal anxiety among students during education had higher values than the parameter of reactive anxiety ($p<0.05$). It is known, that very high personal anxiety directly correlates with presence of the emotional and neurotic failures and with psychosomatic diseases. The dominating degree of reactive anxiety among students was low or moderate, while the personal anxiety was moderate or high. The comparative analysis of the received results during education showed that the greatest level of high personal anxiety and reactive anxiety was registered among students of younger courses (II and III). The estimation of sub-depression level showed that the condition of mild sub-depression at the I course made 5.3% among boys and 10.4% among girls, at the VI course: 3.2% among boys and 5.3% among girls. The greatest share of students with mild sub depression has been revealed among students of the III year of training (15.4% boys and 21.3% girls).

Thus, the obtained data testify that for students of medical university high level of anxiety is characteristic, and propensity to depressions has been revealed in separate persons. Hence, recognition of anxiety and depressions by psychometric methods is necessary for the complete approach to estimation of a health status of young people with the purpose to preserve the emotional stability of mentality, and the prevent psychosomatic diseases development.

INVESTIGATION OF SPATIAL MEMORY IN RATS WITH LOCAL ISCHEMIA

Margaryan T., Ghochikyan A., Topchyan H., Mkhitaryan S.
Department of Pharmaceutical Technology, Yerevan State Medical University, Yerevan, Armenia
E-mail: tigranmargaryan@yahoo.com; **Phone:** 091 01 3481

The most common disorder found in post-ischemic period is memory disturbance. For estimation of that issue many behavioral and memory tests were designed. The Morris water task is one of the most used experimental models to assess disturbances of cognitive functions.

The main purpose of this research was to investigate the changes of spatial memory in condition of local brain ischemia 6 and 12 days after injury.

Forty-five male non-linear albino rats weighing 220-270 *g* were used in this experiment. Local brain ischemia was induced by occluding left middle cerebral artery (MCA) under chloral hydrate anesthesia (400 *mg/kg*).

Investigation of spatial memory was evaluated in rats with the Morris water maze task. Rats were trained during 4 days, 4 trials per day. In each of the trials the rats spent 1 minute in a pool (diameter 108 *cm*, depth 40 *cm*) to locate the hidden platform (submerged 1-2 *cm* below water) that was placed

at the fixed location. The next day after training rats were operated (same operation was performed in control rats, except occluding MCA). In test trial (6 and 12 days after operation) platform was removed from the pool and in 1 minute (1-30 *sec*, 31-60 *sec*, 1-60 *sec*) several parameters were measured: time spent in each quadrant of pool, traveled distance and mean speed. The pool was divided in North West (NW), North East (NE), South West (SW), South East (SE) quadrants; the platform was placed in NW quadrant.

The statistical analysis was performed by Student's t-test.

Results of this investigation showed that control group rats in the first 30 *sec* spent more time in target quadrant than in the second ($p < 0.05$), while the distance and mean speed was increased ($p = 0.01$). Rats fruitlessly spending time to find the hidden platform in a target quadrant moved to find it in another place. Therefore, to assess a spatial memory we analyzed the results of the first 30 seconds.

Time spent in target quadrant of control group decreased at the 12th day compared to the 6th day ($p < 0.05$), while the other parameters were unchanged ($p > 0.05$). Ischemic rats did not show any statistically significant results compared with control rats both on the 6th and 12th days ($p > 0.05$).

Our investigation showed that ischemia induced by occlusion of MCA did not affect spatial memory. Probably, the function of ischemic hemisphere was compensated by the other one. Additionally, it should be mentioned that spatial memory is linked with the vision, which is not affected in this case. These facts could also explain the phenomena that a spatial memory did not change in this paradigm of local ischemia.

THE ANALYSIS OF BACKGROUND IMPULSE ACTIVITY OF THE RIGHT-SIDE SUPERIOR VESTIBULAR NUCLEUS' NEURONS OF THE ONE-SIDE LABYRINTHECTOMIZED RATS IN NORM AND UNDER VIBRATION INFLUENCE

Martinova M., Minassian S., Sarkisyan S.

Department of Human and Animals Physiology, Faculty of Biology, Yerevan State University, Yerevan, Armenia

E-mail: susi.sar@rambler.ru; **Phone:** 077 05 9075

The one-sided neurectomy leads to typical syndrome of the postural and oculomotor [III cranial] disorders. These disorders become weaker with the lapse of time.

At the same time compensatory mechanisms, which are directed to stave off pathological disorders, are involved in the process. The sensor-motor activity and physical exercises have a very important role in a process of vestibular compensation

During acute experiment, registration of background impulse activity (BIA) of the right-side superior

vestibular nucleus' (SVN) neurons was conducted. The labyrinthectomy was done through method of Mocrousova (1980). The background impulse activity was taken aside through glass microelectrodes.

The stereotactic orientation of electrodes was realized through coordinates of atlas (G. Paxinos, Ch. Watson, 2005). The experiments were conducted in 3 series. In the first series the rats with intact labyrinth were used. The second series included rats with one-sided labyrinthectomy. The registration of BIA to rats with one-sided labyrinthectomy was conducted on the second day after operation. The third series included previously labyrinthectomized rats, which were daily exposed to vibration effect on the vibration stand (frequency 60 *Hz*;, amplitude 0.4 *mm*) for 2 hours during 5, 10 and 15 days.

The registration and analysis of impulse activity of SVN were conducted by a particular engineered computer program. The consistent regions of pulse-to-pulse intervals which included to 1200 action potentials were analyzed.

Trough the result of the graph there was appreciated the stationarity of background activity of the neurons; regularity and artifact structure, modality of the impulse flux, as well as frequency diapasons of the neurons (low- , middle- and high-frequency cells) were defined. For stationary units the module of midband frequency of impulsation and pulse-to-pulse interval's coefficient of variation were used.

The analysis of results showed that module of midband frequency and frequency allocation of impulsation did not have significant deviation on the second day after labyrinthectomy with the exception of coefficient of variation 79.5±5.3%, 13.5±1.4 *Hz* (norm: 100.4±3.3%; 14.0±1.4 *Hz* ($p<0.05$). The modules of midband frequency of impulsation had statistically significant variations during all periods of vibration effect. The modules of frequency allocation and coefficient of variation ($p<0.05$) had statistically significant variations after 5 days ($p<0.01$) and 15 days ($p<0.01$) of vibration effect.

So, the vestibular compensation depended upon sensory impulsation, incoming from visual and proprioceptive source, as well as from acting parts of vestibular apparatus. This sensory impulsation, including difficult neurophysiological and adaptive mechanisms, promotes the functional rehabilitation of vestibular analyzer.

STUDY OF HEMOGLOBIN INTERACTION WITH HYPERICIN

Martirosyan A., Babayan B., Ayvazyan G., Zakaryan H.
Biomedical Faculty, Russian-Armenian (Slavonic) University, Yerevan, Armenia
E-mail: alina_mart@list.ru

Photodynamic therapy is a treatment modality involving the photosensitizer, which selectively accumulates in the hyperproliferative target cells, followed by local irradiation with visible light of lesion. Nowadays, the plant pigment hypericin (HY), which can be extracted from plants of *Hypericum* genus, is probably the most powerful photosensitizer found in nature. For *in vitro* evaluation of photosensitizers

efficacy, including HY, a phototoxicity test (Photo-RBC test) may be used that is based on combined evaluation of photohemolysis of red blood cells and hemoglobin (Hb) oxygenation and conversion. Hb is a representative of hemoproteins possessing peroxidase activity in the presence of oxidizing agents, such as hydrogen peroxide. In red blood cells this activity is regulated by the reducing environment and lack of oxidizing equivalents. The free Hb, however, lacks this intracellular regulation and the likelihood for Hb to act as a peroxidase is high. This possibility is markedly increased in the course of influence produced by reactive oxygen species after HY photosensitization.

The aim of this study was to examine the influence of HY on structure and function of Hb, as well as to explore the possibility of improvement of Hb peroxidase activity for construction of Hb-based hydrogen peroxide sensor.

Commercial HY ("Roth", Austria) was dissolved in 96% ethanol and used with a final 5% concentration of alcohol in the reaction mixture. The influence of HY on Hb was studied both under incubation in the dark and after illumination by visible light of filament lamp (100 *W*) by UV-vis (Specord M400, Carlzeiss, Germany) and fluorescence (spectrofluorometer Fluoromax, Germany) spectroscopy, as well as by PAGE disc-electrophoresis. The pH value of Hb solution was monitored by pH 211 Microprocessor pH-Meter (HANNA Instruments, Italy). Peroxidase activity of Hb was determined spectrophotometrically at 610 *nm* by application of benzidine as a substrate. Peroxidase activity of Hb was also determined by substrate staining (5 *mM* benzidine) of protein bands after PAGE disk electrophoresis. Electrophoretic profiles were analyzed by FUJIFILM Image Gauge V4 program. Data were statistically analyzed by a one-way ANOVA for multiple comparisons.

In the present work an absorption decrease of Hb at Soret band (407 *nm*) was revealed under photodynamic influence of HY, depending on HY concentration and irradiation dose. Under incubation of Hb with HY the Soret band intensity decrease in a time dependent manner was also revealed. HY led to a decrease of Hb emission peaks at 334 and 421 *nm*, correlating with the increase of HY concentration, incubation, and irradiation time. An obvious increase of Hb mobility and a pH decrease were observed under the Hb incubation with HY. Structural changes of Hb molecule caused by HY were reflected in an increase of peroxidase activity of the protein. This makes Hb an attractive "recognition" element for construction of third-generation biosensors.

In the present work the interaction of Hb with HY was shown that leads to conformational changes of the protein. These structural changes affect the functional state of Hb, which reflects ion peroxidase activity of the protein. Thus, under the HY influence properties of Hb as a hydrogen peroxide detector could be improved and an effective determination of peroxide formation could be achieved.

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THE LEVELS OF ADENOSINE DEAMINASES 1 AND 2 AND ADA 1 FUNCTIONAL NT22 G>A POLYMORPHISM IN ISCHEMIC STROKES

Martirosyan G.[1], Snkhchyan R.[2], Andreassyan N.[3], Tovmasyan H.[1], Sargissova Ye.[3], Mardanyan S.[3], Arakelyan A.[2], Boyajyan A.[2]

[1] Laboratory of Computer Modeling, Institute of Molecular Biology, National Academy of Sciences, Yerevan, Armenia

[2] Laboratory of Macromolecular Complexes, Institute of Molecular Biology, National Academy of Sciences, Yerevan, Armenia

[3] Laboratory of Metabolism of Adenylic Compounds, H. Buniatyan Institute of Biochemistry, National Academy of Sciences, Yerevan, Armenia

E-mail: g_martirosyan@mb.sci.am

Adenosine deaminase (ADA, EC 3.5.4.4) is a key enzyme of the purine salvage pathway that catalyses the deamination of (deoxy)adenosine to (deoxy)inosine. In mammalians, the enzyme is presented in two isoforms, ADA1 and ADA2. The genetic variations for both ADA1 and ADA2 was found in both normal subjects and individuals with immunodeficiency disease. The both isoenzymes are implicated in several diseases characterized by altered immune response.

The aim of the current study was to investigate involvement of ADA1 and ADA2 isoenzymes in post-ischemic systemic inflammatory response and the association of functional ADA1 nt22 G>A polymorphism with ischemic strokes.

Overall 53 patients with ischemic stroke were involved in this study. Diagnosis of ischemic stroke was based on clinical history and neurological examination and was confirmed by brain computer tomography (CT) imaging and basal laboratory tests. Forty-four of physically and mentally age and sex-matched subjects served as a control group.

ADA1 and ADA2 activities were determined by evaluating ammonia liberated in the enzymatic reaction of adenosine deamination. One of two identical enzyme assay mixtures contained a selective inhibitor of ADA1, erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA). Ammonia amount was measured using the phenol-hypochloride colorimetric method registering the absorption of an assay mixture at 625 *nm*. The index for the EHNA containing mixture has been taken as the activity of ADA2. Subtracting this index from the value registered in the parallel mixture without EHNA, the activity of ADA1 isoenzyme has been received. Solution of ammonium sulfate was used as a standard.

Genotyping for ADA1 nt22 G>A was performed using restriction fragment length polymorphism (RFLP) utilizing TaqI restrictase.

Statistical analyses included Mann-Whitney U test, c2-test and Fisher's exact test.

Data are presented in M ± SE.

We observed the significantly elevated (p = 0.03) level of ADA2 in the blood of stroke patients compared to the control group: respectively, 10.71 ± 0.56 and 8.54 ± 0.77. The level of ADA1 increased also:

3.27 ± 0.52 and 2.28 ± 0.19, respectively, for IS and control groups. However, this difference did not reach the statistical significance (p = 0.09). Genotype frequencies for nt22 G>A did not differ among studied groups. Correlation between genotypes, ADA1 and ADA2 levels in stroke patients was analyzed.

Data obtained indicated that, probably, ADA1 is not, but ADA2 might be involved in the pathogenesis of ischemic strokes. Our future research will be focused on the evaluation of the association of ADA2 polymorphism with the development and progression of stroke.

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GENOTOXICITY OF WATERS FROM HRAZDAN RIVER OUTSIDE YEREVAN CITY

Matevosyan M., Agadjanyan E., Atoyants A., Varjapetyan A., Arevshatyan S., Aroutiounian R.
Department of Genetics and Cytology, Yerevan State University, Yerevan, Armenia
E-mail: mara_matevosyan@hotmail.com

The mutagenic properties of water specimens from Hrazdan River outside Yerevan were studied with the sensitive test of *Tradescantia* stamen-hairs (Trad-SHM) of *Tradescatia* (clone 02) plant test-system. The samples of waters were collected around Hrazdan town (1st point), Lusakert (2nd point), Masis (3rd point) and at the outside border of Yerevan city (4th point).

The determination of mutagenic activity of waters from Hrazdan River with application of *Tradescantia* test-system SHM was performed during three months (October, November, December).

In October, at the samples of waters from Hrazdan River the frequency of pink mutational events (PME) increased around the town of Hrazdan (1st sampling point) surpassing the control level 7.5 times. Another pattern was observed, while determining the frequency of genetically undetermined colourless mutational events (WME), the high frequency of it was observed in samples of waters flowing through the Lusakert town (2nd point) and on the border of the Yerevan City (4th point) (surpassing the control level 4.6 and 3.0 times, respectively).

In November, an increase of PME frequency was observed at all points of the Hrazdan River. Moreover, the maximal frequency of mutations induced the samples of waters from the same 1st point (8.9 times). Beside PME, the frequency of WME also increased (1.4-5.0 times). It should be noted that the maximum level was observed (likewise October) in water samples from the 2nd point, surpassing the control 5.0 times.

In December, the frequency of PME in samples of waters from Hrazdan River increased at all points, surpassing the control 3.2 -11.5 times. It should be noted that in December (like in October and November) the maximum yield of PME was observed in water samples from the 1st point. In a number of samples the frequency of WME also increased in 2.5-39.6 times, compared with the control.

A positive correlation of WME induction with manganese concentration for all analyzed points was demonstrated. More likely described chronological variation of genotoxicity of waters was determined by seasonal changes in the concentration of the genotoxic agents in the water. It was assumed that these changes have local origin and are associated with the accumulation of genotoxins in the river after summer rains and autumn season as a result of washout.

FUNCTIONAL ACTIVITY OF ISOLATED AORTA IN PARATHYROIDECTOMIZED RATS

Minasyan A.

Department of Physiology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: minasyanann@yandex.ru; **Phone:** 010-234940, 091-756888,010-560733

There are data in literature that parathyroid hormone (PTH), one of the main calcium-phosphor metabolism regulatory mechanisms, has an impact on hemodynamic indices. Although these data are very contrary and do not allow to draw a final conclusion, particularly during the specific disturbances of calcium homeostasis, evident hemodynamic changes and the conditions provided by these processes. Moreover, there is no data concerning the experiments performed in different part of vessels, which can give a chance to assess the PTH direct effects on vessels functional activity.

The purpose of the study was to investigate contractile activity of isolated aortal rings in parathyroidectomized hypocalcemic rats.

The parathyroid glands were removed by surgical method, which brought to the parathyroid hormone deficiency-specific hypocalcaemia. The indicator of hypocalcaemia was not only a decrease of ionized and free calcium levels in blood, but also an increase of phosphor levels. The functional activity of isolated aortal rings in control and experimental groups was registered on "Isotonicho Mosseinpichtung" (Germany) equipment, by the method of isolated organs (R. Blattner et al., 1983). As an incubation medium we used aired carbogene, normocalceamic buffer solution of Krebs-Henseleit, at t=37° ± 0.5°C and pH=7.38-7.42. The contractile and dilation effects were induced by injection of correspondingly epinephrine (10^{-5} *M*) and papaverine (10^{-4} *M*). The contractile effects were assessed by absolute numbers (*cm*) graded from initial zero level and relaxation by papaverine from maximal contractile level, which was accepted as 100%. The duration of effects occurrence was assessed by minutes. Recorded data was presented in M ± m (Standard Error). The statistic reliability of data was assessed with the help of Student's t-criteria.

The experiments showed that in aortal rings of parathyroidectomized rats with the significant decrease of ionized calcium level (0.16 ± 0.015 *mmol/L* in contrast with 0.32 ± 0.055 *mmol/L* in control) and an increase of phosphor level in blood (3.6 ± 0.37 *mmol/L* in contrast with 1.51 ± 0.18 *mmol/L* in control) the reliable decrease of contractile activity of aortal rings was induced by epinephrine in contrast with intact group. Thus, if in control group the average amplitude of contraction was 14.87 ± 1.1 *cm*, then in the experimental group it decreased to 9.2 ± 1.9 *cm* ($p< 0.05$). The results of relaxation induced by papaverine in experimental rats giving evidence that the vessels of rats showed insignificant decrease of dilation amplitude (84.36 ± 4.61 % in contrast with 84.77 ± 3.21 in control; $p> 0.05$). In addition, prolongation of contractile and dilation effects in isolated aortal rings was also examined in response to addition of epinephrine and papaverine, correspondingly up to 23 ± 3.8 *min versus* 13 ± 1.36 *min* in control ($p< 0.05$) and 24 ± 3.*5 min versus* 18.3 ± 1.4 *min* in control ($p> 0.05$).

In condition of specific hypocalcemia, the analysis of contractile effects and relaxation of isolated aorta rings in response to addition of epinephrine and papaverine to the incubation medium indicated, correspondingly, a reliable decrease of contractile and insignificant reduction of relaxation effects, with the evident prolongation of both effects as well. The possible mechanisms of revealed effects can be conditioned by hormonal disregulation of the calcium homeostasis.

THE TOPOGRAPHICAL PECULIARITIES OF THE PISOHAMATE CANAL OF THE CARPUS EXISTED IN NORM

Minasyan M.

Morphology Department, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

Phone: +374 91 994981/ +374 10 574744

Comparison of the original description of the Guyon canal with definitions emanating from scientific papers, anatomical handbooks, and internet websites reveals challenging differences, which may create clinical confusion. The dissection and bibliometric survey suggest that the canal of Guyon has received multiple denominations including confusion with the "canalis Pisohamatum" the tunnel for the deep palmar branch of the ulnar nerve towards the hypothenar. Over the years this confusion is to be found in various sources e.g. at the internet sites, in scientific/clinical papers and in the "classical anatomy literature".

The purpose of the investigation was to give the anatomic – topographical description of the deep palmar branches of the ulnar artery and nerve in medial palmar aspect of the carpus with determination of the Guyon ulnar canal and pisohamate canal. According with the purpose several tasks were suggested:

1. To examine the parts of the carpal tunnel;
2. To give the complete description of the walls of the ulnar Guyon canal and pisohamate canal;

3. To unify terminology using the term *pisohamate canal* for description of the existed in norm separate anatomical canal in the pisohamate region;
4. To determine differences of Guyon ulnar canal and pisohamate canal;
5. To examine the contents of Guyon ulnar canal and pisohamate canal;
6. To elaborate the new clinical and educational anatomical classification.

Several methods were used:

1. Dissection of the 24 wrists (12 cadavers: 8 male and 4 female bodies of different age) and prepare the materials;
2. Staining of the materials with eosin and BT-200 contrast paints;
3. Use of the 3D computer animation program.

The pisiohamate canal of the carpus is oriented medially and distally from the ulnar Guyon canal. The medial wall of the pisiohamate canal is formed by the pisiform bone, the lateral – by the hook of hamate bone. The roof or the ventral wall is formed by the pisiohamate ligament, the distal edge of which is fused with the *fascias of flexor digiti minimi* and *abductor digiti minimi* muscles, actually converted the passage under the pisiohamate ligament into the really existed canal; but the proximal edge of this ligament reminding the fibrous arch was bounded by the inlet of the canal. The floor or the dorsal wall was formed by the fascial lamina stretches between *abductor digiti minimi* and flexor digiti minimi muscles. The pisiohamate canal continued distally between ventrally located *m. flexor digiti minimi* and dorsally - *m. opponens digiti minimi*. The pisiohamate canal was separate, independent, anatomical osteo-fibrous canal with its own topography. Through the pisiohamate canal definite anatomic structures passed: the deep motor branch of the ulnar nerve and the deep palmare branch of ulnar artery.

Thus, the term "pisohamate canal" makes the anatomical terminology clear understandable, and in practical and clinical points of view it is necessary to include these data into the corresponding literature. The *canalis ulnaris* should not be confused with the *canalis pisohamatum*, which is another tunnel with other entrapment possibilities. The suggested unified terminology of the carpal palmar aspect of the wrist and original clinical anatomical classification allows the practicing doctors to correctly orient during objective examination of the patients and classify them according to the different nosological forms.

THE BACKGROUND PULSE ACTIVITY OF THE *LOCUS COERULEUS* NEURONS OF RATS IN NORMAL CONDITIONS, IN *PARATHYREOPRIV TETANIA* AND AFTER INTRACISTERNAL INJECTION OF PARATHORMONE

Mirzoyan E.

Department of Physiology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: aryavartuni@rambler.ru; **Phone:** +374 77 103128,

Locus coeruleus (LC) performs a key role in integration processes of CNS due to multiple afferent connections with practically all the structures of the brain. It is known that the main mediator of the LC is noradrenaline, moreover there is a cross-regulation between the catecholaminergic and calcium-regulating systems. Particularly calcium ions play a pivotal role in the activation of the secretion of noradrenaline. Therefore, it is actual to assess the character of the interaction between the catecholaminergic and calcium-regulating systems.

The aim of our investigation was to elucidate the peculiarities of the influence of Ca^{2+} level in the brain on the background pulse activity (BPA) of the neurons in the LC.

The experiments were conducted on the anesthetized (uretan) albino rats (males) weighing 180-220 *g*. For the extracellular registration of BPA we used glass microelectrode with the diameter of 1 *μm*, filled with 2 *M* sodium chloride solution. Parathyroid glands were removed for the change of the calcium level in the organism. After the operation, the rats were kept in the isolated conditions during 3-5 days. Then the BPA and calcium level in the blood were registered. In another group 0.7 *μL* parathyroid hormone was injected intracisternal for change of the calcium level in the liquor.

The degree of regularity and the character of dynamic activity of the neurons in LC have been analyzed. The average frequencies of the neurons pulsation were calculated. Student's test and X^2 criterion were used for the assessment of the validity of the BPA changes in various series of experiments.

In accordance with our results, the main neurons of the LC had the intermediate degree of the activity of the BPA: 48.7%. In LC predominate neurons with local and monotone flow of the discharges changes: 47.9% and 23.3%. Under normal conditions in LC predominate polymodal neurons made 50%, monomodal ones: 26.9% and bimodal cells: 23.1%. In accordance with frequency intervals the neurons of the LC with high, middle and low frequency made, 82.1%, 12.5% and 5.1%, accordingly. The average frequency of the discharge in LC was 24.9 *Hz*.

After the parathyroidectomy the number of the nonregular neurons was increased more than 2 times. The number of the mono- and bimodal neurons was decreased, but the number of the polymodal neurons was increased 2 times.

According to the above-mentioned data, reduction of LC activity after injection of PTH can support the idea on participation of calcium regulating system in adaptation processes via inhibition of several centers, which are under the influence of LC.

THE PECULIARITIES OF DAILY COMPLAINTS AND REGIMEN AMONG CHILDREN OF DIFFERENT AGES STUDYING IN THE FIRST GRADE

Mkrtchyan A.

Department of Hygiene and Ecology, Yerevan State Medical University after M.Heratsi, Yerevan, Armenia

E-mail: armenmkrtchyan76@gmail.com; **Phone:** Tel: 374 10 560101

The research aimed at studying the impact of school life on the health of the growing organism and done in the last 20 years have found out that, if in the sphere of school education there were implemented innovations not based on pedagogical, medical and psychological systematic standards, or if as the result of these changes the hygienic norms of school schedule were violated, then this had a negative impact on the health of the students.

As a result of educational reforms it is already several years that in Armenia the school education starts at the age of 5.8 (5 years and 8 months). It is known that in the organism of a child from 5 to 6 years old functioning indicators are significantly lower than those in a child of 7, therefore the education of these children should be organized due to the functioning abilities and readiness of their organisms.

The purpose of our work was to study the peculiarities of daily complaints among children of different age studying in the first grade of Yerevan schools.

The research was carried in 5 different schools of Yerevan City among 352 children of the first grade. The Questionnaire designed by us was the main tool to carry the research, targeting the parents of these children. The daily schedule and the complaints were studied.

The children were divided into 3 groups: 5.5, 6 and 6.5 years old age groups. The first group made 21%, the second one made 39.6 % and the last group made 39.3% of the whole group. The extended-day group school schedule was attended by 14.9 % of the entire group, meanwhile 21.4% of the first group, 2.4% of the second and 17.4 % of the third group. To the question "does your child wake up relaxed in the morning?", the group of 5.5 gave 50% positive answer, while 85% of the other two groups gave the positive answer ($p<0.001$). In the 5.5 age group 51 % slept in daytime, while among 6 and 6.5 age group relevantly: 38.6% and 27.5 %.

Due to demanded norms, this age group is not given homework, but although to the question "is the time allocated for homework?" almost everybody gave positive response and half of them said they give more than 1 hour time to do homework.

The physical activity in an organized way (they attend sport clubs or dance clubs) meets 1/3 of the group. The study of complaints showed that headaches are less frequent for the children of 5.5 group (see the decrease in numbers: 28.6%, 15.5% and 10.1%; $p<0.05$). In the group of 5.5 compared with 6 and 6.5 years old age group there are more children, who point out the tiredness after school (numbers: 35.7%, 25.65% and 14.5%; $p<0,05$).

The nervous system functional disorders are mentioned in 1/3 of the group.

Thus, in the same class among different age groups registered complaints are significantly different and overweigh among 5.5 years old age group.

THE ROLE OF BACTERIAL TRANSLOCATION IN CASE OF PROLONGED EXPERIMENTAL CRASH SYNDROME

Mkrtchyan G., Sahakyan K.
Department of Morphology, Yerevan State Medical University after M.Heratsi, Yerevan, Armenia

In the latest thirties the process of bacterial translocation was of great importance in pathogenesis of polyorganic deficiency due to a source of persistency residential conditionally-pathogene microorganism besides intestine new microorganisms begin to function. The established complex of symptoms: sensibilization, endotoxicosis, bacteriemia, which are conditioned by the process of migration allows to consider the phenomenon of bacterial translocation as a consistent polyorganic deficiency syndrome component.

The investigation of this process from the bacteriological position was the aim of the present study, as endotoxins of Gram-negative residents of microorganisms can serve both as an independent source of intoxication and a modulator of synthesis of exact pro-inflammatory immunocytokins.

The experiments were done in 280 puberal mice with 40-50 *g* weight. The animals were divided into 4 groups. The control group was formed by intact animals. On a special device the experimental group animals underwent squeezing (pressing) during one hour. The area of affection was on the internal surface of the thigh. The pressure on the mentioned area was 280 kDa. The experimental animals were taken away from the experiment 1 hour, 24 hours and 7 days later after decompression. Bacteriological investigations were carried out by a generally accepted scheme, with the content inoculation of the large and small intestines, blood, regional (mesenterial) lymphnodes, lungs, pancreas, liver on Endo's and Ploskirev's medium. Simultaneously the bacteriological analysis to define *E. coli* growth in colonies was carried out.

The bacteriological and bacterioscopic investigations allowed to determine that one-hour exposure of the posterior extremity of the mice provoked a translocation process of residential Gram-negative microflora from econiches of distal parts of the gastro-intestinal tract (large and iliac intestine) into the internal medium of macroorganism. The positive results of sowing Gram-negative microorganisms, including *E. coli* as well, from the regional mesenterial lymphatic node tissues, blood and a number of parenchimatous organs testify in favour of this circumstance.

Already at the early stages of long-term crush syndrome (LCS) in 1 hour after decompression "retrograding" translocation of Gram-negative microorganisms within the gastro-intestinal tract is quite

precisely observed. The degree of bacterial dissemination increased 10^8 CFU, on the background of the decrease in the number of colonies in the ileum- 10^7 CFU. It is worth mentioning that Gram-negative microflora began to get sowed from the pancreas in the mentioned period of observation (10^4 CFU), whereas inoculation in control mice was sterile. Gram-negative microflora with the presence of *E. coli* began to get sowed from blood- 10^3 CFU. The data of bacteriological investigations obtained at the relatively early stages of LCS, though indirectly testified that the econishes of residential Gram-negative microorganisms localized in the corresponding parts of the large intestine (10^7 CFU against 10^9 in the control) served as a possible source of increased dissemination of the small and duodenal intestines and colonization in the pancreas.

In 24 hours after decompression the following picture was observed in the studied organs of the gastro-intestinal tract. The number of colonies kept decreasing (10^5 CFU) even compared with the control indices ($5x10^7$ CFU). We must note that such nature of the process of intestinal gram-positive microflora translocation was observed in mice in the conditions of their 4-hour rough immobilization

According to the bacterioscopic investigation results, *E. coli* made 8-10% of the general number of iliac Gram-negative microorganisms.

We should mention that in that period of LCS the pancreas kept to serve as a source of colonization of microflora translocated from small and large intestines. The general number of colonies practically did not differ from the colony content of the pancreas in mice of the previous experimental group. However, in bacterioscopic investigation among the Gram-negative microorganisms *E. coli* were revealed quite often: in 20% cases. On the 7^{th} day of observation further "colonization" of the pancreas took place by Gram-negative microorganisms (10^5 CFU), among which *E. coli* were defined in 35-40% of cases. A tendency directed to the normalization of bacterial landscape (10^8 CFU) was observed in the ileum. An analogous tendency was observed in the small intestine (10^6 CFU).

Rather low dissemination (10^3 CFU) was registered in blood inoculation in nutrient medium of Endo and Ploskirev. On the 14^{th} day of observation bacterial landscape (we mean only the resident Gram-negative microorganisms, which are revealed in econiches of exact parts of the gastro-intestinal tract) got normalized: in the small intestine $5x10^7$ CFU, in the ileum 10^9 CFU.

The degree of bacterial dissemination in the pancreas significantly decreased (10^2 CFU) when the Gram-negative microflora in its subsequent bacterioscopic analysis was exceptionally presented by a monoculture: *E coli.* The bacteriological analysis showed that the process of bacterial translocation at the early stages of LCS was accompanied by colonization of liver and lungs by Gram-negative microflora. In 15-20% of cases *E. coli* was identified. In an hour after decompression only single punctate colonies were defined in lungs and liver; 24 hours later Gram-negative microflora (10^5 CFU) was intensively disseminated from the lungs. A tendency directed to the decrease of dissemination of the bronchopulmonary tissue (10^3 CFU) was observed quite distinctly in the subsequent period of observation. Moreover, on the 7^{th} day, as well as in 1 hour after decompression only single punctate colonies. were determined in the nutrient medium.

Unlike lungs, colonization of liver by Gram-negative microorganisms took place only in 24 hours post decompression (10^3 CFU). In the subsequent period of observation (on the 7th and 14th days after decompression) only single punctate colonies were disseminated.

It is worth mentioning that in regional (mesenterial) lymphnodes at all the stages of LCS there was observed a picture, analogous to that in bacterioscopic analysis of bronchopulmonary tissue.

On the base of our investigations and comparing them with the latest literature data, we can draw a conclusion, according to which LCS can be considered a syndrome of polyorganic insufficiency (SPOI). Our complex investigations enabled us to determine that both lungs and liver, as well as the pancreas are very early involved in the general pathological process of systemic inflammatory reactions predetermining the character and the course of LCS and the degree of severity of increasing intoxication.

THE ROLE OF COMPLEMENT SYSTEM IN FAMILIAL MEDITERRANEAN FEVER

Mkrtchyan G., Hovhannisyan L., Boyajyan A., Ayvazyan A., Nazaretyan E.

Laboratory of Macromolecular Complexes, Institute of Molecular Biology, National Academy of Sciences of Armenia, Yerevan, Armenia

E-mail: g_mkrtchyan@mb.sci.am; **Phone:** 099456019

Familial Mediterranean fever (FMF; MIM 294100) is the most prevalent member of autoinflammatory diseases worldwide. FMF primarily affects populations originating from the Mediterranean basin, mainly Armenians, Sephardic Jews, and Arabs. The FMF gene (*MEFV*) located on the short arm of chromosome 16, encodes a leukocyte- and monocyte-specific inflammatory regulator called marenostrin or pyrin, and its mutations cause the autoinflammatory phenotype of FMF. The disease is characterized by periodic attacks of fever accompanied by serosal membrane inflammation at the affected sites, such as peritoneum, pleura or synovium, with a massive influx of polymorphonuclear neutrophils. One of the most devastating complications of FMF is amyloidosis, which may affect the kidneys and other organs and tissues. Chronic subclinical inflammatory processes were evidenced in FMF patients between attacks, and several immunological abnormalities, including changes in T and B cells and cytokines, increased levels of acute phase proteins, were reported. However, the clear picture of molecular events involved in the immune system abnormalities in FMF is not well-defined yet. The human complement system is an important branch of innate immunity and in FMF that system might be involved in the uncontrolled inflammation and contributing to the development of this disease.

The present study evaluates the role of complement system in pathogenesis of FMF. We examined the functional activities of classical and alternative complement cascades and their relationship in FMF. As the indicators of the inflammatory response, the hemolytic activities of alternative (AH50) and classical

(CH50) pathways of the complement and the activities of the key complement components C3 were determined in the serum of 28 FMF patients without colchicine treatment. Being a converge point of all three complement activation pathways, C3 is an initial point of the alternative pathway at the same time.

The hemolytic activities of the complement classical and alternative pathways (CH50 and AH50, respectively) and of the complement component C3 (C3H50) in the blood serum of FMF-affected and healthy subjects were measured according to earlier described procedures. Measuring AH50 rabbit erythrocytes were used as target cells. For CH50 and C3H50 assays, sheep erythrocytes sensitized with rabbit anti-sheep erythrocyte antibodies were used as target cells. The hemolytic activity was expressed in *units/ml*. One unit of hemolytic activity is defined as an amount of serum that causes a 50% hemolysis of erythrocytes in the reaction mixture. C3-depleted serum was obtained by treatment of guinea pig serum with zymosan. Data were analysed by Student's unpaired two-tailed t-test and Pearson's correlation analysis. The value of $p< 0.05$ was considered significant.

The mean value of the AH50 in the blood serum of FMF patients was 1.7 times significantly lower ($p<0.0001$) than the mean value of the AH50 in the blood serum of the healthy subjects. The mean values of CH50 and C3H50 in the blood serum of patients with FMF were 1.7 ($p<0.02$) and 1.3 ($p<0.05$) times significantly higher than the mean values of CH50 and C3H50 in the blood serum of the healthy subjects. It is remarkable that in the group of FMF patients we observed statistically significant positive correlation between the levels of CH50 and C3H50 ($p<0.035$). In addition, statistically significant negative correlation was detected between AH50 and CH50 ($p<0.04$). No significant correlation was detected between AH50 and C3H50 in FMF-affected subjects and between the above-mentioned parameters was detected in the healthy subjects.

The results of the present study clearly demonstrate that pathogenesis of FMF is characterized by complement dysfunction, including hyperactivation state of the complement classical pathway and hypoactivation state of the complement alternative pathway. Different complement regulators are present to tightly regulate the complement system. During the acute phase of FMF a multitude of systemic events are triggered, most of which promote an autoinflammatory state, which characterizes this disease. Here, the increased blood levels of acute phase proteins, such as CRP and SAA, detected in FMF patients might be responsible for hyperactivation of the classical pathway during this disease. The alternative pathway is activated following the spontaneous hydrolysis of native C3 generating the efficient alternative pathway C3 convertase. The detected decline in functional activity of the alternative pathway in FMF may reflect either increase in utilization of its initial C3 component through the terminal pathway as a result of hyperactivation state of the classical cascade, or suppression of C3 spontaneous hydrolysis, which is necessary for initiation of the alternative pathway, or implication of both proposed mechanisms. A more detailed study is needed to come to clearer conclusion on molecular mechanisms responsible for complement dysfunction in FMF

THE MORPHOMETRIC CHANGING OF MICROCIRCULATION OF THE LOWER LIMB DURING ATHEROSCLEROSIS AND ENDARTERITIS

Mkrtchyan H.

Department of Morphology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

Phone: + (374) 10 570 680;+ (3740) 91 501 811

The aim of research was to analyze the peculiarities of microcirculation and its structural changes in skin and soft tissues in case of chronic atherosclerosis and endarteritis, as well as to describe blood supply in skin and soft tissues of lower extremities on the level of microcirculation

The objectives of the study were:

1. To find out angioarchitecture of microcirculation level in skin and soft tissues of lower extremities and to give its quantitative description, taking into account the extent of a lesion.
2. To characterize any structural changes in case of atherosclerosis and endarteritis and to find out vascular and tissue correlation in skin and soft tissues of lower extremities.
3. To draw a parallel of morphofunctional changes in microcirculation stream of skin and soft tissues during the mentioned diseases.
4. To find out pathological and compensatory adapted changes in microcirculation stream and cellular texture in conducted research.

The following methods were used:

1. Preparations were made of tissues (skin and soft tissues) taken from 21 patients of operative field (11 male and 10 female).
2. Preparations were stained by hemotoxin in the way of Erzinol, Yenner-Gimza, Van-Gison and Gramm-Veygert
3. Preparations were examined with the microscope and their morphometric data processing was conducted.

The research showed that the changes of microcirculation in skin and soft tissues significantly affected pathogenesis of the above-mentioned diseases.

Proceeding from microcirculation stream changes data, few offers are made to take into account the extent of lesion of microcirculation stream in case of atherosclerosis and endarteritis treatment.

SYNTHESIS OF NEW FUNGICIDES ON THE BASE OF CYCLOPROPANE CARBOXYLIC ACIDS AND TESTING THEIR ACTIVITIES

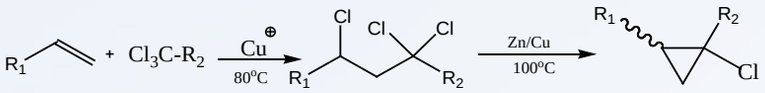
Mnatsakanyan A., Mikaelyan A., Goginyan V., Kocharyan D., Pogosyan M., Torosyan G.
General Chemistry and Chemical Processes, State Engineering University of Armenia, Yerevan, Armenia
State Microbial Depository Centre of National Academy of Sciences of Armenia, Yerevan, Armenia
E-mail: armenuhi-mnacakanyan@rambler.ru

The functionally substituted cyclopropanes are an important class of compounds, because of their occurrence in numerous natural products and drugs, and because of their value as synthetic building blocks in molecules of biologically active compounds.

Some derivatives of cyclopropane carboxylic acids are known as potent fungicide. So, their amides were widely used in agriculture for treatment of fungal disease, 2-oktylcyclopropane-1-carboxylic acid is known as active ingredient of pharmaceutical preparation Octicyl: a cure for such disorders as dermatophytosis, erythrasma, simple (pityriasiform) lichen. Derivatives of polycyclopropane fatty acids (FR-900848 and U-106305, abstracted from microbiological sources) are selective fungicides and owing to their low-toxicity for mammalians have high pharmacological interest.

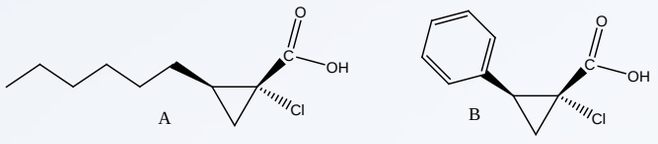
The methods for synthesis of these compounds are mostly based on addition of carbenes to olefins. These reactions need special requirements and are unattractive for manufacturing application.

Earlier we reported stereo-selective method for synthesis of substituted cyclopropanes based on 1,3-dehalogenation of poly-chloroalkenoic acids (Zn/Cu couple in polar aprotic solvents). The obtained 2-alkenylcyclopropane-1-carboxylic acids were successfully used in syntheses of pyrethroides: efficient insecticides. Designed scheme of synthesis can serve as more available and stereo-controlled method for preparing substituted cyclopropanes. On the base of interaction between trichloroacetic acid derivatives and styrene (or 1-oktene) in presence of Cu (I) complexes the following syntheses were carried out.



R_2=CN, CO_2CH_3, $CO_2C_2H_5$ R_1= C_6H_{13}, C_6H_5.

The obtained esters of 1-chlorocyclopropane carboxylic acids were transformed to corresponding acids.



The structure of these compounds was confirmed via NMR and X-ray crystallography studies. As obtained compounds are structural analogues of active compound Octicyl, we investigated them for determination of the biological activity.

The preliminary estimation of fungicide/fungostatistic activity of compounds **A** and **B** was done in 6

different conventional pathogenic strains (*Aspergilus fumigatus, Penicilium chrisogenum, Aspergilus versicolor, Ulocladium botrytis, Penicilium auranitogriseum, Penicilium melinil*). Spores of these strains in a form of aquatic suspension were uniformly superimposed on the 6 Petri dishes with nutrient medium. At the same time 6 sterile filter papers (each with a diameter of 4 *mm*), were soaked into 1.5 *mL* of 10%, 1% and 0.5% ethanol solution of compounds **A** and **B**. After evaporating of ethanol, filter papers with compounds were inputs into any Petri dish on 6 zones, by the same distance.

Starting from the fourth day of cultivation at 25°C fungicide/fungostatistic activity was estimated according to radius of non-strike zones at the round of filter papers.

The obtained results demonstrate that compound **A** displays a stable fungicide influence (radius of influence was kept more then 70 days) and after further testing can be used as a fungicide medicine remedy.

THE STUDY OF PARATHYROID HORMONE ACTION IN DYNAMIC DEVELOPMENT OF AMYLOID BETA 1-42-INDUCED MODEL OF ALZHEIMER'S DISEASE

Mnatsakanyan V.

Department of Physiology, Yerevan State Medical University after Heratsi, Yerevan, Armenia

E-mail: vazgenmnatsakanyan@yahoo.com; **Phone:** +374 93 545819

Despite the intense research into the causes of Alzheimer's disease (AD), only marginal clinical progress has been made and the disorder remains incurable. The calcium hypothesis of aging and neurodegenerative disease postulated that sustained intracellular calcium disturbances are the proximal causes of aging and neurodegenerative disorders, including AD. It allows considering calcium signaling pathways as a therapeutic target. Parathyroid hormone (PTH) has an influence on neuronal functions, regulating intracellular calcium levels.

The aim of research was to investigate the effect of PTH on hippocampal electrical activity and morphological alterations in Aβ1-42 injected rats.

Complex electrophysiological analysis and histochemical and morphological study by detection of Ca^{2+}-dependent acidic phosphatase activity were performed in: 1) intact animals; 2) Aβ1-42 bilateral intracerebroventricular injected animals and 3) Aβ1-42 + PTH treated rats. PTH was administered in a day after Aβ1-42 injection for 7 days (0.7 *mL* 10^{-9} *M* daily). Electrophysiological studies by extracellular recording of hippocampal single-neuronal spike activity under high frequency stimulation of ipsilateral entorhinal cortex was performed in the course of 7 to 20 weeks after Aβ1-42 injection.

During the electrophysiological study a variability of post stimulus excitatory and inhibitory tetanic (TP, TD) and posttetanic (PTP, PTD) potentiation and depression was performed by means of programmed

analysis. Under high frequency stimulation of entorhinal cortex the effects were as follows: in norm group TD+PTP 1.5 times exceeded those of TP+PTP in Aβ1-42 injected group TD was poorly expressed and 4.3 times exceeded TP+PTP during the 7th week and 5.5 times during the 14th week. In PTH treated rats during the 7th week TD was 2 times more frequently encountered than TP+PTP and during the 16th week TD+PTD was recorded 4 times more often than TP+PTP. During the 20th week the effects were poorly expressed in both groups, but comparatively preserved in PTH-treated group. In histochemical study on acute hippocampal slices the swelling of pyramidal neurons with central chromatolysis were observed. In *dentate gyrus* shapeless dark neurons were marked in some areas and the chaotic distributed neurofibrils were observed in cytoplasm. The data testify to hyperphosphorylation that is typical for tau pathology. In PTH-treated group the restoration of shape, size and processes of some neurons in CA1 field took place. In *dentate gyrus* the big neurons started to react and an intensive vascularization was observed.

During the study of different periods of the given model of AD, the modifications of correlations between excitatory and inhibitory tetanic and posttetanic responses were revealed with the prevalence of inhibitory ones, which could have a protective effect as a result of PTH usage.

SYNTHESIS OF NEW AMPHIPHILIC (METALLO)PORPHYRINS AND INVESTIGATION OF THEIR *IN VITRO* ANTICANCER ACTIVITY

Movsisyan[a] L., Babayan N.[2,3], Margaryan K.[3], Tovmasyan A.[1,4]

[1] Department of Organic Chemistry, Yerevan State Medical University, Yerevan , Armenia

[2] Group of Cytology, Institute of Molecular Biology, National Academy of Sciences, Yerevan, Armenia

[3] Department of Genetics and Cytology, Yerevan State University, , Yerevan, Armenia

[4] Department of Radiation Oncology, Duke University Medical Center, Durham, NC ,USA

E-mail: movlevon@gmail.com 055237924

Porphyrinic compounds are used in practical medicine for tumor diagnosis and treatment as photosensitizers. They are extensively studied and described in the literature as a perspective new class of chemotherapeutics. The effect of porphyrins is generally based on their selective uptake and retention by tumor tissues.

Earlier the structure-activity relationship of new water-soluble cationic pyridyl-porphyrins and their derivatives as potential anticancer agents were studied *in vitro*. Metalloporphyrin that includes Ag as a metal atom and allyl functional group (Ag-TAll4PyP) at pyridine ring of *meso*-position was shown to be the most effective (cytotoxic) among them. New porphyrins with lipophilic groups were synthesized to increase bioavailability and cytotoxicity toward cancer cells.

They are characterized by TLC, UV/vis, and NMR spectroscopies and the amphiphilic one (Ag-Tri-

All4PyMVan) tested for the following effects: genotoxicity (CBMN and Comet assays), influence on the cell cycle (flow cytometry) and tissue specificity in five human cell lines derived from blood (KCL22), liver (HEP-3B), cervix (HeLA), breast (MCF-7), and brain (LN-308).

Ag-TriAll4PyMVan was demonstrated to be non-genotoxic. A dose-dependent cell cycle delay was detected after exposing cells within the sublethal doses of the porphyrin. Ag-TriALL4PyMVan induced G1 and S phase arrests in KCL22 cells during 24 *h* of incubation. The most sensitive tissue was blood (IC_{50}=4.8 μM), and the least was cervix (IC_{50}=9 μM). The sensitivity of other cell lines was nearly the same (IC_{50}=5.5-6.4 μM). It was also shown that the porphyrin studied was more toxic for cancer cells than known chemotherapeutic Cisplatin. The metal-free analog of the amphiphilic Ag-TriAll4PyMVan porphyrin synthesized was demonstrated to possess the photodynamic activity higher than the known photosensitizer Chlorin e_6. At the same time, it has no dark toxicity within the phototoxicity concentration range.

The results suggest amphiphilic metalloporphyrins' promising potential as antitumor agents.

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IMMUNOMORPHOLOGICAL SHIFTS IN ORGANS OF IMMUNOGENESIS OF EXPERIMENTAL ANIMALS IN HYPOKINESIA

Muradyan D.[1], Vardanyan L.[1], Alekyan N.[2], Shekoyan V.[1]

[1] Department of Microbiology, Yerevan State Medical University, Yerevan, Armenia,

[2] Scientific-Research Center, Yerevan State Medical University, Yerevan, Armenia

E-mail: dmuradian2001@yahoo.com; **Phone:** 094 47 1823

One of the topical problems in modern medicine is that of motor activity restriction (hypodinamics, hypokinesia), because the hypokinetic syndrome is detected as a risk factor of cardiovascular diseases, motor-supporting apparatus, endocrinopathia and immunodeficiency conditions.

At the same time, immunopathological shifts in central and peripheric organs of immunogenesis in different periods of hypokinesia are not studied completely.

Data about structural and immune shifts in the thymus, spleen and lymph nodes of experimental animals exposed to 3-, 7- and 14-day hypokinesia are presented in this article.

Experimental animals were exposed to partial restriction of motor activity. Animals were decapitated in 3, 7 and 14 days after hypokinesia, in compliance with all the conditions, demanded by the ethic committee of YSMU in euthanasia. Structural changes in the thymus, spleen and lymph nodes were studied in slides stained by hematoxylin and eosin, which were prepared from paraphin blocks. Shifts in B-

lymphocytic population count and immunocompetent cells producing IL-6 were studied consequently in the reactions of direct and indirect immunofluorescence using commercial reactive agents produced by Sigma (USA).

The initial stages of lymphoid tissue hypoplasia were detected in central and peripheric organs of immunogenesis in 7 days of partial restriction of motor activity.

The structural changes of the thymus cortical part were observed in 14 days of hypokinesia, which is known as a "thymus accidental involution". In this observation period the expressed hypoplastic processes of lymphoid tissues and spleen were observed, which were characterized by remarkable decrease of immunocompetent cells of both organs in all the zones and layers, uncovering the reticular stroma.

A cytoangioarchitectonics of lymphoid follicles of spleen and lymph nodes was destructed.

Thus, particularly, periarterial, mantle and marginal zones of spleen follicles were in the stage of discomplexation.

It was established that at the relatively late stages of hypokinesia (14 days of observation) there was inhibition of humoral immunity response, which was expressed by a significant decrease of lymphoid cells in B-dependent zones of spleen and lymph nodes.

Our research found out that IL-6 positive lymphoid cells were revealed in the thymus and T-dependent zones of the spleen and lymph nodes both in intact animals and in those exposed to 3-, 7- and 14-day motor activity restriction.

At the same time, the number of IL-6 positive cells in T-dependent zones of the spleen and lymph nodes significantly increased in 7 and 14 days after hypokinesia.

Taking into consideration the multidirectional properties of IL-6, its role is very disputable in the induction of immunopathological processes during hypokinesia.

On the one hand, the increase in IL-6 positive lymphocytes concentration in peripheral organs of immunogenesis is considered a provoking factor, which can cause hyperthermia and synthesis of acute phase proteins, which can affect negatively on the function of integrative system of experimental animals exposed to hypokinesia.

On the other hand, it is necessary to evaluate the increase in IL-6 positive lymphocytes concentration as a formation of adaptive mechanisms, since it is determined that IL-6 provides the proliferation and differentiation of T- and B-lymphocytes population in a dose- dependent way.

PREVALENCE OF METABOLIC SYNDROME IN ZAHEDAN, SOUTHEAST IRAN

Narouie B., Keykhaei M., Shikhzadeh A., Jahantigh M., Shirzaei E., Rezazehi B., Hoseinian M., Yousefi Sh., Masoudian S., Hashemi M., Hanafi-bojd H.

Clinical Research Development Center, Ali Ebne Abitaleb Hospital, Zahedan University of Medical Sciences, Zahedan, Iran

E-mail: b_narouie@yahoo.com; Telefax: 00985413414103

The present study determines a prevalence of the metabolic syndrome (MES), as well as cut-off points for waist circumference (WC) for diagnosis of MES in Zahedan, southeast of Iran.

The aim in this study is to survey the prevalence of metabolic syndrome in Zahedan.

In 1802 individuals (735 men and 1067 women) MES was determined according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) and International Diabetes Federation (IDF) criteria.

The prevalence of MES was higher in women than in men. In both sexes, the prevalence increased with age. According to NCEP ATP III and IDF the prevalence of MES among 1802 individuals aged ≥19 years were 21.0% (15.4% in male, 24.9% female) and 24.8% (20.0% in male and 28.1% in female), respectively. Using obtained the WC cut-off values (93.5 *cm* in men and 85.5 *cm* in women) in our population the prevalence of MES was 22.8% (20.0% in male and 24.6% in female). The results showed that reduced high dencity lipopropteins–C (HDL-C) (60.6%) and WC (43.3%) was the most common components of the metabolic syndrome, followed by high triglycerides (32%), elevated glucose (17.1%), and high blood pressure (13%).

This study shows a high prevalence of MES in Southeast of Iran.

DETERMINATION OF SENSITIVITY AND SPECIFICITY OF ULTRASONOGRAPHY IN DIAGNOSIS OF FREE ABDOMINAL FLUID IN PATIENTS WITH BLUNT ABDOMINAL TRAUMA

Narouie B., Rohani Z., Agha-teimori M., Shikhzadeh A., Hanafi-bojd H., Soltanpour N., Ghasemi-rad M.

Clinical Research Development Center, Zahedan University of Medical Sciences Ali Ebne Abitaleb, Zahedan, Iran

E-mail: b_narouie@yahoo.com; Telefax: 00985413414103

Nowadays, trauma is the fourth cause of mortality and morbidity in developed countries, with major cause of these mortalities being intra-abdominal hemorrhage due to blunt trauma. Therefore, every action for early diagnosis of intra-abdominal hemorrhage could effectively save the lives of more patients.

The aim of this study was to determine the sensitivity, specificity, positive and negative predictive values and accuracy of ultrasonography (US) compared with CT-scan in detection of free fluid and parenchymal injury in adults and children admitted with blunt abdominal trauma.

Ninety-eight patients were surveyed, 66% were adults and 34% children (< 13 years old); 80% were males and 20% were females. The most common presenting symptoms in both groups were abdominal pain followed by abdominal tenderness. All patients with blunt abdominal trauma admitted to emergency department of our referral hospital were enrolled in this study. Demographic data with results of US and CT-scan were collected in the questionnaire and analyzed. Using CT as the gold standard, we used sensitivity, specificity, positive predictive value, negative predictive value, and accuracy as statistical tools for comparison.

Sensitivity, specificity, positive predictive value and negative predictive value and accuracy of US in the diagnosis of free abdominal fluid in all patients were 84%, 86%, 91%, 75% and 83-85%, respectively.

In the children group, the results were 80%, 77%, 84%, 71% and (81-79%), respectively; while in adult group, the results were 86%, 91%, 95%, 78% and (84-88%), respectively. However, the same values were 54%, 90%, 65%, 79%, and 76% in the diagnosis of intra-abdominal parenchymal damage, respectively.

Ultrasonography has acceptable sensitivity, specificity, and accuracy to detect free fluid in blunt abdominal trauma compared to CT-Scan in children and adults age groups. By combining abdominal parenchymal abnormalities, these parameters will increase sensitively especially in children's age group**.**

CYTOGENETIC CONTEMPORARY METHODS AND THEIR IMPLEMENTATION IN ONCOMORPHOLOGY

Papyan A., Dabagyan V., Avoyan A.

Pathological Anatomy Department, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: andrew_papian@yahoo.com; **Phone:** +374 10 581263

Several methods are used to reveal tumors genetic abnormalities in contemporary medicine. Immunohistochemistry is remaining the most widely implemented method with high clinical significance. Immunohistochemical method is based on antibodies binding to the cellular proteins. It allows to assess genetic abnormalities in cells indirectly.

Fluorescent *in situ* hybridization (FISH) and chromogen *in situ* hybridization (CISH) methods reveal cells nucleotides. A strong hybrid formation between cellular DNA and DNA probes (nucleotides chains complimentary to nuclear DNA) is the basis of *in situ* hybridization method. DNA probes are labeled by fluorescent or chromogen particles to be revealed in different detection systems (luminescence or light microscopy).

At 20-30% of the breast cancer persons with the aggressive course and poor prognosis HER2 gene (17q21) amplification is detected. Immunohistochemistry, FISH and CISH methods are currently used to assess the HER2 status.

The aim of this study was HER2 gene status investigation by CISH method in cases of transitional cell carcinoma of the urinary bladder and its comparative characteristic to other detection methods.

Tissue samples were fixed in 10% neutral buffered formalin at room temperature during 24 hours. Samples were embedded in paraffin blocks by the routine technique. Chromogen *in situ* hybridization was done with Zymed SPOT-Light HER2 kit. Denaturation was conducted for 5 minutes at the 95°C temperature. Hybridization was done within 12 hours at 37°C. Results were assessed by light microscopy.

The results of immunohistochemical methods were assessed by the following scoring system: 1+ means absence of hyperexpression, 3+ indicated presence of hyperexpression, 2+ needed extra FISH or CISH investigations. Complimentary labeled DNA-probe was used in order to detect HER2 gene amplification by CISH method. Brown spotty pigmentation was revealed under the light microscopy. Six and more spots in singe cell showed positive HER2 gene amplification, 1-2 spots meant negative amplification, and cases with 3 to 5 spots needed extra investigations.

Immunohistochemical method detects HER2 protein hyperexpression, but it cannot show abnormalities at the gene level. HER2 protein overexpression may be detected in cells with lack of gene amplification (false positive results). FISH method is a very sensitive method to reveal HER2 gene amplification. Now it is considered to be a "gold standard" in molecular pathology, but in most cases it remains technically difficult and very expensive. The advantage of CISH method implementation is in preserving morphological structures of samples and an allowance of simultaneously assessment of both structural and genetic changes. On the other hand, results of this method assessed under the light not luminescence microscope and, finally, samples with CISH may be preserved for a long time. It is not possible in case of FISH method due to the fast loss of fluorescent signals.

ANTIBIOTIC SUSCEPTIBILITY OF POTENTIALLY VAGINAL PROBIOTIC *LACTOBACILLUS CRISPATUS MH08*

Pashayan M.

Department of Drug Technology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

Bacteria of the genus *Lactobacillus* were proposed as probiotic microorganisms to restore the ecological equilibrium of the intestinal, respiratory, and urogenital tracts. They were also increasingly considered for use in women to prevent genital and urinary tract infections The knowledge of antimicrobial susceptibility or resistance is of interest to predict the behavior of an exogenously applied probiotic

formula in patients subject to any type of chemotherapy, as well as to consider the concomitant use of the probiotic and antibiotics for the restoration of the normal urogenital flora. On the other hand, the antimicrobial susceptibility of exogenously applied microorganisms needs to be known for treating eventual collateral effects. In this regard, the performance of antimicrobial susceptibility testing may be considered as both a necessary selection criterion for probiotic cultures and an effective guide for specific antimicrobial therapy.

The present study was conducted to determine the antibiotics susceptibility of candidate probiotic *Lactobacillus crispatus* MH08 isolated from the human vagina of a woman from Yerevan, Armenia, and identified by biochemical profiles and sugar fermentation patterns *(deposited as INMIA 9612 in the Center of Microbiology and Microbial Depository of National Academy of Sciences, Armenia)*.

LAPTg and MRS agars are suitable media to study antimicrobial susceptibility of *INMIA 9612*.

The Minimal Inhibitory Concentrations (MICs) were determined in LAPTg broth. Solutions of each antibiotic at 10 to 50 *mg/mL* concentrations were prepared and then serially diluted in LAPTg broth and added to LAPTg broth to obtain final concentrations of 1 to 1000 *μg/mL*. Fifty *μL* of exponential growth phase microorganisms at concentration of 10^7 to 10^8 *CFU/mL* were inoculated in LAPTg with antibiotics. The culture was incubated up to 48 hours at 37°C and the inhibition of growth was spectrophotometrically determined at 540 nm performed in LAPTg broth.

MICs with different antibiotics in LAPTg broth were determined. Inhibitors of the cell wall synthesis (vancomycin, kanamycin, streptomycin, chloramphenicol, norfloxacin, ciprofloxacin, and metronidazole) were employed for inhibition tests.

The MICs showed that *L. crispatus* strain grew at concentrations above 10 *μg/mL* of chloramphenicol, norfloxacin, streptomycin, kanamycin, and was resistant to 1000 *μg/mL* of metronidazole and vancomycin susceptible (MICs <4 *μg/mL*).

The effect of antimicrobial substances on autochthonous *Lactobacillus* is of interest in understanding the development of genital and urinary tract infections related to the lack of these bacteria. Resistance to high concentrations of metronidazole suggests that *L. crispatus* MH08 could be simultaneously used with a bacterial vaginosis treatment to restore the vaginal normal flora.

ZINK CONTENT IN PHENYLKETONURIA PATIENTS AND THEIR PARENTS

Petrosyan A.[1], Davtyan A.[1], Moryan G.[1], Kostandyan N.[2]

[1] Therapy Department, Laboratory Diagnostic Center, Yerevan State Medical University, Yerevan, Armenia

[2] Republic Medical Genetics Center, Yerevan, Armenia

E-mail anahitp@ymail.com; **Phone:** (+374 10) 45 55 35/ 093 558 919

Zn as a coenzyme and part of metalloproteids takes are involved in a more than 200 enzyme reactions regulation, development of androgen sexual glands and sperms, keeping structure of estrogen receptors. As an active center of superoxide dismutase Zn takes part in protective and antioxidants regulation reactions. Zn is a neurotransmitter and neuromodulator of CNS. It is proved that Zn organizes creating of bridge of cells' membranes potential, as an inhibitor Zn inactivates NMDA receptors of glutamate. Zn deficiency can be caused by autosomal-recessive diseases when the absorption of microelements, synthesis of amino acids and biopolymers are disturbed. Zn activates movement of Fe^{2+} - Fe^{3+} by creating dipoles due to hydrolisation of tyrosine-248 NH-group peptide compound. For Zn assimilation an organism needs enough amount of Vit A, B_6. Cellulose, phytin, phosphates, calcium, and copper decrease Zn absorption, but glucose, amino acids, peptides increase Zn absorption. Zn deficiency leads to hyperactivity, sleep disturbance, stomatitis, dermatitis, dry skin, eczema, Prasad disease (severe Fe deficiency anemia), dwarfism, hepatosplenomegaly, etc.

There is no any information about Zn metabolism in Phenylketonuria (PKU) patient and cause- consequence link in autosomal-recessive disease. Therefore, the goal of this research was to find out changes of Zn level in PKU patients and their parents, verification and evaluation of the cause-consequence link.

The. content of Zn was investegated in blood serum using ELITech Co. kit, by 5-Br-PAPS special complex creation method, fluorospectrometry.

According to our results, Zn concentrations in PKU patients werre between 6.0-17.0 *μmol/L*, in parents: 7.8-9.0 *μmol/L*, namely in mothers: 7.8-9.0 *μmol/L*, in fathers: 6.2-7.8 *μmol/L*.

Children who do not use cellulose and use MD MIL PKU-1 (special amino acids mixture for PKU patients) have normal or close to normal results, since them get enough amount of Zn.

Children up to 2 years who use MD MIL PKU-3 (special amino acids mixture for PKU patients) and other products, which disturb Zn absorption, have Zn deficiency, probably because of enzyme mechanism deficiency.

Moderate deficiency of Zn is registered in parents of PKU patient, that is probably connected with adeficiency of metalloproteids absorption and using low level of Zn-containing products in their nutrition.

INFLUENCE OF MEFV MUTATION ON NEUTROPHILS PHAGOCYTIC ACTIVITY

Petrosyan L.[1], Ghazaryan D.[1], Avetisyan S.[1], Davtyan T.[2], Hakobyan G.[3]

[1]Department of Pathophysiology, Yerevan State Medical University, Yerevan, Armenia

[2] Laboratory of Immunology and Virology, "Armenicum" Research Centre, Yerevan, Armenia

[3]Department of Internal Medicine Yerevan State Medical University Yerevan, Armenia

E-mail: lousine.petrosyan@yahoo.com, davitghazaryan@yahoo.com; **Phone:** +374 10 58-75-27/ 091 18 98 18

The most common auto-inflammatory disease in Armenian population is Familial Mediterranean fever (FMF): the carrier rate is 1:5 and disease rate is 1:500 among Armenians, which is the highest frequency compared to other populations. The high disease rate of FMF among Armenians is a serious stimulus to investigate its pathogenesis. The mutation is located in MEFV gene (this gene is expressed only in innate immune system cells), which causes structure and properties changes of the MEFV-encoded protein pyrin. Pyrin, in addition to its other functions, interacts with cytoskeleton proteins to regulate chemotaxis and phagocytosis. Taking into account the above-mentioned, we can say that there is a relationship between MEFV mutation and disorders of these functions in innate immune cells.

The aim of this study was to examine neutrophils and monocytes cytoskeleton-depended functions, such as phagocytosis in FMF patients in relation to normal donors. We expected that discovering the specifity of phagocytosis during FMF will certainly illustrate later the attacks periodic and self-limited nature, the inflammation and injury intensity in serous tissues during attacks, the subclinical inflammatory activity in attack-free periods, and thus allow us to improve the effectiveness of pathogenetic therapy.

Peripheral blood samples were obtained from 9 patients with FMF and 4 normal donors.

Each sample was divided into two groups: control group and chemoattractant-activated (with fMLP) group and then each of them was divided into 5 parts. A sample from each group was left on ice during the whole experiment. The others were incubated in a water bath at 37°C for 1, 3, 5, 10 *min*, respectively. Then latex beads with fluorescence activity were added to all blood samples. Phagocytosis was monitored using flow cytometry method by determining the relative fluorescence intensity (MCN).

As expected, phagocytic activity of neutrophils in FMF patients was higher than in normal donors. The period of neutrophil phagocytosis oscillation for FMF patients was 3.65±0.03 *min* and the amplitude 274.9±37 MCN. Whereas, in normal donors 6.06±0.12 *min* and 68.3±19 MCN, respectively.

It was interesting to reveal that fMLP-stimulation caused an increase in neutrophil phagocytosis oscillation period (till 6.03±0.05 *min*) in FMF and a linear time-dependence in normal donors.

The results of this study prove once more the importance of "mutant MEFV gene – change in pyrin properties – change in neutrophils behavior" relation. The increased phagocytic activity can be one of the factors, which play a role in subclinical inflammation development. The increase in neutrophil phagocytosis oscillation period (but not the loss of periodicity, as it was observed in neurtophils of normal donors) caused by fMLP-stimulation might be considered as a manifestation of cellular activation-deac-

tivation program plasticity impairment and a potential mechanism of attacks generation and duration in FMF.

MONITORING OF CONGENITAL ANOMALIES AND THE HEMOMICROCIRCULATORY SYSTEM OF THE HEART IN NEWBORNS, WHICH WERE DEAD IN THE EARLY NEONATE PERIOD IN THE REPUBLIC OF ARMENIA DURING THE LAST 10 YEARS

Petrosyan R.[1], Petrosyan A.[2]

[1] Department of Morphology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

[2]Department of Pathanatomy, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: rupetrossian@yahoo.com; **Phone:** 094499506

Monitoring of congenital anomalies of the perinatal child mortality among newborns of the Republic of Armenia during the last 10 years was performed at the Republic Child's Pathanatomical Centre, and the research work on hemomicrocirculatory bed of the heart was done.

The statistic analysis of 3981 histories of pathanatomic dessections was performed. Circulation system of the heart was studied in autopsies of 48 newborns, which were dead in early neonatal period: at 7 days of age.

The retrospective study was based on the pathanatomical histories of the newborns.

Studying of statistic materials is one of the actual problems of modern medicine. One of the accepted methods of the permanent control of the hereditary changing in human population is the frequency of congenital anomalies. Congenital anomalies are a major cause of neonatal and infant mortality.

Monitoring of congenital anomalies among newborns of the Republic of Armenia during the last 10 years was performed. On the basis of official papers of pathology departments nineteen forms of congenital anomalies, according to the European Register, were registered retrospectively (1998-2007).

During the study period, 814 newborns and fetuses were found to have malformations, inclusive of 174 with the congenital heart malformation.

As a result of analysis of the statistic data it can be said with confidence that there is a tendency of increasing of number of congenital anomalies of central nervous (134%), multiple congenital anomalies (103%), and the congenital anomalies of the heart (15%) in newborns, during the period of study and it emphasizes the necessity for serious studies of the problem. It is interesting that the number of male newborns with congenital heart anomalies is higher (64.6%) than the number of female newborns. Majority of the newborns were premature (54%). Among newborns with the congenital defects 65.2% made stillborns.

The pathological alteration and capillarotrophic failure in the microcirculatory system of the newborns with the congenital heart defects, which were dead in the early neonatal period may be due to true capillary deficiency in the microhemocirculation system results in centralization of tissue circulation and reduction of its metabolic efficiency. Such type of circulation is microhemodinamic centralization.

CAN MOUSE EMBRYONIC STEM CELLS DIFFERENTIATE UNDER THE INFLUENCE OF RECOMBINANT PROTEIN LIF?

Petrova R., Mezhevikina L.

Laboratory of Reception Mechanisms, Institute of Biophysics of Cell, Russian Academy of Sciences, Pushchino, Russia

E-mail: rushanapetrova@gmail.com

The increased attention to mammalian embryonic stem cells (ESC) is caused by the reason that these cultures are the source of differentiated cells used in fundamental and applied researches. Pluripotent embryonic stem cell lines are capable for spontaneous differentiations into other type of cells and tissues . Various techniques of cultivation on the feeder layers and chemical inductors are used for the certain phenotype of ESC obtaining. As a rule, these ESC differentiations *in vitro* are connected with formation of embryoid bodies (EB).

It is worth mentioning that induction of differentiation into EB is connected with the formation of heterogeneous population with a little part of certain phenotype cells. At present, the heterogeneity of ESC is an essential limitation for their use in applied medicine.

The aim of our work was to obtain cardiomyocyte type from ESC without the EB formation.

For that purpose the ESC were prolonged cultivated with the recombinant LIF protein from Cos-1 cells transfected by pcDNA3 plasmid (Invitrogene) with mouse *lif* gene. The commercial mouse LIF recombinant protein (ICN) was used as a control. Transfection of cell line Cos-1 was carried out in accordance with the protocol of the Lipofectamine2000 (Invitrogen), with selection of transfected clones in medium with G418 (300 mkg/mL). As a control, marker vectors pVax1/lacZ (Invitrogen), carrying a bacterial gene lacZ, and pEGFP, encoding gfp (*Clontech*) were used.

In contrast to LIF (ICN), the recombinant Lif-Cos has a distinctive feature: it increases the ability of mouse ESC to survive in form of colonies without formation of embryoid bodies.

At prolonged cultivation with Lif-Cos, the autonomous contractive activity foci of about 1.55±0.4 mm^2 ($1.6 \times 10^4 \pm 0.29$ cells, n = 6) size, were appearing in pluripotent colonies on days 20-26 of cultivation. At cytochemical determination of endogenous alkaline phosphatase (AP) activity, nonuniform coloration of such colonies was shown. The AP activity in contractive region was reduced that pointed to the process of cell differentiation.

According to our observations, cells of interface between more compact central mass of pluripotent cells with high AP activity and peripheral monolayer are those that differentiate into contractive activity ones. Furthermore, several contractive foci were registered in the same colonies with different sizes and frequency and amplitude of contractions.

To identify the type of differentiated cells we used the cardioactive substance: isoproterinol (IP) (10^{-7}-10^{-5} *M*). In mammalian embryonic cardiomyocites isoproterinol selectively acts on β-adrenergic receptors and affects contractive rhythm by activation of ion channel, membrane transport systems and myofilament proteins.

The maximal response of differentiated mouse ESC on β-adrenergic stimulation appeared 2-3 minutes after addition of 10^{-7}*M* isoproterinol to cultivation medium. At isoproterinol influence the contractive frequency was increased from 19 ± 1.2 to 58 ± 2.3 beats per minute. This reaction was typical for cardiac cells with β-adrenoreceptors expression. Determination by monoclonal antibodies to mouse cardiac α-actin proved cardiomyocite differentiation cell type. More early predecessors of cardiac cells had small size and round shape. The specific cells with sarcomere organization and ability for contraction were formed in the course of their development.

The presence of β-adrenoreceptors and myofilament cardiac α-actin in mouse ESC is an evidence of activation of cardiac differentiation processes occurring after prolonged cultivation with the recombinant LIF-Cos protein. According to results of LIF regulation investigations, LIF protein effects on different transduction ways, including those responsible for differentiation of pluripotent cells. The mouse ESC colonies with retractive activity are an experimental model for revealing of cell and molecular mechanisms of cardiac differentiation, for electrophysiological investigations, as well as cardioactive drugs screening.

In prolonged cultivation conditions of ESCs with mouse recombinant protein LIF-Cos an experimental model of cells with contractile activity of the type of cardiomyocytes was obtained for screening cardioactive drugs. It was established that the secretory protein LIF-Sos activates the differentiation of ESCs β-adrenoceptor and the intensity of cell contractions in response to the action of izoproterinol.

CD4 EXPRESSION ON ACTIVATED HUMAN MONOCYTES AFTER DIFFERENT WAYS OF STIMULATION

Poghosyan D., Tadevosyan G., Nersisyan L., Arakelyan A.

Research Group of Immunoregulation, Institute of Molecular Biology, National Academy of Sciences of Armenia, Yerevan, Armenia

E-mail: davpog@yandex.ru

CD4 is a characteristic marker of T-helper cells, but it is also expressed by other white blood cells including monocytes/macrophages. CD4 was shown to play certain role in the functional activity of monocytes, e.g. antigen-presenting capacity and inflammatory response. Blood-derived monocytes expressing CD4 were found at the site of inflammation in experimental model of myosin-induced myocarditis. CD4 crosslinking in monocytes was shown to induce the production of proinflammatory cytokines TNF-α and IFN-γ as well.

The few data about CD4 expression on monocytes/macrophages after cultivation are controversial, although more recent studies have shown that the CD4 expression is down-regulated during monocyte cultivation. This could be explained, for example, by differentiation of blood monocytes into tissue macrophages and/or by CD4 endocytosis. CD4 down-regulation is also detected after macrophage activation *in vitro*: the LPS treatment causes down-regulation of total CD4 at the level of transcription.

The aim of our study was to investigate changes in $CD4^+$ monocyte subpopulation after cultivation *in vitro* with and without treatment with colchicine (Col) and activators: bacterial lipopolysaccharide (LPS) and peptideglycan (PGN).

The peripheral blood mononuclear cells (PBMCs) of five 20-40 years old healthy donors of both sexes were studied. Cells were collected by Histopaque gradient centrifugation of whole blood, and moved to RPMI-1640 medium, containing 10% FCS, 5 *mM* HEPES, 2 *mM* L-glutamine, 1 *mM* sodium pyruvate, 100 *U* of penicillin, and 100 *ug* of streptomycin per 1 mL. Then cells were treated with LPS, PGN or Col with final concentrations 10 *ng/mL*, 2.5 *ug/mL* and 2.0 *ug/mL*, respectively, and cultivated for 3 days. After cultivation the cells were pelleted, washed 3 times with BD CellWash washing solution and incubated for 30 *min* with FITC-labeled anti-CD4 and PE-labeled anti-CD14 monoclonal antibodies according to the protocol. Matched isotype controls were used to exclude non-specific binding of antibodies. After the incubation CD4 and CD14 expression, as well as cell counts were measured by BD FACScan flow cytometer. Non-parametric ANOVA test analog (Kruskall-Willis test) was used to compare changes in $CD4/CD14^+$ cells, as well as levels of CD4 expression on the cells in the studied groups.

It was shown that the number of $CD4^+$ monocytes on the first day (without any treatment) was significantly higher than after cultivation in LPS-, PGN- and Col-treated, as well as in non-treated samples ($p<0.05$). It was also observed that on the third day the number of CD4-expressing monocytes was approximately 2.5 fold lower after the treatment with LPS, PGN and Col ($p<0.05$) compared to non-treated samples, and this difference did not depend on treatment type.

No significant difference in the levels of CD4 expression on cells was found in the studied groups. It could be explained by the increase of cells autofluorescence after cultivation that does not allow to distinguish the fluorescent signal from the background "noise". However, cells treated with Col showed slightly decreased CD4 expression compared to cells treated with LPS and PGN and even to non-treated cells.

Our data suggest that the expression level of CD4 on monocytes does not change after the cultivation and treatment with activators (LPS and PGN) or colchicine. Although this could be a methodological problem, because of dramatically increased levels of autofluorescence of monocytes after cultivation, we found that the number of $CD4^+$ monocytes decreases compared to the first day, and that in treated species it decreases more dramatically regardless of treatment type. We can speculate that the mechanism of impairment of $CD4^+$ cell number differs in case of treatment with activators and Col.

THE INFLUENCE OF PIRACETAM ON BEHAVIORAL REACTIVITY OF RATS UNDER THE CONDITIONS OF HYPOKINESIA

Poghosyan V.
Department of Pharmacology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia
E-mail: varduhikarapetyan@yahoo.com

The breaches of the different links of homeostasis under the conditions of hypokinesia promote the development of cardiovascular, respiratory and excretory systems' diseases, depress immunoreactivity of an organism, and induce the formation of different psychoneurological disorders.

The aim of this study was monitoring of rats behavioral reactivity under the conditions of hypokinesia and under the influence of Piracetam, taking into consideration its wide use in treatment of various functional and organic affections of brain.

The experiments were performed in 64 white mongrel male rats, weighing 200-220 *g*. Rats were divided into the following groups: control (rats were kept in similar conditions of vivarium), hypokinetic (rats were placed in the individual narrow Plexiglas cages during 15, 30 and 45 days), hypokinetic and taking Piracetam (rats were placed in the individual narrow Plexiglas cages during 15, 30 and 45 days and were intraperitonealy injected Piracetam in the dose of 100 *mg/kg* during the last 10 days of hypokinesia). Behavioral reactivity of animals was tested in "Open Field". General locomotor activity was defined as a sum of peripheral, central crossings and number of rearings. Number of faecal granules was calculated as an index of emotionality. Rats were tested before placing in the individual cages and on the 15^{th}, 30^{th} and 45^{th} days of hypokinesia.

Statistical data manipulation was performed by Microsoft Excel 2000 using Student's t-test.

The investigations of behavioral reactions in "Open Field" showed that on the 15th day of hypokinesia general locomotor activity of hypokinetic animals decreased by 66.72% (p<0.0001) in comparison with control group, on the 30th day of hypokinesia it decreased up to 79.32%(p<0,0001) and on the 45th day up to 91.6%(p<0.0001). At early periods of restricted movement activity a decrease of rearings was more expressed than the decrease of peripheral and central crossings, and in late period of hypokinesia significant decrease of both crossings and rearings was observed. Besides,, on the 30th day of hypokinesia the reinforcement of emotional reaction of fear was observed, which was reflected by the increase of faecal granules number up to 50.2%(p<0.05). Monitoring of rats behavior with Piracetam injections showed that their general locomotor activity was higher in comparison with hypokinetic rats not injected Piracetam. Thus, on the 15th day of hypokinesia general locomotor activity of this animals decreased by 61.07% in comparison with control group, on the 30th day of hypokinesia by 67.62% and on the 45th day by 60.05%(p<0.005) compared with control. But Piracetam displayed a most significant effect on increased emotional reactivity of rats decreasing it by 25% on the 30th day of hypokinesia and by 40% on the 45th day.

The present results reveal that Piracetam affects the emotional reactivity more significantly than general locomotor activity of experimental rats. The influence of Piracetam was much more expressed in late periods of restricted movement activity.

INFLUENCE OF HYDROSTATIC PRESSURE ON VOLTAGE-CURRENT CHARACTERISTICS OF A BILAYER LIPID MEMBRANE

Potikyan G.[1], Arakelyan V.[2], Gevorgyan H.[2], Yeghiazaryan K.[1]

[1] Department of Medical and Biological Physics, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

[2] Physics Department, Yerevan State University, Yerevan, Armenia

Email: pgagik@yahoo.com; **Phone:** +37494 963737

The investigation of the electric characteristics (electric capacitance, conductivity, etc.) of biological membranes presents an important aspect in membranology. It is known that both electric forces and hydrostatic pressure (P) frequently act on the membrane, changing the magnitudes of electric characteristics of biological membranes. In view of exceeding complexity of biological membranes, it is reasonable to investigate the influence of hydrostatic pressure on electric parameters of the membrane in models of biological membranes: in a bilayer lipid membrane (BLM).

Instrumentally cyclic voltametric characteristics were chosen for experimental study of the influence of hydrostatic pressure on electric capacitance and electrical resistance of BLM.

The experiments were performed in BLM obtained from phosphatydilserine, suspended earlier in non-

ane. BLM was formed by the method of Muller et al. on a hole with a 1 mm diameter in a polytetrafluorrethylene (PTFE) cell. On the both sides of the membrane a 0.1 *M* NaCl, pH=6.1 solution was positioned. All experiments were performed at 20°C. Cyclic voltametric characteristics obtained on voltametric analyzer (CH Instruments 600), measuring process was controlled by computer. The hydrostatic pressure difference from 0 *Pa* to 6.8 *Pa* was used. Hydrostatic pressure was created by addition of NaCl solution into one of two compartments of the cell.

Cyclic voltage-current characteristics (VCC) of BLM were measured using a computer program at different magnitudes of hydrostatic pressure on the membrane. Cyclic (VCC) allowed simultaneously to define the electrical resistance (R) of BLM, as well as electric capacitance (C):

$$\frac{1}{R} = \frac{\Delta I}{\Delta U}, \; C = \frac{\Delta I}{2\alpha},$$

where ΔI and ΔU increments of current and voltage correspondingly, α - speed of development of voltage.

The electrical resistance (R) and electric capacitance (C) of BLM were calculated from (VCC) in different magnitudes of hydrostatic pressure on membrane. With growth of the magnitude of hydrostatic pressure the electric capacitance (C) increased from 0.14 nF (at P=0 *Pa*) to 0.83 nF (at P=6.8 *Pa*), but the electrical resistance (R) decreases from $8.3 \cdot 10^{-9}$ to $1.4 \cdot 10^{-9}$ *Ohm* at the same magnitudes of hydrostatic pressure.

Assessments of the magnitudes of electrical resistance (R) and electric capacitance (C) of BLM for that case, when there is no difference of hydrostatic pressure on BLM is consistent with literary data. It is visible that with the growth of magnitude of hydrostatic pressure the electric capacitance (C) increases. It can be explained as follows: as the electric capacitance (C) of BLM is directly proportional to the area, then increasing of the hydrostatic pressure must bring to an increase of the electric capacitance (C). On the other hand, increasing of the area must bring to a decrease of the electrical resistance (R) of BLM.

THE CONTROL OF CHRONIC NON-COMMUNICABLE DISEASES IN BORDERING REGIONS OF GEORGIA

Raminashvili D., Bejanov G.

Public Health Department, Tbilisi State Medical University, Tbilisi, Georgia

Phone: 995994623 12

The causes of chronic disease range from proximal causes, such as the lifestyle of the individual, to more distal causes such as the socioeconomic environment. The burden of chronic non-communicable disease is rising rapidly and has now become a major challenge to global development. Georgia has wit-

nessed an epidemiological transition with increasing prevalence of chronic non-communicable diseases with their contributory risk factors. Integrated prevention and control strategies are most effective-focusing on the common risk factors and cutting across specific diseases.

Our aim was to look for ideas to implement changes in care delivery adapted to the needs of chronically ill patients.

Methods of expert analysis were used.

There is little effort to inform adult patients and provide them with skills to self-care or to adhere to an appropriate diet, apart from occasional advice by endocrinologists during visits to facilities.

Health care professionals are familiar with traditional medical treatments but have no knowledge of approaches and interventions to inform patients and to change their behavior.

Health promotion actions are the best known way to achieve a long-term healthy life of population and communities. Health promotion is a core responsibility of a government partnering with civil society and the private sector. Primary care providers play an important role in promoting health, they are ideally placed to carry out health promotion programs and see all group members of community of different age and sex, various educational backgrounds.

Despite these minor successes, there is still an enormous amount to be done and many of the changes that are needed will be even more difficult to achieve than the control of tobacco use. Diet and physical activity must be key components of any strategy to promote healthy life and avoid the chronic diseases of lifestyle, but these lifestyles are very difficult to address. Difficult though it is, the control of tobacco is easy compared with changes in what people eat and whether, when and how they are active.

Health promotion enables community to increase control and to improve health status, to be involved in better functioning of service provision. It is significant to evolve best practice actions and key evidence-based policies mandatory to improve health. The latter calls for health promotion at its most difficult and most powerful acting to build healthy public policy.

ECOLOGY OF THE PROFESSIONAL ENVIRONMENT, SAFETY OF EMOTIONALITY OF THE MEDICAL PERSONNEL OF PSYCHIATRY

Ruzhensky E.

Public Health Services Official Body, Regional Clinical Psychiatric Hospital "Bogorodsky", Ivanovo, Russia

E-mail: elena37r@yandex.ru

The term "burning out", "combustion" has been offered more than 35 years ago for the description of demoralisation, disappointment and extreme weariness, which are observed at workers of psychiatric facilities. Now we regard it as an essential component of professional health of employees.

The research objective was to study the level of emotional burning out of different professional groups of the medical staff working in psychiatry.

By a technique "Diagnostics of level of emotional burning out" 2503 employees of psychiatric service were tested. Among them were experts with the higher and average medical education working in all establishments of a psychiatric profile of 4 areas of the Central Russia. The research embraced 603 doctors (182 organizers of public health services and 421 clinical physicians) and 1900 persons of paramedical staff (237 organizers of a nursing care and 1663 practicing nurses).

Three quarters (75.61%) of experts working in psychiatry had no generated syndrome of emotional burning out, 13.78% had a beginning burning out, and in more than 6.7% of employees it was generated. Among doctors 80.1% psychiatrists had no syndrome of burning out, thus authentically differing from the general results (p <0.02), and from the data on average medical staff (p <0.01). The experts directly contacting with mentally sick, i.e. the practical doctors and the nurses were less protected from burning out. Among the organizers of public health services the tendency was positive: 81.87% (p <0.5) had no syndrome of emotional burning out, among the organizers of a nursing care: 81.02% (p<0.05). The essential distinctions between the general indicators of emotional burning out of men and women were not revealed, however there were differences in phases and symptoms syndrome formation The Formation of a syndrome of burning out began in a group of employees of 40-49 years old. The occurrence of statistically significant decrease in a share of the personnel without burning out was 71.29% (p<0.05), an increase in quantity of the personnel with beginning burning out made 17.03% (p<0.05), and the percent of persons with the generated burning out was the highest: 7.26.

The mentioned period becomes critical for the professional work continuation in psychiatric service. The majority of employees with a negative indicator leave psychiatry and the following age group (50-59 years old) is already characterized by a decrease in the level of burning out.

Employees from the age of 60 and above also showed an optimum profile from the overall sample: there was the greatest number of experts without burning out (82.32%; p<0.01), the least with beginning syndrome (10.14%; p<0.05), while the generated syndrome of emotional burning out made 3.48% (p<0.05). Similar results were received at the analysis of different length of service groups of the experts working in psychiatry. The most positive infrastructure of the personnel on emotional burning out wass among employees with a long-standing medical activity: 40 years and more. At comparison of research results in experts of a different departmental accessory, the essential distinction of indicators of experts working in the system of public health services and social protection of the population was revealed. In public health services the lack of burning out was more often recorded in the medical personnel: 81.23%. Among organizers of public health services it made 85.46%. Among medical nurses the indicator decreased to 72.95%, whereas in organizers of a nursing care it made 81.28%. At the medical staff working in psychiatric facilities of the system of social protection of population, medical staff (paramedical workers) showed the greatest safety in concern of emotional burning out (80.98 %), while among the practical nurses it was above (81.20%) than in organizers of a nursing care (79.4%). The medical personnel had no burning out only in 63.16%, and among the organizers of public health services this

indicator was 47 %, in practical doctors-psychiatrists of boarding schools it achieved 76.06%. The most disturbing ones were indicators of emotional burning out of the medical personnel STUBS: burning out began in 21% of experts of this group, the syndrome was generated among 5.26%. In research the direct interrelation between an indicator of emotional burning out of employees and level of work satisfaction was revealed. The high safety from a syndrome of emotional burning out of the personnel, completely satisfied with the work was traced and statistically confirmed in all investigated groups.

It is necessary to include research of levels of emotional burning out of the experts of the psychiatric service in monitoring of a condition of their professional health, to use the target (address) psychological support of the physicians, both on the part of the administration of establishments, and within the limits of programs of continuing education, preparation of psychiatric service.experts

THE INFLUENCE OF ELECTROSTATIC FIELDS ON THE FUNCTIONAL STATE OF RAT EPIDIDYMAL SPERMATOZOIDS

Sahakyan G.

Scientific-Research Center, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: gogs@doctor.com

The sharp increase of mail infertility has been reported in last years: it increased by 30%-50%. According to many researchers, this fact is conditioned by appearance of new anthropogenic ecological factors. Among them electrostatic fields (ESF) with the tensions exceeding the natural background are widely distributed and continuously acting ones. Data obtained in our previous studies show that both the short-term and the long-term influences of ESF lead to the changes of hormonal regulation of spermatogenesis and the structural and functional state of rat testes.

The aim of present study was investigation of the influence of ESF on the process of epididymal maturation of spermatozoids.

The 1-hour and fractional (for 6 hours during 6 days) influences of 200 *kV/m* ESF on the processes of epididymal maturation of spermatozoids and the structural and functional state of mature gametes were investigated. For avoidance of circadian rhythms, the animals were sacrificed in the same time of day immediately after the influence of field. The epididymal spermatozoids of rats were used as an object of investigations. The concentration of morphological normal, viable spermatozoids, their acrosomal status and the cholesterol/phospholipids ratio in their membranes were determined.

Determination of the functional state of spermatozoids was carried out based on optical estimation of sperm functional activity using vital dye resazurin. The essence of the method consists in resazurin reduction assay, which depends on the ability of metabolically active spermatozoids to reduce dark blue resazurin redox dye to pink resorufin. It is a proven fact that the quantity of reduced dye, conse-

quently the optical density, is in direct proportion to the amount of morphological normal and viable spermatozoids in suspension, as well as to their motility. Phospholipids were extracted by method of E. Bligh and W. Dyer (1959) and then they were separated using the method of thin-layer chromatography. The quantity of membrane phospholipids was determined by method of L. Ernster et al. (1950). The concentration of cholesterol was determined by the method of A. Courchaine et al. (1959). All estimations were done per 1 *mg* protein. The quantity of protein in membranes was measured by O. Lowry's method (1951). After the one-hour influence of field, the acrosomal status of gametes was estimated by the quantitative immunomorphological method using FITC-PNA.

The spectral analyses carried out using resazurin showed that the short-term influence of field led to the reliable increase of the optical density of spermatozoid-containing solution by 8.57%, but the long-term influence decreased it by 9.71%. Determinations of the quantity of total phospholipids and cholesterol in sperm membranes isolated from cauda epididymis showed that under one-hour ESF influence the quantity of total phospholipids increased 3-fold, while cholesterol levels did not change. After ESF fractional influence a 2-fold decrease of cholesterol was observed, while the quantity of total phospholipids did not change.

The results of immunomorphological analysis showed that one-hour influence of field brought to the significant decrease of the intensity of specific emission in the acrosomal locus's of spermatozoids (19.21±2.3 a.u. against to 28.5±4.2 of control).

It was shown that one-hour influence of field results in an increase of the amount of viable spermatozoids, but changes the acrosomal activity of gametes. On the other hand, the decrease of cholesterol/phospholipids ratio in sperm membranes is observed due to increase of quantity of phospholipids by 3-fold. It may lead to the decrease of membrane permeability and fusion, thereby interrupting the process of fertilization.

The fractional influence of field leads to decrease in the amount of viable spermatozoids. In this case the revealed decrease of cholesterol/phospholipids ratio is a result of cholesterol quantity decrease by 2-fold, which, in all probability, will lead to the changes of membrane fusion and gamete surface charge, which are necessary for transmission of genetic information and egg fertilization. It allows us to suggest that the fractional influence of ESF results in the irreversible decrease of functional activity of spermatozoids and as a result they loose their fertilizing capacity.

RISKS AND BENEFITS OF ORAL STEROID CONTRACEPTIVES

Samkharadze S.
Department of Public Health, Tbilisi State Medical University, Tbilisi, Georgia
Phone: +99599462312

The history of contraception is a long one, dating to ancient times; however, the voluntary control of fertility is even more important in a modern society with each woman expected to have two or three children. Effective control of reproduction is essential to a woman's ability to accomplish her individual goals. From a larger perspective, the rapid growth of the human population in this century threatens the survival of all.

The Quality of Health in female population was studied.

Methods of sociological investigation were used.

For the individual and/or the planet, reproductive health requires careful use of effective means to prevent both pregnancy and sexually transmitted diseases.

So, it is very important for us to have improved information about contraceptives.

An attempt was done to represent the type of oral steroid contraceptives, the risks and benefits of which are relative. .

By the 1930s, scientists had isolated and determined the structure of the steroid hormones and found that high doses of androgens, estrogens or progesterone inhibited ovulation. A number of economic, technological, and social obstacles had to be overcome before the development of the first hormonal contraceptive, the combined oral contraceptive pill (COCP). Because of their extremely high rate of effectiveness and ease of administration, within a few years COCPs intake became the most widely used method of reversible contraception among both married and unmarred women. The major effect of the synthetic estrogen is to maintain the endometrium and prevent unscheduled bleeding as well as inhibit follicular development. Most oral contraceptives (OCs) formulations contain a combination of a synthetic progestin and a synthetic estrogen in a single tablet. The initially marketed formulations of OCs contained 150 *mg* of the estrogen component, mestrand, and 9.85 *mg* of the progestin component, norethynodrel. With the high doses of steroids in the original formulations, minor side effects such as nausea, breast tenderness, and weight gain were common and frequently were of such magnitude as to cause discontinuation of use.

During the past 46 years, many other formulations were developed and marketed with steadily decreasing dosages of both estrogen and progestin. The use of these lower steroid dose formulations is associated with very low pregnancy outcomes, similar to those for formulations with higher doses of steroid and a significantly lower incidence of severe adverse cardiovascular effects and minor adverse symptom

There is also epidemiologic data that indicate that OCs reduce bone fragility particularly in perimenopausal women with oligomenorrhea. Michaelsson and co-workers recently reported that OC use by

women after the age of 40 decreased the risk of subsequent hip fracture. There are non-contraceptive health benefits associated with continuing OC use after the age of 40 into the perimenopausal years. There are limited data regarding metabolic risks of OC use by women older than 40, but Goldland and associates reported there were no changes in cardiovascular risk markers with long-term OC use. Because the estrogen given for hormone replacement is not as thrombophilic as the estrogen dose currently used in OCs, it is best to switch therapy approximately at the age of 50. To avoid discontinuing OC use when the woman is still ovulating, measurement of the FSH and E_2 levels on the last day of the pill-free interval provides information about ovarian follicular activity. If the FSH level is elevated and the E_2 level is low, OCs may be discontinued and estrogen therapy begun.

MORPHOLOGICAL CHARACTERISTICS OF THE PERIODONTAL LIGAMENT NERVE FIBERS IN HEALTHY CONDITION AND IN CASE OF PERIODONTAL DISEASES

Sanosyan G.[1], Hovhannisyan S.[2,3], Torgomyan A.[3]

[1] Hospital Prosthetic Dentistry Department, Moscow State Medicine and Dentistry University, Moscow, Russia

[2] Dental Department, CCMH, Ministry of Defense, Yerevan, Armenia

[3] Prosthodontics Department, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

[4] Morphology Department, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: adelinatorgomyan@yahoo.com; **Phone:** 093 515015

According to findings, the periodontal ligament contains different type nerve endings, which transmit information into CNS about masticatory forces degree and distribution in different parts of periodontal ligament. In connection to this, the periodontal sensory receptors can be considered as barrier that prevents teeth and jaws overload and regulates masticatory forces in combination with the temporomandibular articular receptors and proprioreceptors of tendons and muscles.

The aim of this study was to investigate peculiarities of nerve terminations and their distribution in periodontal ligament of different groups of human teeth.

The material for morphological evaluation was mandible and maxillary fragments with teeth. These fragments were taken from people, who died accidentally after casual traumas. In total, we studied a material from 27 persons. We investigated 32 blocks, including incisors and canine, as well as 8 blocks including premolars and molars of mandible and maxilla from right and left sides. In total 40 blocks were studied. For revealing nerve fibers the sections (30-70 *μm* in thickness) were treated by Ag salts by Bilshovski-Gross staining method in modification of L.I. Falin, as well as by Kampos method with subsequent staining with hematoxylin. The nerve fibers myelin sheaths were revealed by staining with black sudan by Lizon method.

The morphological research of the teeth periodontal ligament apparatus revealed several receptors. All receptors of human periodontal ligament belong to the group of free nerve endings. The greater part of them has coiled or tree like shape. The last ones can be simple or compound-brunched. The simple tree-like nerve endings are characterized by 3-4 terminals, which end on bundles of collagen fibers in periodontal ligament very close to each other. The compound branched tree-like nerve endings had completely different appearance. In this case, the nerve trunk gave off side branches, which penetrated collagen fibers continuing branching and terminated amongst them. These branches were found in different planes and occupied significant part of periodontal ligament. In both cases we revealed tree-like nerve endings, which were partly connected to the collagen bundles of periodontal ligament, partly established contact with blood vessels found in the area of their branching, thus forming polyvalent (vessel – tissue) receptors. The coiled nerve endings formed separate group of periodontal ligament nerve terminations. Coiled nerve endings are found in a small area. Usually they are set perpendicular to the periodontal ligament collagen bundles as well as in layers of loose connective tissue. In contrast to tree-like nerve endings they do not branch, so they occupy smaller areas than tree-like nerve fibers. Parallel with single coiled nerve terminations in periodontal ligament the double ones are found, when the nerve fiber divides into two branches and each of them becomes coiled. Another difference between tree-like and coiled nerve endings is their location. Tree-like nerve endings are found in neck and at the lateral apical regions of periodontal ligament. The coiled nerve terminations are present predominantly in the apical part. With regard to distribution of nerve endings in the various areas of periodontal tissues, it should be noted that the apical part of the periodontal space had the richest innervations and the neck region was less innervated. Tree-like nerve endings with many branches predominate in lateral parts of periodontal ligament. Interestingly, their branches are arranged across the periodontal space. We did not find any nerve terminations in close relation to root cementum. The comparative analysis regarding distribution of nerve endings in periodontal tissues of different group teeth showed that incisors had more developed nerve endings than molars. Tree-like nerve terminations were mainly found in molars. We did not determine any difference at nerve fibers distribution for men and women. The study on periodontal ligament nerve fibers of patients with periodontal diseases revealed thickenings of neurolemma in terminal branches, local enlargements of nerve fibers, destruction and fragmentation of nerve fibers and their terminations. These changes were found predominantly in compound tree-like nerve fibers. At the same time, simple tree-like and coiled nerve terminations retained their structure. Interestingly, previously mentioned changes coincided with the decrease in number of young undifferentiated cells. Thus, the results of our research allow to conclude that the periodontal ligament has well developed, compound sensory apparatus. Based on morphological studies differences in distribution of nerve endings of various types of teeth were identified. The healthy periodontal ligament of frontal teeth contains a variety of nerve endings. The periodontal ligament of incisors and canines contains more nerve terminations than the periodontal ligament of molars and premolars. In case of periodontal diseases compound tree-like nerve fibers are destroyed, while simple tree-like and coiled nerve terminations remain preserved.

COMPUTER BASED PLEOPTIC METHODS IN THE TREATMENT OF CONSERVATIVE DISBINOCULAR AMBLYOPIA

Sargsyan A.
Ophthalmologic Clinic, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia
E-mail: hovanush@gmail.com; **Phone:** (+374 99) 90 29 00

Traditional pleoptic methods are not always effective in the treatment of amblyopia. Thus, according to different authors, the efficiency of traditional pleoptics does not exceed 30 to 50% as a rule if considered disbinocular amblyopia..

The purpose of the present research was to realize clinical approbation and determine optional treatment features of computer based methods in conservative disbinocular amblyopia.

For the research, 45 children aged 5 to 12 years developing monolateral high and severe degrees of conservative disbinocular amblyopia were observed. All the patients had previously been treated rather ineffectively with traditional pleoptic methods during 1.5-2.0 years (monocular occlusion, local illumination of retinal fovea by Avetisov, exercises on amblyotrainer, stimulation of retina with helium-neonal laser by Kuppers). Patients had undergone 2 courses of computer based pleoptic treatment with the use of "Chasing" and "Shooting ground" exercises of ECM adopted "Eye" program. These exercises had been prescribed in the absence of traditional contraindications. Each treatment course comprised 10 sessions being conducted every day and rarer with every other day schedule. The average duration of one session was 25 minutes and the course interval was 3/4 – 5/6 months.

Treatment results were evaluated according to the increase of corrected visual acuteness in amblyopia affected eye. After the first course of the treatment visual acuteness had increased 0.05-0.2 in 23 patients. Meanwhile, in the beginning of the second course the visual acuteness remained in 20 patients. At realizing the second course of computer-based treatment the visual acuteness increased to 0.1-0.2 in 11 more patients (total in 31 patients). The stability of results was observed in 5-6 months after the termination of the treatment course. The increase of visual acuteness in amblyopia affected eye was found to be in 29 patients (2/3 of patients), if compared with the initial rate.

The trial results of implementing computer-based pleoptic treatment testify to the optional features of approbated method concerning disbinocular one-sided amblyopia, which had previously been treated rather ineffectively with traditional pleoptic methods.

The above-mentioned serves a ground for using computer-based treatment in children developing conservative disbinocular amblyopia.

DIFFERENT METHODS OF OPHTHALMO-ELECTROPHYSIOLOGICAL INVESTIGATION AS THE EFFICACY RATE OF PLEOPTIC TREATMENT

Sargsyan I.

Ophthalmologic Clinic, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: ingasargsyan@yahoo.com; **Phone:** (+374 91) 33 01 45

Treatment of patients developing disbinocular and refractive amblyopia is effective in 30 to 60 % of cases according to different authors.

The prospect of efficiency of the prescribed treatment, as well as the efficiency of further pleoptic treatment courses still remain to be insufficiently studied in paediatric ophthalmology.

Attempts had been made to use electroretinography (ERG) and the results of visual evoked potentials (VEP) as the efficiency estimation of the treatment of amblyopia.

The purpose of the study was to estimate the efficiency of the applied traditional and contemporary treatment methods in children developing moderate and high degree of monolateral disbinocular and refractive amblyopia by means of ERG and VEP results.

Twelve patients (6 girls and 6 boys) were examined with maximum correction of visual acuteness 0.3 in amblyopia affected eye without ophthalmoscopic changes of the eye ground and norm rate of visual acuteness of the other eye.

All the patients were examined with traditional methods (visometry, biomicroscopy, ophthalmoscopy, static and dynamic refractometry, deviametry, etc.), including the examination of retinal electrogenesis (scotopic, mixed, photopic and rhythmic ERG). Binocular EPR was carried out without cycloplegia. Functional disorders of the visual analyser's upper areas were diagnosed by means of pattern and/or flash VEP.

Registration of the electrophysiological research was carried out according international standards of ISCEV by means of electrodiagnostic system LKC (USA).

The degree of visual acuteness of 6 patients among 12 increased after pleoptic treatment. It is worth mentioning that in all patients the increase in ERG rate was detected up to 15 % and higher. No changes were detected in the results of VEP. For the rest of the patients (6 patients) pleoptic treatment was ineffective. In this group of patients no significant change or decrease (in 3 patients) of initial ERG rate was observed. Thus skipping pathophysiological explanations, it can be proposed that electrophysiological sensitivity of amblyopia affected retina of an eye in pleoptic treatment.

Results of the research indicate that electroretinography is more informative than visual evoked potentials if considered the pleoptic treatment of identified EPR traditional methods. Thus, comparative analysis of ERG in pleoptic treatment of children developing amblyopia is considered a subject of further research.

DEMENTIA EVALUATION EFFICACY AND SENSITIVITY BY DIFFERENT SCALES

Sargsyan R., Avetisyan A., Aleksanyan A., Manvelyan H.
Department of Neurology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia
Phone: 077694460

Recent data shows that dementive disorders affect about 1% of population worldwide, whereas in Armenia the number of these patients is surprisingly very low. We started the program aiming to reveal and in-time diagnose dementia.

This is one of the first reports in the framework of dementia study in Armenia within YSMU University Clinic. In the context of this study during a year 30 patients (7 men and 23 women) were examined.

The main aim was search, evaluation and medical counseling of patients with dementia.

We used dementia evaluation tests: MMSE, Blessed Dementia Scale, A-screen.

Mini-mental state exam (MMSE) is a fully structured scale that consists of 30 points grouped into 7 categories. Blessed Dementia Scale is a brief behavioral scale based on the interview of a close informant. A-screen checks memory and attention ability to listen three subject names after patient tries to recall 15 names of animals per 30 second, then return three subject names.

According to evaluation by MMSE the results were as follows:

- No cognitive impairment: 3 patients;
- Slight cognitive impairment: 5 patients;
- Slight dementia: 9 patients;
- Moderate dementia: 10 patients;
- Severe dementia: 3 patients.

According to evaluation by Blessed Dementia Scale the results were:

- Slight dementia: 16 patients;
- Moderate dementia: 11 patients;
- Severe dementia: none.

In three patients the result of this test was excellent and coincided with MMSE and Blessed Dementia Scale results; 3 patient did not have dementia (no cognitive impairment).

We received the evaluation results shown above from patients' examination and evaluation by MMSE, Blessed dementia scale, A-screen tests and grouped the patients to severe, moderate, slight dementia, slight cognitive impairment and no cognitive impairment groups using appropriate measurement scales for MMSE 0-30 points, for Blessed dementia scale the score is 0-17, and for A-screen 3/15 per 30 second is the norm. These evaluation data helped us to do statistic analyses and determined the mean statistics

indices for our varieties range.

Using values of obtained indices we determined that varieties ranges were right and there were no missing variants.

Having the indices, dementia evaluation data and the age of the patient we grouped them and evaluated the percentage of the dementia severity in different groups and revealed that

ascending of the patient age tends to ascend the percentage of moderate and severe forms of dementia.

Using the worldwide appreciated scales of Dementia evaluation for different patients we arranged them according to their age, sex and determined the level of cognitive impairment. It is obvious from our investigation that the greater part of our patients are women and there is a strong correlation between the age of the patients and dementia severity.

THE SERUM LEVEL OF NITRIC OXIDE AND ENDOTHELIN-1 IN FAMILIAL MEDITERRANEAN FEVER

Sargsyan Y., Sargsyan A., Davtyan A.

Department of Therapy # 2, Laboratory Diagnostic Center, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

Phone: (37 41 0) 54-58-32

Familial Mediterranean fever (FMF) is an autosomal recessive disease characterized by recurrent inflammatory febrile attacks of serosal membranes. Recently few studies have shown that FMF is associated with pulmonary hypertension. Endothelial cell dysfunction plays an important role in development and progression of the pulmonary hypertension. Nitric oxide (NO) and edothelin-1 (ET-1) are both synthesized in the endothelium, and mediate vasodilatation and vasoconstriction, respectively.

The aim of this study was to examine the levels of NO and ET-1 in FMF patients and, therefore, to investigate whether FMF patients have an increased risk of pulmonary hypertension.

Fifteen FMF patients (male/female: 8/7) during attack-free periods and 9 age- and sex-matched healthy controls (male/female: 5/4) were included into the study. The mean age of the patients was 38 ± 16 years. Total nitrite, a stable product of NO, was quantificated by means of the Griess reaction, while ET-1 was measured by enzymatic immunoassay.

The levels of NO were within normal ranges in FMF group, but the mean level of NO was significantly lower in the FMF group than in the control group (3.25±0.25 *mmol/L* vs 6.19±0.46 *mmol/L*, t=5.596, $p<0.000$). The mean level of ET-1 was high in the FMF group, and it was significantly higher in the FMF group than in the control group (19.45±3.33 *pg/mL* vs 7.52±0.63 *pg/mL*, t=3.52, $p<0.003$).

Our results suggest that patients with FMF tend to have endothelial cell dysfunction. The latter could contribute to pulmonary hypertension in FMF patients.

RISK FACTORS IN PATIENTS WITH VASCULAR DEMENTIA

Sayutina S., Suvorova I.

Department of Neurology and Neurosurgery, Irkutsk State Institute of Postgraduate Education

Russia, Irkutsk

E-mail: ilona.suvorova@mail.ru; **Phone:** +7 985 281 42 41

Vascular dementia and vascular cognitive impairment are important causes of cognitive decline in the elderly. It has now been shown that vascular risk factors have measurable negative effects on the brain and are associated with cognitive impairment. We review vascular factors that might be responsible to cognitive decline in vascular dementia and vascular cognitive impairment and the corresponding interventions that might prevent cognitive impairment as we age.

The aim of research was to study the status of risk factors in patients with vascular dementia.

Patients (n=144) with mild and moderate subcortical dementia (55 male and 89 female; average age = 70.75±3.5 years) and 128 patients with mild and moderate poststroke dementia (46 male and 82 female; average age = 64.9±4.6 years) have been studied. Diagnosis as "Vacular Dementia" was determined in accordance with ICD-10 and NINDS—AIREN criteria. Clinical-neurological and MRI-investigation were performed for all patients.

For patients with subcortical dementia the coronary heart disease and hyperlipidemia were significant risk factors for those aged from 50 to 59 years; coronary heart disease and diabetes mellitus were significant risk factors for the group aged 60-69 years; hyperlipidemia was significant risk factor for the age group of 70-79. In accordance with MRI results the cerebral white-matter lesions in thalamic, basal ganglion and bilateral subcortical leukoaraiosis were significant risk factor for dementia. For patients with poststroke dementia a coronary heart disease was significant risk factor for those aged from 50 to 59; coronary heart disease, diabetes mellitus, overweight and hyperlipidemia were significant risk factors for the group aged 60-69; diabetes mellitus, overweight and hyperlipidemia were significant risk factors for age group of 70-79. The stroke-related factors were cerebral infarction in left hemisphere, frontal and temporo-occipital infarction, thalamic, basal ganglion; cerebral white-matter lesions.

All determined vascular risk factors are potentially eliminated and resolved and timely therapeutic actions performed for patients with vascular cognitive impairment allowing to avoid development of vascular dementia

THE INFLUENCE OF PHYTOECDYSTERONE ON THE CONDITION OF LYSOSOMAL MEMBRANES BY HYPOXIC HYPOXIA

Schulkin A.
Chair of Pharmacology, Ryazan State Medical University after Academician I.P. Pavlov,
Ryazan, Russia
E-mail: alekseyshulkin@rambler.ru

Lysosomal enzymes play the important role in cell life in norm and in pathology. It is established that the labilization (destruction) of lysosomal membranes and release of lysosomal enzymes in cytoplasm are characteristics of pathogenesis of many diseases. Therefore, studies on the ability of medicinal substances (drugs) to stabilize lysosomal membranes are of a great concern. Ecdysteroids are of paramount importance in this research. It is a group of lipophilic poly hydroxylic steroids, which play the important role in the life of many living organisms. Phythoecdysterone is one of these substances.

The aim of our work was to study the influence of phythoecdysterone on the condition of lysosomal membranes during acute hypoxic hypoxia.

In our research we used a liquid extract from *Silene nutans* and *Silene tatarica*, containing 0.1% of phythoecdysterone. The concentration of substance was determined spectrophotometrically with the wave length of 242 nanometers. For comparison we used mildronate as means of metabolic action. The work was carried out in 28 male white rats (not pedigree) with the weight of 250-300 *g*. We carried out 4 series of experiments. The first series was represented by the intact rats (the control group). We simulated acute hypoxic hypoxia in animals of the second series. The third and fourth series consisted of animals preliminary, before the influence of hypoxic hypoxia, injected mildronate in a dose of 25 *mg/kg* into stomach or phythoecdysterone in a dose of 5 *mg/kg* within 7 days, once a day (last time 1 hour before the experiment). After hypoxic hypoxia simulation followed by 30 minutes exposition the animals were sacrificed under the ether narcosis. For our research we took away the heart, liver and brain. Lysosoms were extracted with the method of differential centrifugation. The activity of lysosomal enzyme cathepsin D was determined by the method of M.L. Anson updated by A.J. Barret, J.T. Jingle (1972). Cathepsin D activity was obtained by measuring hydrolysis of hemoglobin at pH 4.0 and expressed in *nmol* thyrosine per *mg* of fiber per one minute. The permeability of the lysosomal membranes was assessed on factor of the lability, which was calculated as the ratio of the cytoplasmic activity of cathepsin D to the lysosomal and cytoplasmic. The results were processed statistically in view of effect of plural comparison with the help of Newman-Keils's criterion.

In rats of a control experiment the factor of the lability of cathepsin D in the liver made 0.59±0.01, in the myocardium: 0.69±0.02, in the brain: 0.63±0.03. After the influence of hypoxic hypoxia the factor of the lability of cathepsin D in the liver increased by 26.72 % ($p<0.05$), in the myocardium by 18.61% ($p<0.05$), in the brain by 26.05% ($p<0.05$). In animals receiving mildronate, in comparison with the rats undergone hypoxic hypoxia, the factor of the lability decreased in the liver by 10.72% ($p<0.05$), in the myocardium by 6.99% ($p>0.05$), in the brain by 6.62% ($p>0.05$). In animals receiving phythoecdysterone

the factor of the lability has decreased in the liver by 12.79% ($p<0.05$), in the myocardium by 9.69 % ($p>0.05$), in the brain by 13.01% ($p<0.05$) in comparison with parameters of rats of the second experiment.

The following conclusions were drawn:

1. hypoxic hypoxia causes the destruction of the lysosomal membranes;
2. phythoecdysterone stabilizes lysosomal membranes in the liver and the brain of rats by hypoxic hypoxia; mildronate stabilizes lysosomal membranes only in the liver.

ALVEOLAR GAS EXCHANGE DISORDERS IN PREVENTION OF DEVELOPMENT OF ADRENALINE-INDUCED EXPERIMENTAL MYOCARDIAL DAMAGE

Semerjyan A.

Medical Biology Department, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: annasemerjian@hotmail.com; **Phone:** (37 41 0) 560323

Myocardial infarction (MI) and sudden cardiac death are often a result of stress-induced hypercatecholaminemia. High amount of catecholamines (CA) have deteriorating effect on both myocardium and lungs. Despite the existing concepts about the primary role of myocardial hypoxia during MI, we suggest a new pathogenetic mechanism of cardiac damage, that is, primary affection of pulmonary tissue by CA leading to generalized hypoxia and myocardial injury.

The present research is focused on studies of adrenaline-induced pathomorphological alterations in the rat myocardium and lungs, alterations of blood gas saturation, as well as prevention of these changes by improving lung ventilation.

Experiments were performed in albino male rats:

- control group 1: intact animals (n=5);
- group 2: six rats injected 0.18% adrenaline hydrotartrate 0.5 *ml/kg i.v.;*
- rats exposed to mechanical lung ventilation (MLV) prior to adrenaline injection under nembuthal anesthesia (n=8).

Macro- and microscopic examination of heart and lungs was performed. Morphology of lung and myocardium was studied by hematoxylin-eosin staining. Blood gas saturation (pO_2, pCO_2) and pH were analyzed by Astroup micromethod. ECG was recorded during experiments. Statistical analysis was realized by Student's *t*-test.

Macroscopic findings showed edema and hemorrhages in lungs and myocardium following adrenaline

injection. Heart sections exhibited cardiomyocyte degeneration zones of necrosis, absence of linear orientation of myofibrilles; lung sections showed interstitial edema, neutrophil infiltration, rupture of alveolar epithelium that were not observed after MLV exposition.

ECG records during adrenaline injection showed that heart rate increased by 80% in 1-3 minutes follow up and the contraction amplitude decreaseds by 60%. Various arrhythmias (extrasystoles, tachysystoles) finally developed fibrillation and cardiac arrest by the 5-7th minutes follow up. Cardiotoxic doses of adrenaline in MLV-assisted animals also caused some arrhythmias, however, no fibrillation and heart arrest were recorded.

The analysis of partial pressures of blood oxygen and CO_2 resulted in decrease of pO_2 opposing increase of pCO_2 after adrenaline injection. Hypercapnia was associated also with acidosis (pH decreased from 7.3±0.02 to 6.95±0.03; $p<0.05$). In the third group of rats oxygen saturation in blood was not significantly changed from that in intact ones.

The results obtained allow concluding that improvement of alveolar gas exchange by mechanical lung ventilation prevents lethal arrhythmias and heart arrest induced by cardiotoxic doses of adrenaline, as well as decreases hypercapnia and acidosis simultaneously increasing blood oxygenation. Moreover, it is concluded that myocardial injury caused by adrenaline injection is mostly mediated by disruption of alveolar gas exchange leading to myocardial hypoxia.

INDUCTION OF HUMAN STROMAL STEM CELLS TOWARDS NEURAL LINEAGE: 2D VERSUS 3D MICROENVIRONMENTS

Shakhbazau A.[1] Petyovka N.[2], Goncharova N.[2], Kosmacheva S.[2], Potapnev M.[2]

[1] Laboratory of Molecular Genetics, Institute of Genetics and Cytology, National Academy of Sciences, Minsk, Belarus

[2] Republic Center for Hematology and Transfusiology, Minsk, Belarus

E-mail: shakhbazau@gmail.com

Autologous stem cells are expected to play essential role in treatment of neurotraumas and neural degenerative diseases. Many researchers indicate the possibility of obtaining neuronal precursors from human stromal stem cells, also known as mesenchymal stem cells (MSCs) from bone marrow, with subsequent differentiation into various nervous tissue types (neurons, astrocytes, oligodendrocytes).

We aimed to estimate the potential for neurogenic induction of stromal stem cells (MSCs) from human bone marrow in adherent culture and 3D cultures in biodegradable fibrin-based scaffolds.

hMSCs were isolated from healthy donors and cultured on plastic and in fibrin 3D clots with the use of EGF and FGFb-containing neurogenic medium (Neurocult). Stem cells were also cultured on poly-L-

lysin and poly-L-ornithin layers. Neuronal differentiation was assessed by morphological studies, immunolabeling (including screening by flow cytometry) and RT-PCR analysis for expression of early neural markers.

Human bone marrow MSCs cultured in neurogenic medium exhibited notable phenotype changes, as well as immunopositivity for β-III-tubulin. RT-PCR analysis proved expression of neural progenitor/early neuronal markers nestin and NSE. After 2-week incubation, expression of neural and oligodendroglial markers MAP-2, dm-20 and MBP was also observed. The majority of fibrin-embedded stem cells showed massive outgrowth of branching neurite-like processes. Neural induction in adherent culture resulted in 18% of neuron-like β-III-tubulin-positive cells, whereas 3D culture in biodegradable fibrin-based scaffolds lead to appropriate phenotype change in over 57% of hMSCs.

3D culture of human adult MSCs in fibrin 3D clots with use of neurogenic medium resulted in higher rate of conversion to neuron-like phenotype compared to adherent culture. The chemical composition and architecture of the microenvironment act together to provide the necessary cues directing cell differentiation and function. Proper combination of 3D matrix and neurogenic factors may give significant insights into the potential of neurogenic differentiation of human adult stromal stem cells. This might open the opportunity for the clinical use of hMSCs in treatment of neural degenerative diseases and neurotraumas.

STRUCTURE-FUNCTION ANALYSIS OF MICROCIN B17

Shkundina I.[1], Ghilarov D.[2], Ershov A.[2], Pashvikina G.[2], Severinov K.[1,2,3]

[1] Institute of Molecular Genetics, Moscow, Russia

[2] Institute of Gene Biology, Moscow, Russia

[3] Waksman Institute, Rutgers, NJ, USA

E-mail: irina_shkundina@mail.ru

Microcins are potent antibacterial agents produced by enterobacteria. Microcins are excellent candidates for drug development because virtually limitless numbers of their derivatives can be generated by genetic engineering. Microcin B17 (McB) of *E. coli* targets DNA gyrase, capturing it in a complex with DNA containing a double-stranded cut. McB is synthesized from pro-McB: a product of the *mcbA* gene. Pro-McB undergoes post-translational modifications carried out by the McbBCD enzyme. Mature McB is a polypeptide of 43 amino acids containing two oxazole, two thiazole heterocycles, and two bis-(oxazole-thiazole and thiazole-oxazole) heterocycles. The two bis-heterocycles form, respectively, the A and the B-site. The exact mechanism of McB function and the role of individual McB amino acids and heterocycles in McB maturation and target enzyme inhibition and McB transport across the cell membrane are unclear.

The aim of research was to perform:

1. Systematic structure-activity analysis of McB.
2. Investigation of the inhibition mechanism of DNA gyrase by McB.

Site-directed mutagenesis of *mcbA* gene has been employed for creation of mutant McBs. Antibiotic activity of mutant McBs has been assessed in cell growth inhibition bioassay. Production and maturation of mutant McBs has been analyzed by MALDI-TOF mass-spectrometry. DNA gyrase inhibition by mutant McBs has been estimated in DNA gyrase *in vitro* assays.

Minimization of the McB molecule was carried out. The minimal fragment of pro-McB able to be processed by McbBCD *in vivo* comprised 59 amino acids. It was shown that the deletion mutants of McB, which lack C-terminal amino acids, were capable of inhibiting DNA gyrase but were devoid of bactericidal activity. We hypothesize that the C-terminus of McB includes amino acid residues, which are necessary for its interaction with the inner and outer membrane transporters of *E. coli*. Thus, C-terminally truncated McB mutants cannot pass through the membranes of sensitive cells. Using saturated mutagenesis of cloned mcbA gene, we have constructed a complete collection of A and B-site McB point mutants. Our analysis indicates that the A-site bis-heterocycle is not critical for production and bactericidal activity of McB.

INFLUENCE OF BLOOD SUPPLY TO THE KIDNEYS ON PACEMAKER ACTIVITY OF THE URETERAL TWO EDGING ZONES OF CAT

Simonyan L., Kazaryan K.

Smooth-Muscle Tissue Physiology Department, L.A. Orbeli Institute of Physiology, National Academy of Sciences of Armenia, Yerevan, Armenia

E-mail: luiza@neuroscience.am/ luizasimonyan@hotmail.com; **Phone:** 077 055 007

Research of the spontaneous electrical activity of ureteral smooth-muscle tissue of cats, rats, and guinea pigs revealed along with the perirenal pacemaker the presence of rhythmogenesis on peribladder zone as well. In contrast to the main pacemaker of the upper, which provides the peristaltic activity of the whole organ, automatism of the marked area has a relatively low frequency. It is known that the nervous and humoral control is necessary for the modulation and coordination patterns of activity of both the whole organ and its local areas. The important role in the humoral regulation of blood is due to its transport function.

Obtained data signify to the spontaneous activity of edging zones, thus indicating to the rhythmogenetic autonomy of its peribladder zone.

The experiments were performed in adult cats anesthetized by Nembutal. Ureter was denervated.

Spike burst activity was allotted by bipolar electrodes, slow-wave activity was recorded as the introduction of the ball electrode in the region of pieloureteral zone and in the region connecting the ureter to the urinary bladder. Vascular system of the cats ureter was detected by using calcium-adenozine triphosphate method.

Getting the influence of circulatory disorders on pacemaker activity of ureter was carried out by clamping the artery *renalis*. It was a sharp inhibition of electrical activity in the perirenal zone during the 10-12 *min*. The automatism of peribladder area in the described conditions displays in a slightly other way. At different phases of waves spikes propagating to this area could be traced. In contrast to the pieloureteral anastomosis oscillatory process in the studied area, there was even an increasing slow-wave activity against the full inhibition of the upper renal rhythmogenesis. These results were fully consistent with data obtained by cutting the cat ureter, and showed the independence of genesis of pacemaker activity from one to another of the ureteral edging zones. Blood supply of the renal area provided a functional activity only in the upper part of the ureter.

Morphological studies on the identification of vascular beds supplying blood to studied two ureteral edging zones may also be indicative of their activity autonomy.

On the basis of denervation of the ureter it might be concluded that the slow-wave activities of the ureteral edging zones are provided by endogenous mechanisms.

All experiments were carried out in accordance with the regulations of the Yerevan State Medical University Committee on Ethics on Care and Use of Laboratory Animals. Every effort was made to minimize animal usage and discomfort.

THE INFLUENCE OF DEPRESSION ON COGNITIVE FUNCTIONS AND QUALITY OF LIFE IN PATIENTS WITH ISCHEMIC HEART DISEASE

Stadnik S.

Neurology Department, Vinnitsa National Medical University named after N.Pirogov

Vinnitsa, Ukraine

Phone: 0673377076

High frequency of affective violations, mainly of depressed spectrum is a feature of development of cognitive disorders of vascular genesis in patients with ischemic heart disease. As generally known, depression is the independent risk factor of vascular dementia development. At anxious and anxiously-depressed disorders a change to activity of sympatho-adrenal, hypothalamo-pituitary-alrenal and renin-angiotensin-aldosteronal systems of organism is appropriately accompanied by the change of all types of exchange, procoagulanting changes of hemostasis, parafunction of endothelia of vessels. All these changes can result in strengthening of progress of atherosclerosis, increase of instability of athero-

thrombotics name-plates, increase of tone of coronal vessels, by propensity to trombogenesis and cardiocerebral embolism, instrumental in making of flow by ischemic heart disease and growth of cerebral hypoperfusion heavier.

The aforesaid defined a research purpose: as a study on influence of depression on cognitive functions and quality of life of patients with ischemic heart disease.

The research was conducted in 25 patients with ischemic heart disease. A basic group was made by 13 patients with the anxious-depressed disorders. The control group involved 12 patients with ischemic heart disease and anxious disorders without the signs of depression. In research patients of both sexes at the age from 55 to 85 years, sufferings ischemic heart disease without cardiac insufficiency or cardiac insufficiency-I; duration of disease made from 10 to 15 years inclusive. Groups were comparable in weight and duration of cardiovascular pathology. The level of anxious disorders in basic and control groups did not differ significantly. Patients enrolled in research had no sharp violations of cerebral circulation of blood and heavy craniocerebral trauma in anamnesis, chronic somatopathies in the stage of decompensation, psychical disorders. Persons accepting psychotropic preparations at the moment of research were not observed.

The research on cognitive functions was conducted with the use of minimum scale by the estimations of psychical status (MMSE), batteries of tests on frontal disfunction (BTFD), 5-word test, the clock drawing test, Shulte test, test of verbal associations (lit and category). For estimation of the state of emotional sphere we applied the hospital scale of alarm and depression. The research on quality of life was conducted by the scale of SF – 36.

Patients with the anxious-depressed disorders had more expressed cognitive disorders, from data of MMSE than persons with the "isolated" alarm (22.77±1.16 and 26.58±1.26, accordingly). Most difficulties arose in tests on research of vocal functions (6.85±1.57 and 8.42±0.79, accordingly; $p<0.05$) and concentration of attention, account (2.54±1.39 and 3.33±1.15. accordingly; $p<0.05$).

In a basic group significantly, heavy frontal dysfunction came to light more frequent than in control group (46% and 33%, accordingly; $p<0.05$). From data of BTFD, a middle bulk-tanker of a basic group was 13.61±1.28, in control: 15.42±1.21. Patients with depression executed the simple reaction of choice (1.84± 0.55 and 2.25±0.62, accordingly; $p<0.05$) and the test on dynamic praxis (2.3±0.48 and 2.66±0.49, accordingly; $p<0.05$) definitely worse.

The presence of depression in patients with ischemic heart disease was associated with the low self-appraisal of the state of health, pessimistic prognosis of ischemic heart disease course, inclination to a restrictive physical and social conduct, less adherence to therapy.

Thus, in patients with ischemic heart disease combined with anxiously-depressed disorders more heavy cognitive disorders were observed than in persons with the "isolated" alarm. Cognitive violations are presented mainly in form of frontal dysfunction, violations of concentration of attention, account, and easily expressed vocal disorders. The presence of depression in patients with ischemic heart disease was associated with more expressed decline of quality of life than in persons without depressive semiology.

In the inspection of patients with ischemic heart disease it is recommended to include research of higher cerebral functions with the purpose of early revealing cognitive violations, anxious-depressed disorders and setting the proper therapy.

CLINICAL EFFICACY OF PROLONGED COURSE OF CEREBROLYSIN THERAPY IN PATIENTS WITH POSTSTROKE VASCULAR DEMENTIA

Suvorova I.

Department of Neurology and Neurosurgery, Irkutsk State Institute of Postgraduate Education, Irkutsk, Russia,

E-mail: ilona.suvorova@mail.ru; **Phone:** +7 985 281 42 41

Vascular dementia and vascular cognitive impairment are important causes of cognitive decline in the elderly. The main aim of dementia treatment is to avoid and prevent the progression of cognitive impairment and progression of vascular dementia.

The aim of the investigation was to study efficacy and safety of prolonged course of cerebrolysin therapy in patients with poststroke vascular dementia.

Forty-eight patients with mild and moderate poststroke vascular dementia (20 male and 28 female; average age = 64.3±5.9 years) have been studied. Diagnosis as "Vacular Dementia" was determined in accordance with ICD-10 and NINDS—AIREN criteria. Clinical-neurological and MRI investigations were performed for all patients. The study on clinical efficacy and safety of prolonged course of cerebrolysin therapy in poststroke vascular dementia was conducted witin the open randomized clinical study. All patients with dementia were divided in two groups with mild and moderate dementia. The infusion of cerebrolysin was prescribed for them. The study period was 36 months and during the study period five courses of cerebrolysin therapy were performed with each course duration for 4 weeks.

A potential of 3-year course of cerebrolysin therapy to slow down the progression of cognitive impairment in patients with poststroke dementia has been studied in the open study. The efficacy and safety of cerebrolysin were assessed clinically and with a battery of widespread scales and neuropsychological tests. The improvement of cognitive, functional, and motor activities in patients with mild and moderate dementia indicates the high effectiveness of cerebrolysin. A prolonged neuroprotective therapy allows to prevent the progression of cognitive impairment in patients with dementia, so by the end of 3-year study in 8 (34.8%) patients with mild dementia the MMSE summary point was more than 24 points, consequently the moderate cognitive impairment was diagnosed for these patients; in 6 (26.1%) patients with moderate dementia the MMSE summary point was more than 19 points, thus the mild dementia was diagnosed for these patients.

A prolonged neuroprotective cerebrolysin therapy allows to prevent the progression of cognitive impairment and development of poststroke dementia.

ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE POLYMORPHISM RELATES TO VASCULAR DYSFUNCTION

Sydorchuk L., Ursuliak J., Sydorchuk R.
Family Medicine Department, Bucovina State Medical University, Chernivtsi, Ukraine
E-mail: lsydorchuk@ukr.net

Nitric oxide (NO) is the most important vasorelaxant counteracting to the vasoconstrictive and vasoproliferative hormones of the endothelin and RAAS family. NO production depends on activity of endothelial NO synthase gene (eNOS). An impaired NO synthesis as a result of eNOS gene polymorphism has an important part in appearance of target-organs alteration.

The study was aimed to investigate the T894G polymorphism of eNOS gene influence on NO (nitric oxide/nitrite/nitrate) production and hemodynamic in patients with coronary artery disease (CAD).

The study involved 70 patients with CAD (27 with acute coronary syndrome (ACS); 43 with stable angina (SA)), including 26 female and 44 male patients; mean age was 53.7±6.9 years old. ECG, Troponin-test, Echo-CG and biochemical analyses were performed. Blood pressure (BP) at baseline was assessed with 24h ambulatory monitoring. NO (nitrite/nitrate) total plasma concentration was defined by IEA. Categorical results of genotypes were compared with c^2. Genes' polymorphism of eNOS (G894T) was assessed with PCR based method.

T894G polymorphism of eNOS3 gene distribution in CAD patients was as follows: the TT genotype was revealed in 4 (5.7%) patients, heterozygous TG genotype in 35 (50%) and GG genotype in 31 (44.3%) (T allele: in 21 (30%), G allele: in 49 (70%). In T-allele carriers of eNOS gene (TT/TG) systolic BP (SBP) was higher than in G-allele homozygous patients (168.3±10.2 *mm Hg* vs. 147.1±5.0 *mmHg*; $p<0.05$). Impaired NO production was related to systolic and less diastolic pressure ($r=-0.3$, $p<0.05$ and $r=-0.22$, $p<0.05$, respectively), ST segment depression ($r=0.45$, $p=0.038$), left ventricle mass index (LVMI) ($r=-0.6$, $p<0.01$), and associated more often with SA, than ACS (79.1% vs. 48.1%, $p<0.01$). Plasma NO metabolites level circulation was lower in T allele's carriers' of eNOS (TT/TG) than in G allele homozygous patients: 21.5±2.2 *mmol/L* vs 29.7±1.8 *mmol/L*, $p<0.05$.

.In patients with CAD endothelial dysfunction, vasoconstriction and endothelial alteration are associated with 894T allele carriers of eNOS gene. T-allele of eNOS gene plays role in inhibition of NO production and may be predictive risk-factor of cardiovascular diseases development.

INFLUENCE OF NONTHERMAL ELECTROMAGNETIC RADIATION ON COEFFICIENTS OF SURFACE TENSION OF WATER AND DNA SOLUTION

Tadevosyan A.

Department of Medical Physics, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: anitad@mail.ru/ ysbaba@ysmu.am; **Phone:** +37410589614

There is a great number of investigations, which try to explain the mechanism of nonthermal influence of millimetric electromagnetic waves (MEMW) on biosystems. In spite of multitude of similar literature the present results are condradictory. There is a point of view that in some biosystems MEMW penetrates into the deeper layers of tissues by means of water molecules, which is the cause of their influence on biosystems.

To check this aspect we have measured the coefficients of surface tension of twice distilled water/water-salt solution and DNA solution, which were non-irradiated and irradiated by nonthermal MEMW.

Samples were irradiated by G4-142 and G4 141 generators. The density of stream power at the sample was ~50 *microwatt/cm*^{2}. Values of the coefficients of surface tension were measured by SITA science t60 tensiometer.

The results of experiments showed that coefficients of surface tension of twice distilled water/water-salt solution and DNA solution irradiated by MEMW of 64.5 *GHz* and 50.3 *GHz* frequencies, which coincide with the resonant frequencies of oscillations of molecular fractions of water structures and with 48.3*GHz* nonresonant frequencies do not change, as compared to the nonirradiated samples.

Consequently, we can suppose that MEMW both at resonant and nonresonant frequencies penetrate into the deep layers of solution. Therefore, densities of the solutions change due to the deeper penetration of the radiation and not because of absorption in the surface areas of solutions.

CHANGES IN A SYSTEM OF MONONUCLEAR PHAGOCYTES AND ULTRASTRUCTURE OF CARDIOMYOCYTES DURING CRUSH SYNDROME

Torgomyan A., Hartenyan N.

Department of Morphology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: adelinatorgomyan@yahoo.com; **Phone:** 093 515015

The mechanical traumas at earthquakes or explosions, at transport accidents, frequently have specific features, when the significant part of the injured remains under the rubble for a long time. Decompression of pressed down extremities, without rendering assistance, results in a condition of the injured with fall of blood pressure, loss of consciousness, acute renal failure, etc. Such condition was defined as crush syndrome. The possibility of natural disaster is high, that is why the problem of studying crush syndrome has been a matter of current research.

We studied changes in macrophages of liver, spleen, lungs and lymphatic nodes, blood neutrophils and ultrastructure of cardiomyocytes as well.

The experimental animals (rats) were exposed to squeeze over one pelvic extremity during 1 hour.

During the investigation on macrophages and myocardium, as well as blood neutrophils in 1, 7, 20 days after decompression we revealed the following ultrastructural and microscopical display of cell pathology:

- quantitative changes (decrease in number) of macrophages in spleen, liver, lungs and lymph nodes in 1, 7, 20 days after decompression;
- histochemical changes of macrophages, particularly decrease in activity of acid phosphatase, of the above-stated organs in the same terms of experience;
- influence of thymalin on the structural changes in macrophages;
- decrease in activity of cationic proteins in neutrophils of peripheral blood during crush syndrome (in 1, 7, 20 days after decompression);
- ultrastructural changes in cardiomyocyes, foremost in mitochondria and myofibrils.

These changes come early, then persist and do not vanish during at least one month after decompression. The introduction to immunomodulators to experimental animals in the early period makes it possible to reduce the severity of the pathological process to some extent.

BINDING OF SOME ANTITUMOUR COMPOUNDS WITH THE DNA-RADIATED MILLIMETER ELECTROMAGNETIC WAVES

Ulikhanyan G., Tadevosyan A., Sngryan H., Grigoryan G.

Department of Medical Physics, Yerevan State Medical University, Yerevan, Armenia

E-mail: ysbaba@ysmu.am/ gretau7@mail.ru; **Phone:** +37410589614

At present, millimetric electromagnetic (MEM) waves of low intensity are successfully used in clinical medicine despite the fact that mechanism of their action on biological objects is not clarified yet.

In the present work the thermodynamic parameters of binding of intercalating compounds of mitoxantrone (MX), ametantrone (AM), doxorubicin (DX) and nonintercalating-nitropsin (NP) with the calf thymus DNA and Sarcoma 45 DNA previously radiated by the millimeter coherent electromagnetic waves of nonthermal intensity *in vitro* were studied.

The DNA water solutions prepared for the spectrophotometric titration were radiated for 60 and 90 minutes. The DNA solutions were radiated at resonant water structure frequencies (64.5 *GHz* and 50.3 *GHz*) and nonresonant one of 48.3 *GHz* . The VHF generators were applied for radiation. The density of the stream power at the sample was ~50 *microwatt/cm*^{2}.

The experiments showed that for both radiated and nonradiated DNA the same pattern of the changes of DNA solution absorption was observed, which is a result of binding with MX, AM, DX and NP. Consequently, under the investigated conditions they interact with both radiated and nonradiated DNA by means of intercalation. The binding constant (K) and the stoichiometry of MX, AM, DX and NP absorption (n) with the radiated DNA were calculated from the stoichiometry titration spectrum. Calculations show that MX, AM, DX and NP formed a more stable complex (K increases) with the DNA radiated at resonant water structure frequencies of 64.5 *GHz* and 50.3 *GHz*. When the same DNA solutions were radiated at 48.3 *GHz* frequency, the thermodynamic parameters of MX, AM, DX and NP binding with the radiated DNA changed insignificantly, as compared with nonradiated DNA and were within the range of experiment error.

Consequently, as a result of DNA radiation at water structure resonant frequencies, there occur such changes in the hydrate shell of the DNA that intercalating antitumour compounds form more stable complex with them.

EXPERIMENTAL DIABETES MELLITUS AND ITS PREVENTION BY PROTEOGLYCANS OF EMBRYONAL GENESIS

Vahedian V.[1], Aghajanova Y.[2]

[1] Biochemistry Department, Yerevan State Medical University after M.Heratsi, Yerevan, Armenia

[2] Endocrinology Department, Yerevan State Medical University after M.Heratsi, Yerevan, Armenia

E-mail: vahedian.vahid@gmail.com; **Phone:** 077 79 2164

Diabetes mellitus (DM) is one of the most serious problems of modern medicine. In 2000, according to WHO data, at least 2.8% of population of the world suffered from DM. In 2005 an estimated 1.1 mln people died from DM. Its incidence is increasing rapidly and based on current trends more than 360 mln individuals will have diabetes by 2030.

The cardinal manifestation of DM is hyperglycemia, which results from decreased entry of glucose into cells, decreased utilization of glucose by various tissues, and increased production of glucose (gluconeogenesis) by the liver.

Diabetes is a heterogenous group of disorders. Differences between different forms of disease are expressed in terms of ethiology and pathogenesis (genetic, environmental, and immune factors), in natural history, and in response to treatment. Therefore, DM is not a single disease, but a syndrome, which is characterized by dysfunction of endocrine apparatus of pancreas or abnormal responses to its hormones by target tissues that results in serious disturbances in nutrient homeostasis.

DM is known to have numerous dangerous complications, as micro- and macrovascular diseases, renal failure, blindness, neuropathy, heart diseases, gangrene of the feet, etc.

There are different ways of DM prevention and treatment, but all of them are not effective enough.

The aim of investigation was to reveal under conditions of experimental DM the possible preventive action of proteoglycans of embryonal genesis (PEG) created by Prof.L. Mkrtchyan.

Fifty white adult male rats with the average weight of 250-300 *g* were used.

The rats were divided into 3 groups:

1. Intact rats;
2. streptozotocin (STZ) injected diabetic rats;
3. diabetic rats previously injected PEG. Experimental DM in rats was created by single *i.p.* injection of STZ (Sigma) in dosage 50 *mg/kg.*

PEG was administered as a single *i/p* injection 7 days prior in dosage of 1 *mg/kg*. The amount of food and water consumption was measured regularly. The rats were decapitated 10 days after STZ injection under 50% nembutal anesthetization.

The amount of glucose was measured in blood by glucose oxidase method. The content of insulin in blood was measured by IFA with monoclonal specific antibodies for insulin. The pancreas was treated

by 10% buffered formaldehyde. The 4 *mcm* microscopic sections were dyed by hematoxylin/eosin and studied by light microscopy.

Our investigation revealed that the amount of glucose in rats previously treated with PEG was approximately 2 times lower than in diabetic rats without PEG. As to insulin, its content in blood of PEG-treated rats was 12% higher than in diabetic group. The results of morphological study testify that PEG injection restricts discompensation of acini. Dystrophic changes of pancreocytes were limited by vacuolization of some cells. The islets apparatus practically does not differ from those in intact rats.

The calculation of water and food consumption revealed obvious dependence on the animals group and amount of used product. So, during the last 10 days of the experiment the consumption of water by each rat in diabetic group increased approximately 6 times, whereas in preventive group the increase was only 4-fold. As to food consumption, it increased 2.4 times in diabetic group, and only 1.4 times in the preventive group.

Preliminary injection of PEG in STZ diabetes mellitus revealed a definite preventive action.

THE POSSIBILITY OF RECONSTRUCTION OF BRAIN IONIC CHANNELS IN S-BLM

Vardanyan A.[1], Sahakyan A.[2], Hambarzumyan A.[1], Mikaelyan A.[2], Martirosyan S.[2]

[1] CJSC "Scientific-Research Institute of Biotechnology", Yerevan Armenia.

[2] State Engineering University of Armenia, Yerevan, Armenia.

E-mail: *avardanyan82@yahoo.com*

The selective conductance of sodium ions across the plasma membrane by the voltage-gated sodium channel underlies the propagation of action potentials in neuronal cells of both vertebrates and invertebrates. Reconstruction of a functional ion translocation system from the minimal number of components required to mimic a chemically activated membrane would certainly foster our understanding of membrane excitability in general. One of the most useful approaches, in this respect, is the reconstruction of the simple membrane system using self assembled lipid bilayers, such as planar bilayer lipid membranes (BLMs) and liposomes. Conventional BLM is ideal system for basic research but is very sensitive to electrical and mechanical disturbances. So, we studied the possibilities of reconstruction of ionic channels in both planar BLM, and in metal supported BLM (s-BLM).

The aim of study was to develop methods of obtaining planar BLM and s-BLM with reconstructed ionic channels, to study the biological activity of newly synthesized cyclopropane and cyclobutane compounds.

All mesurments were performed on CH Instruments Model 600 voltamperometric analyzer.

Newly synthesized pyrethroid insecticides, which were developed by us, underwent a study for reveal-

ing the molecular mechanism of their interaction with Na channels of insects and mammalian species according to our modification of s-BLM technique developed by Tien and co-workers.

It follows from obtained data., that s-BLM received on the basis of fraction of the general lipids of bovine brain, extracted and purified by Folch's chloroform-methanol method showed a typical curve of Tien's cyclic voltamperometry, with parameters: $C=4.83\times10^{-9}$ *F* and $R=1.36\times10^{9}$ *Ohm*. At immersing received s-BLM in a solution of membrane proteins the capacity increased more than 16 times and resistance of BLM decreased nearly 60 times ($C=7.83\times10^{-8}$ *F* and $R=2.27\times10^{7}$ *Ohm*).

Obtained data showed that maximum changes in s-BLM characteristics occured at 0.15 *mg/mL* membrane proteins application, and future increase of protein concentration slightly influence on mentioned characteristics.

The used channel proteins being of transmembrane nature produced polar side pieces on BLM surface. They cause the sorption and accumulation of existing ions and increase their concentration immediately on BLM surface. This increases the membrane capacity. The characteristics of planar BLM and s-BLM were as follows: s-BLM – 4.8310^{-9} *F*, 1.36 10^{9} *Ohm*; s-BLM +15 *mg/mL* protein – 783.3 10^{-9} *F* 0.0227 10^{9} *Ohm*; s-BLM +1.5 *mg/mL* protein – 658.3 10^{-9} *F*, 0.025 10^{9} *Ohm*; s-BLM +0.15 *mg/mL* protein – 441.6 10^{-9} *F*, 0.0357 10^{9} *Ohm*; planar BLM – 3.33 10^{-9} *F*, 1.8 10^{9} *Ohm*.

On the base of physical measurements the membrane electrical parameters were calculated, demonstrating the existence of reconstructed ionic channels in BLM.

GARDENING AS AN IMPORTANT CONDITION FOR HOSPITAL AREA OPTIMIZATION

Vardanyan K.

Department of Family Medicine, Yerevan State Medical University after M. Heratsi, Yerevan Armenia

Phone: (37 41 0) 581 794

Human vital activity is defined by the potential of his/her health and environment. Changes in quality of the inhabitant's surrounding brings to a decrease of the population's life comfort, increase of the morbidity level, growth of genetic diseases and life expectancy shortening

One of the directions of improving the state of the environment is greenery planting. Green plantations enable to lead a healthy lifestyle, provide comfort and conditions for real relaxation.

Greenery planting problems become especially actual regarding greenery planting of hospitals, as the hospital garden has a leading role in the formation of thermal-humidity regimen and "psychological" climate of the hospital, as well as decreasing the negative influence of the external surrounding factors on the patient in order to provide more favourable conditions for treatment and rehabilitation.

The formation of comfortable surrounding of medical institutions is one of the most important sanitary-hygienic goals, as the disease causes deviations from the norm in psycho-physiological state of the patients, exacerbated reaction of treating-preventive process on stress-inducing factors. This is why for creating comfortable surrounding of treatment-prevention institution it is necessary to take into account its main characteristic scientifically sound features: sanitary-hygienic, psycho-physiological, space-anthropometric.

During the last four years activity has been carried out in YSMU devoted to the gardening of the 1-st University Clinical Complex and "Muratsan" Hospital. It is being carried out on the base of starting and evaluating the plant greenery condition in the hospital areas and interiors taking into account the sanitary-hygienic, decorative-esthetic significance of gardening for human health.

The goal of investigation was scientific elaboration of optimal variants of external and internal gardening of YSMU clinical hospitals for bringing into a healthy state the environment and making the hospital institutions more esthetic.

The activity is implemented as follows: the sanitary-hygienic investigation of the external area has been carried out by aspirative, instrumental, methods. Sedimentative method was used for estimation of the sanitary-bacteriological condition.

Expected results include implementation of scientifically pursued complex of gardening reorganization measures aimed at bringing to a healthy state the external and internal areas of medical institution and making them more esthetic.

As a result of the investigation more improved variants of gardening the hospitals are considered by using landscape design, elements of vertical greenery, changes of pedigree composition of plantation, increase of the number of *coniper dendroplant* in the hospital gardens, lawn space and flower garden with the prevalence of assortment of plants with more expressed phytoding activity. Greenery of hospital interiors with specially chosen assortment of indoor plants allows a significant decrease of bacterial sowing and improvement of sanitary-hygienic indices.

THE MORPHOMETRIC INVESTIGATION OF BOVINE PERICARDIUM AFTER GALL BLADDER WALL PLASTY

Varzhapetyan A.[1], Avagyan T.[2], Barseghyan H.[1]

[1] Department of Surgery No.3, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia,

[2] Department of Morphology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

E-mail: wrgo@yandex.ru; **Phone:** 093118835

Recently bovine pericardium (BP) is widely used in surgical practice as a bioplastic material. Experimental and clinical investigations have shown that it has low reactivity and high biocompatibility, which is demonstrated by the fast integration with surrounding tissues, minimal inflammatory reaction, easy formation of connective tissue and ability to provide a lengthy supporting function. It is still possible to doubt regarding the use of BP for the plasty of bile duct, as in this case we are dealing with a fairly aggressive fluid, bile, which may produce its negative effect on the postoperative period.

We have done a comparative study of morphological changes in the gallbladder of rabbits after plasty using bovine pericardium in different times of postoperative period.

Objects of investigation were 40 rabbits. The plasty of the gallbladder wall using bovine pericardium was performed under general anesthesia. The animals were proportionally divided into the following 4 groups: control, early period (14 days), intermediate period (3 months), late period (6 months). During the investigation, surfaces of epithelium and stroma and the ratio epithelium/stroma were detected using the net of Avtandilov.

The morphological investigation of gallbladder in early postoperative period group animals showed, that in contrast to the control group, the epithelium and stroma were noticeably changed. Thus, the surface of epithelium decreased up to 7.8±1.08% (in control group: 30.7±2.95%) and, on the contrary, the stroma surface increased to 92.2±1.08% (in control group: 69.3±2.95%). It is quite characteristic that the ratio epithelium/stroma decreased to 0.09±0.01 (in control group: 0.4±0.06), which points to the initial stage of the inflammatory process.

In the intermediate postoperative period the comparison of morphological condition of this group animal's gallbladder with the gallbladder of control group, shows that the surface of epithelium continues to be reduced until 23.3±1.86%, surface of the stroma remains elevated until 76.7±1.86%. However, it is evident that the surface of the epithelium from the intermediate group, compared with the same index of early group, increases and stroma surface decreases. It is quite important: in the intermediate period the ratio epithelium/stroma increases and makes 0.3±0.03 (in early postoperative period it makes 0.09±0.01).

The analysis of indexes of late postoperative period shows that in comparison with the control group the surfaces of the epithelium and stroma of gall bladder almost unchanged, the ratio epithelium/stroma (0.39±0.04) practically is the same one as in control group (0.4±0.06). The above-mentioned shows that by 6 months the inflammatory process is already complete and morphofunctional

balance of tissues is completely recovered, which is particularly evidenced by the fact that indexes of the ratio epithelium/stroma of late period and in a control group are equal.

Thus, our study reconfirmed that the plasty of the extrahepatic bile ducts with the bovine pericardium is acceptable and feasible.

CONCERNING PREVALENCE OF MIGRAINE IN DIFFERENT REGIONS OF ARMENIA

Vekilyan H., Karapetyan A., Gevorkyan E., Manvelyan H.
Department of Neurology, Yerevan State Medical University, Yerevan, Armenia
E-mail hvekilyan@yahoo.com; **Phone:** +374 91 50 38 17

Migraine is one of the common types of primary headaches. Migraine is disease with hereditarily determined dysfunction of trigemino-vascular system and manifested with paroxysmal headaches of moderate or severe intensity, pulsating, in general, hemicranial localization, with duration from 4 to 72 hours, accompanied with nausea, vomiting (sometimes), photo and phonophobia. Also, migraine is characterized as paroxysms, which involve a group of symptoms, among which headache is more frequent. There are some theories, which explain, illustrate evolution of migraine. Probably, migraine is the result of a combination of genetic predisposition and different factors of environment.

Despite the increased interest to studying the migraine, many questions of this disease remain insufficiently clarified. According to some studies the prevalence of migraine vary from 3-4% to 35%. Meantime, epidemiologic, population researches in developed countries revealed quite similar prevalence. Such big difference cannot be explained only by the difference in definition and study design or methodology, but at the same time there could be different prevalence of migraine in different (urban vs. rural) populations.

In this report we give results of a study on prevalence and particular qualities of clinical forms of migraine in different regions of the Republic of Armenia. Investigation was done by spreading of the Questionnaires based on diagnostic criteria of International Headache Classification (IHS –II, 2003) proposed by the International Headache Society (HIS). Questionnaires include questions concerning age of onset, frequency, headache character, and preceding symptoms (aura), frequency of physicians intermissions.

Mean age of participants involved in this study was 18-60 years. In Yerevan (at the altitude 800 *m* above the sea-level) we questioned 248 persons (179 female/69 male). Migraine without aura was found in 63 cases (50 female/ 13 male) and in 26 cases there was a combination of migraine with tension type headaches (TTH). Migraine with aura was found in 14 females and in 7 of them migraine was associated with TTH.

Among 188 habitants of Ararat valley (130 female/58 male) (altitude above the sea-level corresponds

to all geographic conditions of Yerevan) migraine without aura was found only in 22 cases (21 female/1 male); in 5 females there was a combination with tension type headache; migraine with aura was found in 14 cases (13 female/1 male) in 5 cases of them there was a combination with tension type headache (4 female/1 male).

In Vanadzor, the third largest city in Armenia, which is situated in the mid-mountain region (altitude above the sea-level is 1400-1500 *m*) among 94 inhabitants (84 female/10 male) migraine without aura was found in 24 (22 female/ 2 male) and in 14 cases it was combined with tension type headache (13 female/1 male); migraine with aura was found in 9 (8 female/ 1 male) and 2 females had combination with tension type

In all investigated regions the age of onset was about 20-50 years, with mean value of 30 years. Obtained data show some prevalence of migraine in Yerevan and Vanadzor vs. Ararat Valley, where the majority of population is rural.

Preliminary results of our study show that migraine abundance is higher among urban population than rural (Yerevan and Vanadzor vs. Ararat Valley), and also we found predominance of migraine in women: female/male ratio was about 4/1.

THE BODY TEMPERATURE AND THE SUCCESS OF FREE TISSUE TRANSFER

Yaghjyan G., Nazaryan A.

Department of Plastic Surgery, University Hospital #1, Yerevan State Medical University after M. Heratsi, Yerevan Armenia

E-mail: plastam@yahoo.com; **Phone:** (374) 93 426592

Free tissue transfer is generally associated with a high success rate; however, vascular complications may occur in 12-17% of cases. Involuntary body cooling during these lengthy operative procedures frequently occurs, and core temperatures below 35°C are not uncommon. The effect of hypothermia on the success of free tissue transfer is unknown. There is a theoretical risk of vasospasm, reduced nutrient flow, and ultimately microvascular thrombosis. On the other hand, severe hypothermia can cause a coagulopathy, the correction of which requires replacement of clotting factor even after re-warming has occurred. We surmised that this could theoretically lead to an increased patency rate of microvascular anastomoses. No experimental study using a standardized model has been attempted to determine if core temperature plays a role in the success or failure of microvascular surgery.

The aim of this study was to determine if core temperature has an effect on the patency of microvascular anastomoses and the subsequent success of free tissue transfer in a rat model.

Rats were anesthetized with inhaled isoflurane and randomly assigned to one of the following four core temperature groups: 34°C, 35°C, 37°C, and 39°C (n=10 animals per group). Bilateral groin free flaps were then performed (n=20 flaps per group), while each animal was maintained at the temperature of its assigned group. Flap survival was evaluated on post-op day five by a blinded observer.

The best flap survival occurred in the 34°C group, with an overall flap survival rate of 95%. There was a statistical difference between the survival rate of the combined 34°C and 35°C group (survival rate 90%, n=40) and the combined 37°C and 39°C group (survival rate 67.5%, n=40; p=0.027).

During microvascular reconstructive surgery, large skin surface areas of patients are frequently exposed to the relatively cool intraoperative environment. The magnitude of this type of surgery and the need for blood transfusions are associated with intraoperative hypothermia. Considerable effort may be required to prevent hypothermia through the use of forced-air warming blankets, elevation of the operating room temperature, and warming the inhaled gases and intravenous fluids. Most microsurgeons attempt to maintain patients' core temperatures above 36°C because of a hypothetical fear of thrombosis from vascular spasm. Spasm, it is theorized, causes vascular stasis at the sites of the microvascular anastomoses, and this is thought to be the nidus for thrombogenesis. Investigations dealing with the causes and treatment of vascular spasm in animal models of microvascular surgery are sparse. While it seems clear that static blood in contact with a fresh microvascular anastomosis will cause thrombosis after a certain time limit, it is not known, whether vascular spasm can cause such conditions. On the other hand, hypothermia prolongs the clotting time, and normalization of clotting requires both rewarming and clotting factor repletion. Impaired clotting, both during surgery and in the postoperative period after rewarming, could result in higher patency rates of microvascular anastomoses. Thus, the current strategy of warming the patient during surgery may be flawed.

In conclusion, the results of this experiment indicate that core body temperature does have an effect on the success of free tissue transfer, and that hypothermia improves the patency rates of microvascular anastomoses compared to normothermia and hyperthermia. Hypothermia may have a beneficial effect on the success of free tissue transfer.

INFLUENCE OF MILLIMETER WAVE ON STABILITY OF A BIOLOGICAL LIPID MEMBRANE

Yeghiazaryan K.[1], Potikyan G.[1], Gevorgyan H.[2]

[1] Department of Medical and Biological Physics, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia

[2] Physics Department, Yerevan State University, Yerevan, Armenia, Heratsi,

E-mail: yeghiazaryan_kristina@yahoo.com; **Phone:** (+374)93 54 17 13

The influence of millimeter waves (mm-waves) on biological objects is conditioned by numerous radiation sources in the surrounding world. It is known that biological objects (including a human body) are basic targets of mm-waves exposure: plasma membrane cells, inside the intercellular liquid. Despite the large number of works in this field, a final mechanism of the influence displayed by mm-waves on the biological objects is not established. In connection with this, it is of interest to study the influence of mm-waves on a model system: biological lipid membrane (BLM).

The influence of mm-waves on BLM stability was investigated experimentally. As the capacity of parameter, characterizing the degree of BLM stability a mean lifetime of BLM at the given values of BLM intensity was taken.

The experiments were performed in BLM obtained from phosphatydilserine suspended earlier in nonane. BLM was formed by the method of Muller et al. on a hole with a diameter of 1 *mm* in a polytetrafluorrethylene (PTFE) cell**.** On the both sides of membrane a 0.1 *M* NaCl, pH=6.1 solution was positioned. All experiments ran at temperature of 20°C The potential difference on BLM was provided by silver-chlorine electrodes, which were connected with ADS (NI USB-6008) and controlled by computer. The potential difference was used from 150 *mV* to 300 *mV* with a step of 50 *mV*. The howling cell by two opposite parts had windows, which were closed by transparent organic glasses. A waveguide entered through one window and was connected to the output of mm-wave generator. Power on exit of the waveguide was 30 mcW/cm^2 . The next window was made so that through the binocular one could follow planting and blackening of BLM.

For the first time change of BLM mean lifetime with increasing of the potential difference was investigated. It was derived that an activity of electrostatic field brings a decrease of BLM mean lifetime and was well conformed to literary data. Experimentally the influence of potential difference on the mean lifetime of BLM was investigated after 10 minutes exposure to mm-wave at frequency of 61.22 *GHz* and 65 *GHz*. The value of 65 *GHz* is a resonance frequency for aqua and biological objects. BLM mean lifetime decreased after the exposure to mm-waves. The decreasing of mean lifetime upon resonance frequency was less than in case of non-resonance frequency.

It is known that the loss of stability by free BLM, as well as BLM in electric field is connected with a formation of lipid pore at critical sizes. The analysis of curves and their comparison with theoretical curves show that activity of mm-wave on BLM brings to decreasing of the mean lifetime, though the difference in formation of critical pore for resonance frequency and non-resonance frequency is insignificant.

INVOLVMENT OF MATRIX METALLOPROTEINASE-9 IN EXERCISE INDUCED NEUROMUSCULAR PLASTICITY

Yeghiazaryan M.[1], Slawinska U.[1], Kaczmarek L.[2], Wilczyński G[1].

[1] Department of Neurophysiology, The Nencki Institute of Experimental Biology, Warsaw, Poland

[2] Department of Molecular and Cellular Neurobiology, The Nencki Institute of Experimental Biology, Warsaw, Poland

E-mail: G.Wilczynski@nencki.gov.pl

The neuromuscular junctions (NMJs) in skeletal muscle fibers form complex interactions between motor neurons, muscle fibers, and perysinaptic Schwann cells (PSCs). The NMJs have a high degree of structural plasticity, and it has been reported that increased neuromuscular activity impacts NMJ structure. Ultrastructural changes in NMJ appear following physical training, and are known to be exercise intensities dependent and muscle fiber type-specific. Most, if not all, such processes require a remodeling of the extracellular matrix (ECM), by degradation and regeneration. Matrix metalloproteinases are key regulatory molecules in the remodeling and degradation of ECM. Although several MMPs exist in muscle, two MMPs that are though to play an important role in skeletal muscle adaptation to changing contractile demands and to response to injury are MMP-2 (also known as gelatinase A) and MMP-9 (also known as gelatinase B).

The aim of the present study was to assess, whether MMP-9 can play a role in synaptic function in the peripheral nervous system. We also attempted to determine, whether the changes in gelatynolytic activity of MMP-9 at the NMJs observed are fiber type-specific.

Muscle fibers from the *extensor digitorium longus* (EDL) and *soleus* (Sol) were obtained from young male rats (n = 32) of both legs on several occasions: before and immediately, 2 *h*, 6 *h*, 24 *h* after the first exercise boat; after 5, 14 and 30 days of consecutive days of treadmill running at high intensity (20 *m/min* (-1) to 40 *min* per day). We examined the localization of MMP-9 in normal rat muscles by immunohistochemistry, and *in situ* hybridization, and the gelatinolitic activity of MMP-9 at NMJ of control and trained muscles by *in situ* zymography.

Our results clearly show that MMP-9 is present in perisynaptic part of NMJ. After a high-intensity exercise regime, gelatinolytic activity significantly increased in PSCs of the Sol after 5 days of training (effect of training; $p<0.001$) and remained elevated after 14 days ($p<0.001$) of training. The zymographic activity of MMP-9 in EDL did not change in response to long term training, however basal level of activity was changed after single boat of exercise: it was significantly lower immediately ($p<0.001$) and elevated 2*h* after the first exercise boat.

We conclude that MMP-9 is mainly localized in PSCs of NMJ and synthesized by these cells. MMP-9 is a PSC-derived factor that is involved in physiological adaptation to high intensity physical training. High-intensity exercise is required to promote the expression of MMP-9 at the NMJ in skeletal muscles and the influence of exercise on MMP-9 expression is dominant in muscles containing a high percentage of slow fibers.

ROLE OF EMBRYONAL PROTEOGLYCANS IN ADULT NEUROGENESIS AS A POSSIBLE WAY OF NEURONAL SURVIVAL AT NEURODEGENERATION

Yenkoyan K.
Biochemistry Department, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia
E-mail: enkoyan@yahoo.com; **Phone:** (093) 97 78 78

Up to now different therapeutic approaches are used to prevent and treat a wide spectrum of neurological disorders linked with dementia**,** but recovery is still missed. Among various dementia-linked diseases Alzheimer's disease (AD) is on top. The main trigger of Alzheimer's disease (AD) related neurodegeneration is β-amyloid peptide, which subsequently activates different metabolic disorders in neuron and finally leads to neuronal death. Many of biologically active products are approbated as neuroprotectors, but only few of them demonstrate more or less efficiency.

Neurogenesis, the generation of new neurons from neural precursor cells (NPCs), is a multi-step process that includes the proliferation of NPCs, fate determination, migration, and neuronal maturation. The discovery that neurons and glia arise from neural stem cells located in specific regions (i.e. subventricular zone, hippocampus) of the central nervous system has important clinical implications for the treatment of life-threatening, neuronal disease, including AD and Parkinson's Diseases.

Thus, the aim of current study was to show the survival effect of embryonal proteoglycans (PEG) at neurodegeneration via activation of neurogenesis in brain. For this purpose, intermediate filament proteins of undifferentiated neural stem cells Vimentin and Nestin, dividing cells marker bromodeoxyuridine (BrdU) and signal molecule Wnt were detected. Additionally, we determined the expression of transcription factors of the Sox family that regulate stem-cell maintenance, decide whether neurons or glia are generated, or control terminal differentiation. Particularly, Sox2 - required for stem cell maintenance; Sox10 – essential for terminal oligodentrocyte differentiation and Sox14 – which concomitantly increases the generation of various neuronal subtypes.

Thirty young adult male rats were supplied. Animals, weighing 230–290 *g* at the beginning of the experiment were housed four per cage at 12:12 *h* light/dark cycle (08.00–20.00 *h*) and fed *ad libitum*. The experimental protocol was approved by the Institutional Ethics Committee.

Experimental protocol: The protocol consisted of the following sequence of events. The animals were divided into 3 groups: the control group consisted of vehicle-treated animals; the 1st experimental group was *i.c.v.* injected with aggregated Aβ1-42; the 2nd experimental group was subcutaneously administered PEG (0.5 *mg*/100 *g*) 7 days before Aβ injection and on the 31st day after it. Markers of neurogenesis (Vimentin, Nestin, BrdU, Wnt, Sox14, Sox2, Sox10) were determined in subventricular zone (SVZ) of rats' brain on the 90th day from *i.c.v.* injection of Aβ by immunohistochemical and flow cytometric detection.

Results of our experiment revealed the following changes:

1. after *i.c.v.* injection of Aβ1-42 in SVZ fluorescence intensity of Nestin, Vimentin, as well as Nestin

and Vimentin double-labeled cells, Sox2 and Sox14 cells significantly decreased compared with the control cells, which suggested the inhibitory effect of neurodegeneration on neural stem cells maintenance and survival;

2. after i.c.v. injection of Aβ1-42 in SVZ there were no significant changes in BrdU and Wnt positive cells compared with the control group, on the contrary, the expression of BrdU and Wnt double-labeled cells dramatically increased, which suggested that neurodegeneration could play a trigger role in stem cells proliferation;
3. after i.c.v. injection of Aβ1-42 in SVZ fluorescence activity of Sox10 positive cells increased, which suggested great glial proliferation at neurodegeneration followed by still unknown consequences;
4. after subcutaneous administration of PEG fluorescence intensity of Nestin, Vimentin, as well as Nestin and Vimentin double-labeled cells, Sox2, Sox14, BrdU, Wnt, BrdU and Wnt double-labeled cells significantly increased compared with amyloid and in some cell types (Sox2, BrdU, Wnt, BrdU and Wnt double-labeled) compared with the control groups, which suggested that PEG had a great capacity to activate the proliferation and further differentiation of adult stem cells in brain.

Summarizing this complex investigation we can conclude that activation of the adult neurogenesis and its "correct" regulation could be crucial in the processes of neuroregeneration. The pool of proteoglycans of embryonal genesis has a great potential to be one of the probable agents against neurodegenerative disorders, and AD in particular.

LVV-HEMORPHIN-7 AFFECT DNA STRUCTURE IN PHYSIOLOGY AND PATHOPHYSIOLOGY

Zakaryan H.[1], Sarukhanyan F.[1], Garibyan D.[2], Stepanyan H.[2], Grigoryan I.[3], Dalyan Y.[3], Barkhudaryan N.[1]

[1] H. Buniatian Institute of Biochemistry, National Academy of Sciences of Armenia, Yerevan, Armenia

[2] Institute of Fine Organic Chemistry, National Academy of Sciences of Sciences, Yerevan, Armenia

[3] Department of Molecular Physics, Faculty of Physics, Yerevan State University, Yerevan, Armenia

E-mail: hermine-83@mail.ru

Using calorimetric approaches the thermodynamic investigation of DNA isolated from sarcoma-45 (S-45) bearing rats with intraperitoneal (*i.p.*) administration of LVV-hemorphin-7 (LVV-H7) (1 *mg/kg*, single *i.p.* injection daily) during 8 days, we have shown the increased value of enthalpy (12.8 *kcal/mol*) in comparison with enthalpy of S-45 DNA (9.23 *kcal/mol*). The formation of DNA-LVVH7 complex was detected *in vitro* by differential absorbance measurements. In latter experiments we used DNA isolated

from healthy rats liver. It is proposed that LVVH7, acting as homeostatic agent in severe pathologies (stress, cancer) may modulate transcriptional activity by affecting DNA structure.

Hemorphins demonstrate multiple actions by affecting different receptors function. LVVH7 (LVVYPWTQRF) as other hemorphins was shown to modulate calcineurin activity both *in vitro* and *in vivo* in physiology, as well as in pathophysiology (cancer, stress, etc.). It is well known that calcineurin is a key enzyme involved in signal transduction cascade leading to T-cells activation by involvement in gene expression and production of several cytokines and other regulatory proteins via dephosphorylation and nuclear translocation of NFAT family transcription factors. It is suggested that by modulation of calcineurin activity hemorphins may indirectly affect transcription of those proteins. However, since hemorphins were shown to inhibit DNA methylation, the, direct interaction of mentioned hemorphins with DNA is not excluded. Indeed, for LVVYPW we have already received data supporting that supposition.

The aim of present study was to examine, if LVVH7 may have ability *in vitro* and *in vivo* affect DNA structure in physiology and pathophysiology.

Rats were inoculated with S-45 by the method of W. Chernov (1971) DNA extraction was done by method of W. Vasiliev et al. (1972). Excess heat capacity (ΔC_p) *versus* temperature (T°C) profiles for the thermally induced transitions of DNAs isolated from S-45 and S-45 treated with LVVH7 were done using a differential scanning microcalorimetry (DSM). Measurements were conducted on a DASM-4 microcalorimeter (Russia). In these experiments, the heating rate was 1°C/*min* within the temperature range 10-100°C. Enthalpies (ΔH) were calculated from the areas under the experimental ΔC_p *versus* T°C curves using Scal Dos software. The formation of DNA-LVVH7 complex *in vitro* was detected by differential absorbance measurements, which were conducted on a SPECORD UV/VIS spectrophotometer (Germany).

Using DSM approach we revealed that changes in heat capacity (ΔC_p) of DNAs isolated from S-45 and S-45 treated with LVVH7, depend on T°C. Using automatic data processing software Scal Dos the values of ΔH of studied DNAs were determined. The increased value of enthalpy of DNA isolated from rats treated with LVVH7 (12.8 *kcal/mol*) compared with enthalpy of S-45 DNA (9.23 *kcal/mol*) has been detected. *In vitro* measurement of differential absorbance spectra confirm the formation of DNA-LVVH7 complex. Registration of complex with changed conformation was detected in *μM* concentration ranges of LVVH7. DNA for experiments was isolated from healthy rats liver.

Data obtained give us reason to suggest that, as a result of *in vivo* treatment of S-45 with LVVH7, the hydrophobic interaction of DNA with peptide may take place, which can induce the conformational changes in DNA structure and, thus, affect the transcriptional activity. The *in vitro* registration of DNA-LVVH7 complex formation confirmed our supposition.

PALLIATIVE CARE IN ARMENIA: FIRST REPORT

Zakaryan J.[1], Ohanyan T.[1], Lalazaryan N.[1], Poghosyan S.[2]

[1]Yerevan State Medical University after M.Heratsi, Yerevan, Armenia

[2] "Eurometer" Research Center, Yerevan, Armenia

Phone: (37 41 0) 560405

Palliative care is new to Armenia. Currently this type of medical service is provided by NGOs and volunteers. It is not comprehensive and mostly limited to pain control. Palliative care was included in the official list of types of medical services in December 2009.

The aim of research was to assess the awareness level in population, their perceptions of pediatric palliative care in particular and how much it can be called-for. We were interested to learn, if parents are ready to provide children with hospice care or home care.

Quantitative survey was conducted to reveal public opinion and qualitative experts' interviews were used to study the opinion of doctors caring patients with advanced diseases.

Although nearly one third reported the occurrence of need in palliative care in their families in the past, on average only 13% of respondents had heard about palliative care and 14% known what hospice is. Among those with terminally ill family members more than 80% did not know about palliative care. There was statistically significant association in education level and awareness on palliative care.

Medical doctors agreed that there are several problems relating to palliative and hospice care in Armenia in legal, organizational, institutional areas and in public awareness. The need in palliative care is huge, but the demand is hidden.

An advocacy campaign is needed to improve understanding and support of hospice care. The population often misapprehends hospice care as a way of getting rid of terminally ill person, to free family from severe burden and concerns. The potential demand to palliative care is high in Armenia; nevertheless, it is hidden.

C-REACTIVE PROTEIN GENE SINGLE NUCLEOTIDE POLYMORPHISMS IN SCHIZOPHRENIA

Zakharyan R.[1], Khoyetsyan A.[1], Arakelyan A.[1], Gevorgyan A.[2], Melkumova M.[2], Torosyan S.[2], Mrazek F.[3], Petrek M.[3], Boyajyan A.[1]

[1] Laboratory of Macromolecular Complexes,
Institute of Molecular Biology, National Academy of Sciences of Armenia, Yerevan,
[2] Psychiatric Medical Center, Ministry of Health of the Republic of Armenia, Yerevan Armenia
[3] Laboratory of Immunogenomics and Proteomics, Palacky University, Olomouc, Czech Republic
Phone: +37410 28 20 61

Schizophrenia is a complex psychiatric disease with genetic component. C-reactive protein (CRP) is an acute phase protein produced by the liver and is a marker of systemic inflammation. The involvement of inflammatory response in ethiopathogenesis of schizophrenia has been demonstrated in numerous studies. However, data on implication of CRP in schizophrenia-associated inflammation are controversial.

This study was aimed to estimate whether single nucleotide polymorphisms (SNPs) within CRP gene are associated with schizophrenia in Armenian population.

Totally, 103 patients with paranoid schizophrenia and 105 healthy subjects (control group) were enrolled in this study. All subjects were unrelated Armenians living in Armenia. The genotyping for the CRP rs1417938 (T/A), rs1800947 (C/G) and rs1205 (C/T) SNPs was performed using polymerase chain reaction with sequence specific primers (PCR-SSP). SNPs were selected according to their frequent occurrence in the European population and functionality. Statistical analyses were performed in SNPanalyser web-based environment.

Genotype distributions of all studied SNPs were in correspondence to Hardy-Weinberg equilibrium. For three investigated SNPs no significant differences in mutant allele frequencies were obtained when comparing patients (rs1417938*A: 0.28; rs1800947*G: 0.03; rs1205*T: 0.33) and control subjects (rs1417938*A: 0.26; rs1800947*G: 0.02; rs1205*T: 0.27). However, rs1417938 AA homozygotes (11%) and rs1205*T carriers (56%) were overrepresented in patients compared to controls (AA: 7%, T: 45%, *p*-value was 0.1 in both cases).

Whereas none of the studied CRP gene SNPs is associated with schizophrenia in Armenian population, differences between the genotype distributions of mutant alleles for two SNPs in patients and control subjects were detected.

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THE ROLE OF MELATONIN IN REGULATION OF PERMEABILITY OF MICROHEMOCIRCULATION PATHWAYS

Zargaryan A.
Department of Morphology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia
E-mail: zaranal@yahoo.com; **Phone:** (374 10) 563953/ (374 94) 881832

Comprehensive morphological, morphometric, and fluorescent microscopic methods of research in small laboratory animals (rats, mice, hamsters) were applied to study the state of permeability in mesentery microhemocirculation pathways, loose connective tissues, as well as hamster's cheek pouch under conditions of single intravascular administration of melatonin. All experimental animals were administered rather low doses of melatonin similar to those determined in nocturnal blood serum of intact mammals.

Morphometric studies allowed to reveal that the single intravascular administration of melatonin was not accompanied by the increase of microvessels permeability for colloid coal and FITC-labeled gamma-globulin. However, the histohematic barrier of microcirculation pathways appeared to be permeable for such a relatively low-molecular compound as fluorescein (uranin). It was ascertained by morphometric and fluorescent-microscopic methods of research that such an important extravascular factor for regulation of transcapillary exchange and microvessel permeability as tissue basophiles is not engaged. Thus, after a single administration of rather low concentrations of melatonin, almost no signs of degranulation were observed in tissue basophiles; whereas the quantitative fluorescent microscopy allowed to determine high indices of histamine and serotonin compared to the control group. It is not excluded that under conditions of our experiment there were involved both the receptor mechanisms of direct inhibiting influence of melatonin towards the processes of tissue basophiles proliferation and secretion of histamine and serotonin by these cells. It should be specially emphasized that the cytoarchitectonics of all investigated connective tissue derivates was preserved in case of a single intravascular administration of melatonin.

Therefore, the revealed evidence of enhanced permeability of microvessels for low-molecular compounds should be considered from the point of view of physiology: as a possible mode of functioning at the level of microcirculation pathways of melanin-dependent mechanism for regulation of transcapillary exchange.

ELAEAGNUS ANGUSTIFOLIA L. AS A SOURCE FOR NEW ANTINOCICEPTIVE AND ANTI-INFLAMMATORY AGENTS

Zhamharyan A., Balasanyan M.
Department of Pharmacy, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia
E-mail: zhamgaryan@mail.ru **Phone:** 077373788

Plant extracts as an attractive source for creation of new anti-inflammatory drugs have been intensively developed after demonstration of flavonoids ability to affect the prostaglandins system and produce promising results in treatment of inflammation. Silver berry (*Elaeagnus angustifolia L., f. Elaeagnaceae*) from Armenian flora contains rich flavanoid compounds and can be used for development of new potent anti-inflammatory and antinociceptive drug.

The aim of the study was to perform phytochemical analysis of an extract from semen *Elaeagnus angustifolia* and evaluation of the antinociceptive and anti-inflammatory effects of the extract.

The fruits of *E. angustifolia* werecollected from Ashtarak region of Armenia. The extract from seeds of fruits was obtained after preliminary drying at room temperature. The phytochemical analysis of the received extract on the presence of sapogenins was verified by GC-MS method using HP 6890 PLUS (Hewlett-Packard) with the Mass Selective Detector HP 5973. The antinociceptive activity of the extract from semen of fruit of Armenian flora silver berry was studied by the increase in duration of the latent period of the rat tails drawing back at "tail-flick" test. The anti-inflammatory activity was investigated in the experimental model of rats with induced ear edema acute inflamed by 0.03 *mL* xylene. The anti-inflammatory activity of the preparations was estimated by the rat ear mass difference (the right: inflamed and the left: intact) in 90 minutes after *i.p.* administration of the extract.

The antinociceptive activity of the extracts from semen of fruit in a 1000 *mg/kg* dose expressed by an increase in the duration of the latent period by 56.4% ($p<0.001$, n=8) of the rat tail-flick test.

Investigation on the anti-inflammatory effect of extract from semen of *Elaeagnus angustifolia* in xylene induced ear edema in rats showed that the mentioned extract reduced the inflammation by 58% ($p<0.005$, n=6) compared to 71% ($p<0.005$, n=6) for sodium diclofenac. The received data were confirmed also by the histological analyses.

The phytochemical screening of extract from fruit of *E. angustifolia* of Armenian flora revealed the presence of flavonoids and tannins. Moreover, in the extract triterpenoid sapogenin-germanicol was established, which according literature data possesses the anti-inflammatory activity.

Obtained data indicate that *E. angustifolia* of Armenian flora can be used as a source for new anti-inflammatory and antinociceptive drugs. Thus, phytochemical screening of the extract from semen of Amenian flora *E. angustifolia* testifies that its anti-inflammatory and antinociceptive action is realized not only due to flavonoids and tannins, but treterpenoid sapogenin-germanicol as well

IMMUNOMODULATING EFFECTS OF CORTISOL AND PROLACTIN IN PATHOGENESIS OF ACAID ABOLITION

Zilfyan A.
Scientific-Research Center, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia
Phone: 37 410 58 08 40; **E-mail:** namj@ysmu.am

The term "immune privilege of an eye" implies the entire complex of regional immune cell-mediated and humoral reactions aimed at prevention of development of inflammatory and dystrophic processes in membranes and fluid sections (slices) of an eye under conditions of a wide range of ophthalmologic maladies.

At the same time, this term first of all reflects the state of "local immune homeostasis" under conditions of normal functioning of an eye. The complete cascade of local protective –adaptive mechanisms is aimed to formation of immunological processes, which in total are defined as anterior chamber associated immune deviation (ACAID).

According to modern views (concepts) in specific eye membranes (sclera, corneal membrane, ciliary body) the cells of epithelial genesis and fibroblastic origin besides their main function are also endowed with the hormone producing function: synthesis and secretion of cortisol.

At present there dominates the point of view according to which the fundamental function of cortisol in eye membranes consists in maintenance (ensuring) the optimal level of intraocular pressure.

The role of prolactin appears more disputable, as it is determined in anterior chamber of an eye and retina either in normal state and under cataracts, uveites and retinopathies.

To our mind, the role of *in situ* produced hormones such as cortisol and prolactin in tissues of an eye is more versatile and is far beyond the frames of functions ensuring the processes on regulation of ion (ic) exchange/metabolism and intra-ocular pressure.

We propose the hypothesis, in accordance with which cortisol and prolactin play an important role in formation of ACAID by modulation of immunological processes either in the aspect of activation of T- and B-populations or targeted synthesis of cytokines of adaptogenic spectrum of action.

The IF of patients with senile cataract (Group I) and complicated cataract on the background of medicamentous compensated glaucoma (Group II) was studied.

Using immune-enzyme and immunological methods by 32 IF samples were analyzed in each Group of patients operated at Medical Center "Shengavit". Extraction of chamber humor was performed alongside the surgery under sterility and in compliance with all requirements of YSMU Committee on Bioethics.

The content of Cortisol, CD_4, CD_8 and Prolactin in IF was determined with appropriate kits ("DRG-International Inc.", USA-Germany and "Biotech Research", USA, appropriately). The immune-enzyme analysis was performed on automatic spectrophotometer "Stat-Fax" (USA) in the wavelength range

420-450nm. The content of cortisol was expressed as *pg/mL*; CD_4 and CD_8 were measured as *unit/mL*, while prolactin was expressed as *ng/mL*.

Determination of immunoglobulin G class was done according to generally accepted procedure proposed by Manchini and the content expressed as *UI/l*. The obtained results were analyzed using Student's criteria.

The immune-enzyme analysis revealed that Prolactin and Cortisol levels in IF in Group I patients made 0.34±0.045 *ng/mL* and 12.9±0.64 *pg/mL*, appropriately; in patients of Group II these indices markedly exceeded those in IF of Group I patients amounting 1.1±0.008 *ng/mL* ($p<0.0005$) and 23.38±1.46 *pg/mL* (<0.0005), correspondingly.

In Manchini reaction the results of IF analysis for IgG in patients of Group I were negative. In 25 patients of Group II, IgG was revealed and made 39.7±5.9 *UI/mL*. The immune-enzyme assay allowed revealing relatively high indices of CD_4(26.1±4.3 *U/mL*) and low CD_8 indices (52.7±6.5 *U/mL*) in IF as compared to those in Group I patients: CD_4=10.7±2.1 *U/mL* ($0.005>p>0.0005$); CD_8 = 21.75±5.6 *U/mL*($0.005>p>0.0005$).

The obtained results signify to the fact that in persons with complicated cataract on the background of primary open–angle /wide-angle and closed-angle /narrow-angle glaucoma in immune competent membranes of the eye there occurs activation of reactions of humoral immunity on the background of marked decrease of T-killer lymphocytes activity; to this latter testify relatively high indices of CD_4 and IgG, as well as low level of CD_8 in intraocular fluid.

It was in this contingent of patients that we found highest indices of IgG, CD_4, cortisol and prolactin on the background of decreased killer-activity, which already in the early pos-operative period upon disturbance of the integrity of a capsule might be factors of risk for induction of autoimmune aggression in response to "baring" antigenic determinants of lens.

On the base of obtained data, the role of *in situ* produced cortisol and prolactin in ACAID induction and abolition in case of cataracts should be considered from the qualitatively new standpoint. Apparently, the entire chronological process of surgical intervention on the background of already existing regional immunopathological and endocrine perturbations should be considered as a provoking factor because under operation the antigenic determinants of lens tissues are "bared".