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HOST CELL INTERACTION MECHANISMS OF SEVERAL VIRULENT BACTERIAL VIRUSES

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Molecular organization, biological features and host cell interaction mechanisms of several bacteriophages - ingredients of the therapeutical preparations are studied. Phage DNA resistance against restriction enzymes *in vitro* is investigated. Molecular cloning of phage genes in non-methylated cells with subsequent restriction analysis of the recombinant clones are performed. The recombinant clones reserve the initial resistance to specific endonucleases, thus confirming the absence (counterselection) of these sites in the clones and correspondingly, in the phage DNAs. Molecular cloning of the phage-specific conditionally-lethal genes is performed; the influence of the expressed products of cloned phage genes on bacterial cells is described.

Key Words: *Sewage waters, Dacteriophages, Cloning, Restriction.*

An exceptionally rapid spread of multiresistant strains is limiting the effectiveness of antibiotic treatment of infectious diseases. Consequently, the therapeutic use of bacteriophages remains an interesting alternate approach. High specificity against the infectious agents and harmlessness of virulent bacteriophages supports the possibility of their successful usage.

Specific, polyvalent phage preparations successfully used as a remedies against various infectious diseases have been developed at the Tbilisi Institute of Bacteriophages, Microbiology and Virology. Phi-1 and Phi-5 (host strains E.coli, Shigella), ()ST-I (Pseudomonas aeruginosa), Sb-1 (Staphylococcus aureus) and IRA (Salmonella typhimurium) bacteriophages are ingredients of these preparations. All the five bacteriophages are strictly virulent, thus promoting special interest to investigate early stages of phage intracellular development. Comparative analysis of the interaction peculiarities of phages with different host cells could provide useful information for what is common in these processes, thus enlightening some peculiarities of viral evolution.

MATERIALS AND METHODS

Phages were isolated from the sewage at the Tbilisi Institute of Bacteriophages, Microbiology and Virology. Bacterial strains were obtained from the bacterial collection of the same Institute.

The lytic activity of the phages was investigated by means of standard methodology (Kuhn et al., 1973). The efficiency of plating, adsorbtion characteristics and one-step growth properties of the phages were studied by Adams (1959).

The phages were grown by a standard procedure (Tikhonenko et al., 1966). Large volumes of phage were prepared in Frazer fermenters at 37°C. Bacterial cultures were cultivated to 5ò10^8 cfu/ml, infected with phage and aeration continued during 8-10 hours. The phage lysate were centrifuged at 5000g for 30min. at 4°C. After tangential flow of phage suspension through a Minitan apparatus (Millipore), further purification of concentrated phage particles was achieved by CsCI density gradient centrifugation.

Micrographs of phage particles were obtained using JEM 1200EX electron microscope (JEOL). Parlodion (Mallinkrodt) plates were overlaid by phage suspensions and contrasted by uranylacetate.

Phage and plasmid DNAs were isolated by a standard alkaline-phenol procedure. Restriction and ligation, transformation and electrophoresis (0.8-1% agarose slab gels) were performed by standard methods (Maniatis et al., 1982).

G-C content in DNA molecule was determined by means of thermal denaturation ofDNA by Mandel et al. (1970).

Molecular weight of phage DNA was studied by determination of kinetics of DNA reassociation (Wetmur et al., 1968; Britten, 1968).

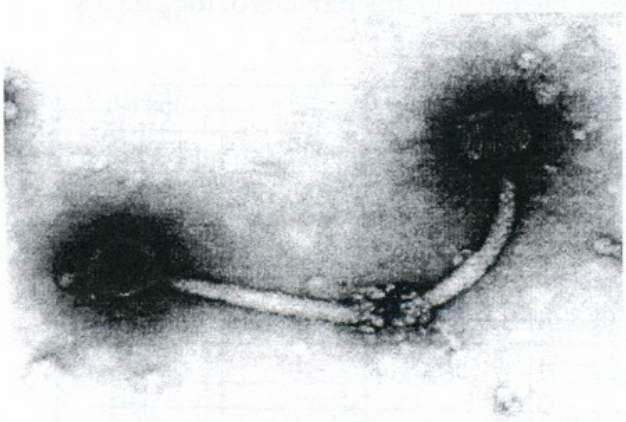
Determination of influence of cloned IRA phage genes on recipient cells: E.coli JM105 was transformed by pKI71 and pKI72 recombinant plasmids in Luria-Bertani medium (Maniatis et al., 1982) containing 30u,g/ml ampicillin. Isopropyl-p-D-thiogalactoside (IPTG) was added to a final concentration of 5mM and growth was continued for 24 hours. Aliquots were taken at various time intervals and viable cell counts were determined. These aliquots were divided into two parts; cellular DNA was isolated from the first sample, while the cells of the second were analyzed by electron microscopy. Transformed E.coli JM105 cells without inducer as well as nontransformed E.coli JM105 cells served as controls.

RESULTS AND DISCUSSION

Host range spectra and main parameters of intracellular development of phages were studied (Table 1). It appeared, that the data for latent period, time of lysis, as well as the yield of the phage particles from the single cell, differs significantly from phage to phage.

The electron microscope studies of the viral particles showed that they have rather similar molecular organization. Virions have well-defined binal symmetry with icosahedral or hexagonal heads and except IRA, complex contractile tails. Ac-

cordingly, Phi-1, Phi-5, Sb-1 and ϕST-1 belong to Myoviridae, while IRA is a representative of the Siphoviridae family (Ackermann, 1992). The dimensions of Phi-1 are almost identical to T4, ϕST-1 is quite a bit larger, while three others are much smaller, (Fig.1-5).



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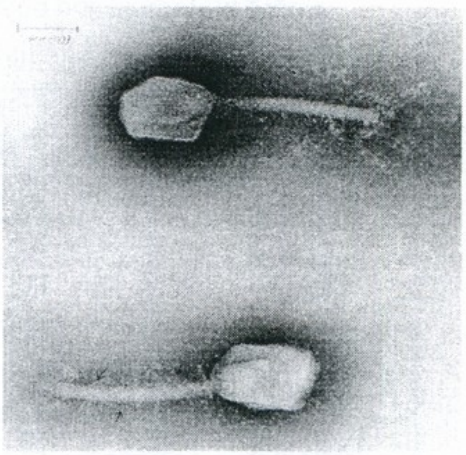

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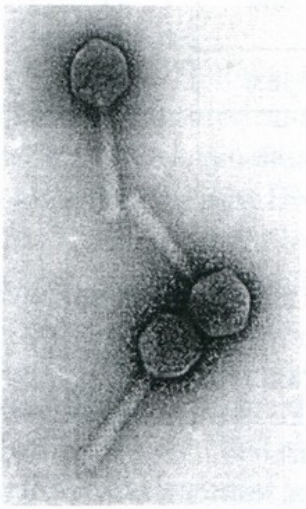

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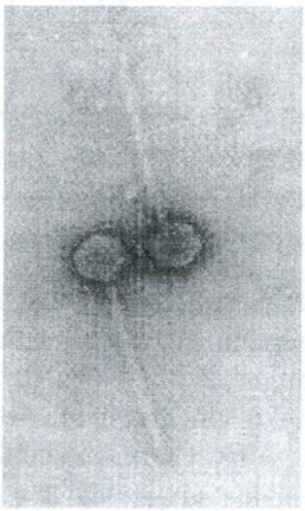

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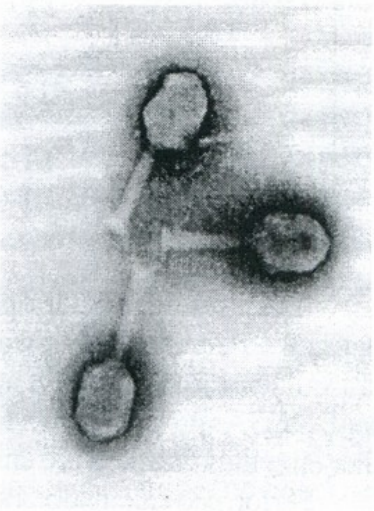


5

Fig. 1-5
1-Eliction micrography of Bacteriophage Sb-1, magh.x102.500
2-Eliction micrography of Bacteriophage ϕST-1 magh.x200000
3- Eliction micrography of Bacteriophage PHI-5 magh.x200000
4-Eliction micrography of Bacteriophage IRA-5 magh.x248000
5-Eliction micrography of Bacteriophage PHI-1 magh.x105000

The genomes of the investigated phages are represented by double-stranded DNAs with standard conformation and do not contain any hydroxymethylated and glucosylated bases. The distribution of G-C pairs in these molecules is of

Gaussian type, that is usual for virulent phages (Falkow, Cowie, 1968).

The neutralization reactions of the investigated phages with T4 anti-phage serum showed that none of them, even Phi-1 which is morphologically identical to T4, have any serological relation to T4 phage.

Table 1

Some biological and physico-chemical characteristics of the phages and their DNA

Parameters	Phi-1	Phi-5	Sb-1	IRA	ϕ8T-1
Head (A°)	1100x900	750x750	850x800	500x500	1400x1400
Tail (A°)	1200x200	1000x200	1800x150	2800x150	2400x300
Adsorbtion (min.)	3	5	5-6	3-4	5-8
Latent period (min.)	18	22	55-60	40	180
Lysis time (min.)	28	35	120-130	20	250
Average burst size	52	145	80-90	300	43
G-C%	41.6	38.1	29.0	38.0	34.9
DNA M.W. (MD)	85	57	80	48	140

As all studied bacteriophages were active against the majority of homologous bacterial strain checked, it was interesting to examine their resistance to restriction endonucleases digestion. It appeared that Phi-1 DNA is fragmented only by BglII, Mspl, Sau3A and EcoRV, while Phi-5 is sensitive to HindIII, EcoRV and Mspl. Twelve other enzymes checked are enable to digest these molecules.

Performed calculations showed that the amount of actual restriction sites on these molecules is much less then predicted by first order of Markov chain analysis (Table 2), indicating thus, partial elimination of the sites.

Restriction analysis of the three other phages showed, that Sb-1 DNA is resistant only to Sau3A (GATC), BamHI (GGATCC) and BglII (AGATCT) endonucleases, (J)ST-I DNA is not restricted by PstI (CTGCAG), Sail (GTCGAC), PaeR7I (CTCGAG) and Pael (GCATGC), while IRA phage DNA is fragmented by all 12 used enzymes.

It is rather evident that, such type of resistance could be caused by the specific modification of restriction sites. To check this possibility cloning of large fragments of Phi-1, Phi-5, ())ST-I and Sb-1 phages DNAs into methylase-negative host cells with subsequent restriction analysis of the recombinant clone DNAs were performed.

The results of these experiments showed that non-methylated phage DNA in recombinant clones reserve the initial resistance to specific restriction enzymes, thus confirming the absence (counterselection) of restriction sites in the clones and correspondingly, in the phage DNAs.

Table 2

Number of cleavage sites for different restriction enzymes in Phi-1 and Phi-5 DNAs

Enzyme	Recognition sequence	PhI-5		PhI-1	
		actual	predict	actual	predicted
BamHI	GGATC	0	22	0	41
BglII	AGATCT	0	58	37	81
EcoRV	GATATC	29	58	29	81
EcoRI	GAATTC	0	58	0	81
HindIII	AAGCTT	22	58	0	81
MspI	CCGG	34	227	>50	482
KpnI	GGTACC	0	22	0	41
Sau3A	GATC	0	600	>50	950
PstI	CTGCAG	0	22	0	41
SmaI	CCCGGG	0	8	0	21
SalI	GTCGAC	0	22	0	41
XhoI	CTCGAG	0	22	0	41

It is well known, that T4 possesses several systems aimed at overcoming of cellular defense mechanisms. First of all, these systems are based on the modification of phage DNA cytosine bases and producing of specific enzymes for the degradation of cellular DNA. As the DNAs of the phages under study did not contain any modifications, it was interesting to examine how these genomes overcome defense mechanisms of host cells, induce inhibition of cellular metabolism and secure their own reproduction.

For this purpose, nuclease activity analyses was performed in E.coli C cells infected with Phi-5 and S.typhimurium cells infected with IRA. Obtained results clearly indicated that phage encoded nuclease activities exist in the infected cells and the maximum of such activity could be observed on 7^{th} min. for Phi-5, and 11^{th} min. after infection for IRA. Exo- and endonucleolytic activities could be observed in both cases. Although these activities could be easily detected, purification attempts performed were unsuccessful. Therefore, we decided to clone DNA fragments of one of the phages, particularly IRA, whose expression would be lethal for the host cell. Such fragment can be cloned only in vectors which provide a regulated expression of foreign genes. pKK223-3 plasmid vector was chosen for that purpose.

PstI fragments of IRA phage DNA were ligated to the pKK223-3 plasmid and E.coli JM105 cells were transformed by these constructions. Recombinant clones were identified in shotgun experiments by parallel plating in the presence and absence of inducer. This approach allowed us to isolate directly recombinant clones carrying genes lethal for the cell.

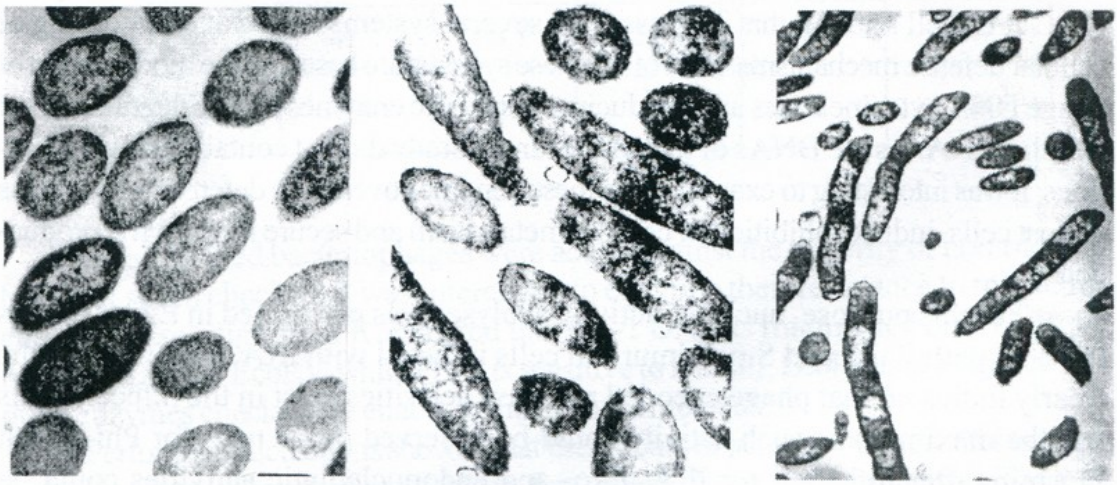
Two clones were selected which formed colonies on the plates without inducer

plasmid which designated as pKI71. This plasmid composed of vector DNA and the 3.6kbp PstI-E fragment of IRA DNA. The second clone carries the plasmid pKI72 which contains the 3.0kbp PstI-G fragment of IRA DNA. Blot hybridization of these recombinant plasmids with the PstI fragments of phage DNA confirmed the phage IRA origin of the cloned fragments.

To investigate the effect of phage gene products on the host DNA, bacterial cells carrying recombinant plasmids were added by inducer, the cells were disrupted and genomic DNA isolated. It appeared that expression of pK.171 causes degradation of chromosomal DNA. In case ofpK.172 no nucleolytic activity was found.

Electron microscopy study was performed to determine the character of morphological alterations that could occur in bacterial cells due to the expression of cloned phage genes. Expression of pK.171 causes significant alterations in cell wall structure. It is difficult to understand the reason, but these changes are more pronounced in cells with rather short exposition to the inducer.

The character of cell morphology alterations provoked by expression of pK.172 is different. The cell wall appeared normal. However, in the vast majority of the cells two or more nucleoids occurred and the length of cells containing expressing pK.172 increases significantly. It seems that products of recombinant plasmids pKI72 genes disrupt normal cell division. The function of these genes during phage replication is as yet unknown. (Fig. 6-8).



6 7 8

Fig. 6-8

6-Normal E.coli jM 105 cells (500 nm)
7-E.coli jM 105 transformed by pKI71 plasmids
8- E.coli jM 105 transformed by pKI72plasmids

As a conclusion, it can be noted, that phages evolutionary diverged by the activity towards host cell range, maintain several common features. It seemed, that the counterselection of the restriction endonucleases sites is more general phenomenon, then it used to be considered. As a rather common feature could be considered also phage induced nuclease activity on the early stages of infection.

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ვირულენტური ბაქტერიოფაგების პატრონ ბაქტერიულ უჯრედებთან ურთიერთქმედების ზოგიერთი მექანიზმის შესწავლა

რ.ადამია, ნ.ბალარჯიშვილი, ლ.კვაჭაძე, მ.ქუთათელაძე

საქართველოს მეცნიერებათა აკადემია, გ.ელიავას სახ. ბაქტერიოფაგიის, მიკრობიოლოგიის და ვირუსოლოგიის ინსტიტუტი

რ ე ზ ი უ მ ე

შესწავლილია სამკურნალო-პროფილაქტიკურ პრეპარატებში შემავალი სხვადასხვა ბაქტერიოფაგის და მათი დნმ-ის მოლეკულური ორგანიზაცია, ბიოლოგიური თვისებები, პატრონ ბაქტერიულ უჯრედთან ურთიერთმოქმედების მექანიზმები. გამოკვლეულია ფაგების დნმ-ის მგრძნობელობა ბაქტერიული რესტრიქციული ფერმენტის მიმართ in-vitro. ჩატარებულია ფაგების გენების კლონირება არა-მეთილირებულ უჯრედებში და მიღებული რეკომბინანტული კლონების რესტრიქციული ანალიზი. დადგენილია, რომ რეკომბინანტული კლონები ინარჩუნებენ საწყის რეზისტენტობას სპეციფიური ენდონუკლეაზების მიმართ, რაც მიუთითებს კლონებში, და შესაბამისად, ფაგები-ს დნმ-ში გარკვეული ამოსაცნობი საიტების ელიმინაციაზე. ჩატარებულია ფაგოსპეციფიური პირობით-ლეთალური გენების კლონირება და ნაჩვენებია რეკომბინანტული პლაზმიდების შემცველი კლონების ზეგავლენა პატრონ ბაქტერიულ უჯრედებზე.

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COMPLEX TREATMENT OF ALIMENTARY OBESITY BY MEANS OF CONTROL AND REGULATION OF PHYSICAL TRAINING

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The work is devoted to elaboration of method for control and regulation of physical load in therapeutic gymnastics procedure in obese patients. Programs of physical load were composed on the basis of determination of tolerance to physical strain, with calculation of optimal, submaximal and maximal heart rates. The results of research showed that weight and number of anthropological data reliably decreased. Data of functional and biochemical investigations have already been improved by the end of the first stage of treatment. Thus, usage of system of control and regulation of physical load in therapeutic gymnastics procedure program of which is composed according to the data of tolerance to physical strain represents therapeutically high – effective and economic method.

Key words: *Treatment of alimentary obesity, Individualization of physical training, Tolerance to physical strain, Human*

Alimentary obesity belongs to a group of social diseases. Significant treatment have been achieved by complex treatment of obesity [1, 2, 3, 5, 6, 7, 8]. Normalization of weight in wide masses of population is one of compounds of healthy life-style, because it represents a risk-factor for number of diseases. Different combinations of complex treatment by diet and treating exercises have been created.

Special variety distinguishes diets. Only one principle remains unchanged – limitation of calories on different levels. Most of the diets are based on prior usage or prohibition of certain products.

As for development of program of physical training, also exist here main principal and different methods: exercises used for treatment of obesity should excess common and mild strains by intensiveness, they should cover possibly big group of large muscles. The majority of authors emphasize importance of carrying out of exercises with special carefulness and gradual increase of strain, because obesity of high grade and long duration in different percent of cases is complicated by disorders of cardio-vascular, respiratory, digestive, and/or muscles and skeletal systems. The issue of individualization of intensity and type of exercise and

their further realization remains unstudied.

The purpose of our work was creation of individual, original, method for treatment of patients with alimentary obesity, that would give us possibility of selection of exercises of optimal intensity on the background of diet and realization of them in the procedure of treating training.

We have investigated and carried out dynamic clinical observation of 180 patients with alimentary obesity of I, II, and III degree. Patients were divided into 2 groups: 121 patients composed basic group, 59- control group. The diet "D.T.-2" was used in both groups. In basic group (121 patients) program of treating exercises and its realization was carried out according to method proposed by us, and in control group (59 patients) - according to generally recognized method of treating training. Patients underwent general and special clinical investigations - anthropological, functional and biochemical.

The process of treatment was divided into 2 periods: the first period implied complex treatment, was composed by procedures of treating exercises and I and II stages diet "D.T. –2" in the second period of treatment only diet-therapy sing III stage of the same diet was carried out. The results of investigations are represented in Table 1.

In the basic group development of program of treating exercise and its realization was carried out according to method proposed by as. In order to select physical training and individualize them in the procedure for determination of critical and optimal pulse definition of tolerance to physical strain was carried out by means of veloergometer. Heart rate registered during tolerant or maximal training represents "critical pulse", 60-70% of which is "optimal pulse", and it is recognized in the procedure of treating exercises as background strain. 90-90% of critical in the procedure of treating training by gradual quickening in type of peaks. Special program envisaged alteration of optimal and submaximal levels of the heart rate at the beginning of treatment, and inclusion of maximal – "critical pulse" in the following period.

In order to achieve critical and optimal pulse and maintain control in the procedure of treating training while treating obesity we used apparatus of guided tachycardia and system "I.K.-I.C", that was created and proposed by Tsverava, Ionatamishvili, and Khabibulin [4].

The apparatus is small device, which announces about deviation of heart rate from programmed pulse by means of sound signal.

Installation of backreverse information connection (biofeedback) by means of apparatus of guided tachycardia and system "I.K.-I.C." gives possibility of real implementation of developed program of physical training.

As table shows (Table 1), data in basic and control groups were same. On the first stage of complex treatment statistically trustworthy decrease of weight and Kettle's index was manifested in the basic group while data obtained by dynamometring and spirometry increased significantly. Tolerance to physical exercise and results of background pulse also improved: mentioned positive changes

were less significant in the control group.

Table 1

Dynamics of investigation results in case of treatment of alimentary obesity with the new method of physical load control in the therapeutic gyimnnastic procedure

On the second stage of treatment, when procedures of treating training was ceases

Investigations	Groups	Number of patients	Anthropometrical tests								Biochemical tests	
			Loss of weight	Kettle's index	Dynamometry of right hand		Spirometry left hand		Background pulse	Tolerance to physical strain	Cholesterol	Glukose
			kg	kg/gr	kg		kg	sm3	1/2min	kgm2/min	mmol/l	mmol/l
Inicial data	Basic	121		530,87+	32,16+		31,78+	2885+	44,00+	620,56+	55,51+	5,75+
				68,43	9,52		11,09	318,07	5,92	107,64	5,56	0,59
	Control	59		530,87+	32,16+		31,78+	2885+	44,00+	620,56+	55,51+	5,75+
				68,45	9,54		11,04	318,02	5,89	107,5	5,42	0,51
	p<			*	*		*	*	*	*	*	*
By the end of first stage of treatment	Basic	121	11,8+	459,33+	41,98+		42,68+	3232+	38,99+	644+	50,32+	5,44+
			5,21	70,73	7,80		7,10	353,3	5,34	93,55	7,1	0,74
	Control	58	6,62+	504,24+	33,30+		35,53+	2856+	42,43+	606,9+	57,12+	5,68+
			7,32	62,13	7,61		7,39	319,08	6,36	109	3,84	0,52
	p<		0,0001	0,001	0,0001		0,0001	0,0001	0,0001	0,001	0,0001	0,0001
By the end of second stage of treatment	Basic	116	7,95+	478,37+	35,52+		35,46+	3433,6+	41,11+	629,65+	45,12+	5,08+
			3,46	65,93	8,17		8,45	198,23	5,27	102,65	8,24	0,80
	Control	54	6,12+	465,86	31+		33,21+	2771,4+	38,29+	650+	52,42+	5,68+
			5,42	124,05+	13,12		13,12	847,24	12,43	170,57	14,16	10,62
	p<		0,001	*	0,0001	*		0,0001	0,05	*	0,0001	*

and only III step of diet therapy was carried out, decrease of weight and index of Kettle was manifested in both groups, and data of pulse and tolerance to physical strain was maintained on the same level.

Besides, data obtained in the control group were behind the results of treatment of patients in the basic group.

Important difference was noted according to results of biochemical testes. Statistically trustworthy difference between results gained in basic and control groups were have been revealed by the end of the first stage of treatment.

Decrease of glucose and cholesterol has been manifested by the end of the second stage of treatment, but they were not statistically trustworthy, that should be explained by of influence of treating exercises.

On the basis of carried out work we can make following conclusions:

Usage of methodology of definition of tolerance to physical strain in treatment of alimentary obesity gives opportunity of implementation of individual programs of physical exercise for each patient, that significantly increases efficiency of carried out measures.

In the process of definition and implementation of procedure of treating exercises gives opportunity of gaining optimal efficiency of procedure, prevention of excessive physical strain or vise versa inappropriately low strain.

Usage of modern methods of physical exercises and control in procedure of treating training gives possibility obtaining much better results, that is proved by appropriate anthropological, functional and biochemical data as compared with usage of traditional methods.

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ალიმენტური სიმსუქნის კომპლექსური მკურნალობა ფიზიკური დატვირთვების კონტროლისა და რეგულაციის მეთოდით

ნ.აფაქიძე

თბილისის ექიმთა დიპლომისშემდგომი დახელოვნების აკადემია

რ ე ზ ი უ მ ე

სტატია ეძღვნება ალიმენტური სიმსუქნით დაავადებულ ავადმყოფთა მკურნალობისას სამკურნალო ფიზკულტურის პროცედურაში ფიზიკურ დატვირთვათა კონტროლისა და რეგულირების მეთოდის შემუშავებას.

ფიზიკური დატვირთვების პროგრამის შემუშავება ეფუძნებოდა ფიზიკური დატვირთვებისადმი ტოლერანტობის განსაზღვრას და მის მიხედვით ოპტიმალური, სუბმაქსიმალური და მაქსიმალური პულსის დადგენას.

გამოკვლევათა შედეგებმა გვიჩვენა, რომ ძირითად ჯგუფში სარწმუნოდ შემცირდა წონა და მასთან დაკავშირებული ანთროპომეტრული მაჩვენებლები. გაუმჯობესდა ფუნქციურ და ბიოქიმიურ გამოკვლევეთა შედეგები მკურნალობის პირველივე პერიოდში. ალიმენტური სიმსუქნით დაავადებულთა მკურნალობისათვის სამკურნალო ფიზკულტურის პროცედურაში ფიზიკური დატვირთვების კონტროლირებისა და რეგულირების პროგრამა, რომელიც დატვირთვისადმი ტოლერანტობის მონაცემებს ეყრდნობა, წარმოადგენს ეკონომიურ და მაღალი სამკურნალო ეფექტის მქონე მეთოდს.

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LYSINE CONVERSION DURING NATURAL ALCOHOLIC FERMENTATION

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In the process of natural alcoholic fermentation Saccharomyces vini-39 is actively assimilated by yeast and is converted to lysine carbon atoms. The 214C-lysine biotransformation products are identified in the yeast and wine amino- and organic acids.

The lysine carbon skeleton undergoes complex conversions during fermentation in anaerobic conditions and is partially oxidized to carbon-dioxide. The basic pathways of lysine conversion is linked with the functioning of a modofied Krebs cycle in anaerobic conditions.

Key words: *Lysine, alcoholic fermentation, yeasts, amino acids, organic acids*

Revelation of peculiarities of energy and constructive metabolism is closely related to the conversion of nitric compounds in yeast [2, 5]. regularities of qualitative and quantitative variation of amino acids in the process of alcoholic fermentation accounts eventually for the wine stability, affect the formation of color, taste, and fragrance of ready product.

From this point of view it is of interest to study the essential amino acids as carbon sources. Distribution of the products of their metabolism has a paramount importance for the management of microbiological and biotechnological processes.

The purpose of the present study was to reveal the possibilities of using the lysine carbon skeleton by yeast during natural alcoholic fermentation.

MATERIAL AN METHODS

Alcoholic fermentation proceeded in natural nutrition medium – grape juice containing 19.3% of sugar, pH=3.8. The yeast industrial strain Saccharomyces vini-39 in 48 hr culture was used as a fermentation agent. The 214C-lysine with 4.6 MBq radioactivity per 200 ml grape juice was introduced into the medium. Fermentation occurred at 14-16oC.

Assessment of the yeast and wine components was done as soon as primary fermentation was over, when the sugar amount remained in the medium did not exceed 1%. Chemical, chromatographic, and autoradiographic methods [3, 8] were used for the isolation of separate fractions and identification of individual compounds. Radioactivity of individual amino- and organic acids was measured on the LKB-type scintillation spectrometer Rackbeta.

RESULTS AND DISCUSSION

Different microorganisms species are known to cause enzyme degradation of lysine with diverse intensity. The lysine catabolic products formed thereat are utilized by cells as carbon and nitrogen source. In Sccharomyces cerevisiae yeast during assimilation of exogenic lysine priority is given to the carbon skeleton [7].

Our results indicate that 30-35% of lysine contained in the fermentation medium is taken up and converted by yeast. The lysine incorporated in the biomass is actively involved in the intermediate exchange of amino acids and participated in the synthesis of yeast protein and free amino acids (Table 1).

Table 1

Distribution of radioactivity (in %) in the yeast individual amino acids during biotransformation of 2^{14}C-lysine

Percentage of identified amino acid radioactivity in the fraction overall activity			
Protein amino acids		Free amino acids	
83.8%		16.2%	
Lysine	54.7	Lysine	41.2
Aspartic acid	14.3	Valine	35.1
Glutamic acid	13.5	Phenylalanine	14.0
Serine	7.2	Methionine	6.8
Glycine	4.1	Proline	2.9
Valine	3.2		
Leucine	2.6		
Alanine	0.4		

As a result of uptake and conversion of 214C-lysine in the overall fraction of yeast proteins radioactivity appeared to be 8 amino acids. Especially high was lysine radioactivity. A complex way of conversion of exogenic lysine is suggested by the existence of carbon atoms in the amino acids, which had been formed through various metabolic ways. At the same time, lysine high radioactivity both in protein and in the pool of free amino acids might be due to a direct assimilation of this amino acid with high intensity, especially in the experimental phase of fermentation process [5].

The pool of free amino acids also deserves attention. Yeast cells maintain a high

content of free amino acids, which, at a certain ratio, are distribited among the cytoplasm and other cell organelles. Though its major part falls on vacuoles [9]. At the same time the pattern of qualitative distribution of proteins and free amino acids is somewhat diverse, which again emphasizes the functioning of various pools of free amino acids [1].

The 214C-lysine carbon skeleton assimilated and converted by yeast is involved in the synthesis of extracellular compounds; major part of 214C-lysine conversion products is transferred to wine in the process of alcoholic fermentation. Among its components the basic ones are amino- and organic acids (Table 2). As a result of regulation of heterogeneous metabolic processes ongoing in anaerobic conditions a dynamic equilibrium is established between the yeast and wine components that manifests itself variously at separate stages of fermentation [6].

Table 2

Distribution of radioactivity (in %) in the wine amino- and organic acids during 2^{14}C-lysine biotransformation

Radioactivity (%) of identified compounds in the fraction overall activity			
Amino acids		Organic acids	
84.7%		15.3%	
Glutamic acid	27.7	Succinic acid	42.8
Aspartic acid	15.2	Fumaric acid	33.3
Serine	14.3	Glyoxalic acid	17.7
Glycine	11.6	Malic acid	4.2
Arginine	6.4	X 2.0	
Proline	5.2		
Valine	4.8		
Leucine	4.2		
Tyrosine 3.9			
Phenylalanine	3.8		
Methionine	2.9		

Almost 85% of overall fractions of organic- and amino acids identified in wine falls on wine amino acids. Glutamic acid is particularly distinguished by its high radioactivity. But the relative picture of qualitative and quantitative distribution of yeast and wine amino acids is diverse. It is clearly seen that the synthesis of extracellular compounds is a result of normal variability of yeast and is not coupled with the lysis processes subsequent to fermentation.

As a consequence of 214C-lysine conversion among the organic acids identified in the medium of alcoholic fermentation succinic and fumaric acids are distinguished by their high radioactivity. Glyoxalic acid being also radioactive. The fragments of lysine carbon skeleton seem to be incorporated into the components of the Krebs modified cycle. However, under our fermentation conditions, no sharp changes were noted in

the qualitative content of organic acids as compared to grape juice.

Of 214C-lysine conversion products in the fermentation medium radioactive appeared to be carbon dioxide as well. Approximate quantitative calculations indicate that the isolated 14CO2 is minute and does not exceed 3-5% of the lysine taken up and converted by yeast.

Thus, in the process of grape juice alcoholic fermentation the carbon skeleton of exogenic lysine undergoes a complex conversion. It participated in biosynthesis of amino acids of various genetic origin, as well as in separate reactions of organic acids synthesis, the products of which, irrespective of restricted anaerobic conditions, are oxidized to carbon dioxide.

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ლიზინის გარდაქმნა ბუნებრივი სპირტული დუღილის დროს

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საქართველოს მეცნიერებათა აკადემიის ს.დურმიშიძის სახელობის მცენარეთა ბიოქიმიის ინსტიტუტი, თბილისი

რეზიუმე

შესწავლილია $2^{14}C$-ლიზინის გარდაქმნის ძირითადი პროდუქტები ყურძნის წვენის ალკოჰოლური დუღილის დროს საფუარსა და ღვინოში. დადგენილია, რომ ეგზოგენური ლიზინის ნახშირბადოვანი ჩონჩხი რთულ გარდაქმნებს განიცდის და მონაწილეობს საფუვრის ცილებისა და თავისუფალ ამინმჟავათა სინთეზში.

ლიზინის ნახშირბადატომები ერთვება საფუვრის ნორმალური ფუნქციონირების შედეგად წარმოქმნილ ღვინის ორგანული მჟავებისა და ამინმჟავების სინთეზში. $2^{14}C$-ლიზინის ბიოტრანსფორმაციას ალკოჰოლური დუღილის პროცესში თან ახლავს რადიოაქტიური ნახშირორჟანგის გამოყოფა.

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RESULTS OF TREATMENT OF ACUTE B HEPATITIS BY PLAFERON LB

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It was shown experimentally that Plaferon-LB modulates immunity index in the damaged cells and increases a number of cells possessing CD4+, CD22+, and CD16+ phenotypes. Influence of Plaferon-LB on the HBs-antigenemy also was found to be significant. It is concluded that application of Plaferon-LB during acute hepatitis C, rapidly alleviates intoxication events, which is followed by correction of biochemical and immunological indices.

Key words: *Hepatitis C, Plaferon-LB, Treatment, Immunity modulation, Correction*

There are no sufficiently effective methods in the treatment of acute B hepatitis as yet. Moreover, there is no unanimity within physicians about suitability of application of any medicine including immunomodulatory drugs in spite of important role of immune system of organism in development of clinical symptoms of acute B hepatitis and chronization of process is proved.

Our work is concerned with treatment of acute B hepatitis by immunomodulatory drug Plaferon LB – medicine, which beside immunomodulatory activity has wide spectrum of pharmacologic action, among them antitoxic and hepatoprotective properties.

Total of 90 patients, by randomized method, has been investigated. They were distributed into groups. I group – basis therapy, II group – basis therapy+Taktivin; III group – basis therapy+plaferon LB. It has been shown that Plaferon LB expressed significant effect on manifestation of intoxication. under influence of therapy disappearance of such symptoms as weakness, anorexia, nausea, vomiting, headache, sleeplessness, duration of jaundice – was showed significantly rapidly in the patients by Plaferon LB ($P < 0,001$).

Normalization of biochemical parameters of liver function have been shown more quickly in group of patients treated by Plaferon LB. Influence of Plaferon LB on total bilirubin's level was obvious, same was true with the level of ALAT ($P<0,001$).

It has been shown in case of acute B hepatitis that there are significant changes in the cellular immunity of patients, which was expressed by decreasing of amount of T-cells with CD3+, CD4+, CD22+, CD16+ phenotypes and increasing of amount of cells with CD8+ phenotype. These alterations are also kept in period of early

reconvalescence (except of amount of cells with CD3+ and CD8+ phenotypes). Plaferon LB modulates these changes in cellular immunity of patients by increasing of amount of cells with CD4+, CD22+ and CD16+ phenotypes ($P < 0,001$).

Influence of Plaferon LB on the dinamic of HBs-, HBe-antigenemia is also evident. Before treatment HBsAg in the compared groups was shown in 100%. After treatment, HBsAg was exposed in control group in 62%, then patients were treated by Plaferon LB it was shown in 36%.

The influence of Plaferon LB on the HBe-antigenemia was the same. HBe-antigenemia from 63% was falled down in the control group till 15%, and under the influence of Plaferon LB – till 4.5%.

In time of discharging from hospital (approximately after month from the starting of treatment) anti-HBsAb were exposed in control group in 11% and in case of Plaferon LB therapy-in 30% of patients. After 3 months accordingly-in 40% and 66% of patients.

It's known that chronisation of hepatitis most often occurs in casw of mild and moderate courses of disease. For prophylaxis of chronic hepatitis patients with mild and moderate current of disease were treated by PlaferonLB.

Only one from 32 patients after 3-5 years of treated by PlaferonLB, has HBsAg, anti-HBe and anti-HBcor IgM. Now indices of livel function of this patient are normal. This patient is under the clinical examination. Anti-HBsAb , indicating existence of immunity, has been shown in 53% of patients.

Thus, administration of Plaferon LB in the patients with acute B hepatitis hastened reverse development of clinical symptoms of disease, wich is followed by correction of both, biochemical parameters of liver function and immunologic indices. These determine faster clinical recovery and prevent chronization of process.

Excellent clinical effect after treatment by Plaferon LB is determined by immunocorrection [1, 3, 4, 6] and hepatoprotective actions of this medicine.

By decreasing of lipid peroxidation intensity and reduction of generation of free radicals Plaferon LB restores antioxidant potential of the organism.By stabilization of mitochondrial microsomal membranes and recovering of electron transport in respiratori chain of mitochondrial and microsomal monooxiganase, Plaferon LB protects mitochondrial damage [2, 4]. Restoration of antioxidant potential protection in damaged mitochondria under the influence of Plaferon LB determine hepatoprotective effect of this medicine.

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მწვავე ჰეპატიტის პლაფერონ ლბ-თი მკურნალობის შედეგები

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საქართველოს მეცნერებათა აკადემიის სამედიცინო ბიოტექნოლოგიის ინსტიტუტი, თბილისი

ნაშრომი ეხება მწვავე B ჰეპატიტის მკურნალობას იმუნომოდულატორ პლაფერონ ლბ–თი – პრეპარატით, რომელსაც იმუნომამოდულირებელი თვისებების გარდა, გააჩნია ფარმაკოლოგიური მოქმედების ფართო სპექტრი, მათ შორის ანტიტოქსიური და ჰეპატოპროტექტორული.

რანდომიზაციის მეთოდით გამოკვლეული იქნა 90 პაციენტი. პლაფერონ ლბ–ს მოქმედების შეფასებისას აღმოჩნდა, იგი მნიშვნელოვან გავლენას ახდენს ინტოქსიკაციის მოვლენების კუპირებაზე. ამასთან ერთად ღვიძლის ფუნქციური სინჯების გაცილებით სწრაფ ნორმალიზებას ადგილი ჰქონდა პლაფერონოთერაპიის ფონზე. ავადმყოფების სისხლის შრატში საერთ ბილირუბინისა და ალანინ-ამინოტრანსფერაზის დონის დინამიკა პლაფერონ ლბ–ს ფონზე სტატისტიკურად სარწმუნოდ განსხვავდებოდა საკონტროლო ჯგუფის ანალოგიური მაჩვენებლებისაგან.

პლაფერონ ლბ მოდულირებას უწევს დარღვეული უჯრედული იმუნიტეტის მაჩვენებლებს და ზრდის CD4+, CD22+ და CD16+ ფენოტიპების მქონე უჯრედების რაოდენობას. პლაფერონ ლბ–ს გავლენა HBs–ანტიგემიაზე ასევე სარწმუნოა.

ამრიგად, მწვავე ჰეპატიტის დროს პლაფერონ ლბ–ს გამოყენების შემთხვევაში სწრაფად იხსნება ინტოქსიკაციის მოვლენები, რასაც თან ახლავს ბიოქიმიური და იმუნოლოგიური მაჩვენებლების კორექცია, ხოლო ყოველივე ეს განაპირობებს ვირუსის ელიმინაციას და გამოჯანმრთელებას.

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IMPROVEMENT OF THE TECHNOLOGY PROCESS OF THE PROBIOTIC PREPARATION *COLIBACTERIN;* CONSTRUCTION OF A NEW PHAGE-FREE STARTER STRAIN

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Colibacterin - a probiotic preparation has been used throughout the whole territory of the former Soviet Union since 30's [5, 15, 19]. The basic strain responsible for its antagonism against the major intestinal pathogens is *E. coli M17*. In late 80's the industry of Colibacterin met a lot of troubles related to spontaneous emergence of an unknown bacteriophage and consequent lysis of the large volumes of the end-product. For detection of the reasons leading to these losses our group performed detailed studies of various starter cultures and the product samples obtained at different processing stages. A number of bacteriophage clones (assigned as *CB1, CB2, CB3...*) have been identified. Comparison of the basic taxonomy features of these clones revealed complete identity among them. Moreover, lysogenic status of the strain *E. coli M17*, temperature-dependent induction of bacteriophage (*CB*) was discovered. These data explained the reasons causing spontaneous lysis of the end product. Due to application of certain genetic methods a stable mutant strain *E. coli M17/CB-7* was constructed. The new strain with improved traits has been successfully applied as a starter for production of Colibacterin by a number of pharmaceutical industries in Georgia.

Key words: *Bacteriophage clones, Temperature-dependent induction, Starter-strain, Phage-free strains*

The troubles in various areas of microbial industry, such as dairy processing, antibiotic and vitamin industry, are often caused by spontaneous phage lysis, which significantly decreases quality of the end-product. This fact is usually accompanied with serious financial losses. Contamination of bacterial starters becomes possible through several ways: 1) as a result of so called monoclonal industry, which is based on the use of a single bacterial strain, and is usually complicated with spontaneous emergence of the large volumes of phage progeny. This event is a common result of induction of an initially lysogenic strain (1); 2) as a banal contamination with foreign phages. Phage decontamination procedure is quite

expensive. Therefore, search for new efficient preventive measures are of the primary importance. One of such approaches is isolation and selection of phage resistant mutants that, at the same time, completely preserve all the features of the initial strain.

During the last decades phage lysis in Colibacterin-producing plants located in different regions became frequent. Colibacterin is a bio-preparation consisting of live bacterial cells of *E. coli M17* - a strain which was first isolated by L.Perez in 1933 [5]. Colibacterin was widely used in the former Soviet Union for treatment and prophylactics of a number of intestinal diseases and restoration of gut ecosystem disturbed by overuse of antibiotics or intensive chemotherapy [5, 15, 19]. Spontaneous emergence of phage progeny during industrial propagation of *E. coli M17* leads to undesirable halting of technology process which affects the quality of the end-product. Application of the standard eradication methods, such as chemical and physical treatment (UV irradiation) of plant spaces appeared to be ineffective. Thus, investigation of contamination sources, reasons of spontaneous lysis and elaboration of a new technological strategy for combating of this problem was of great importance. The present study focused on the following goals: a) to determine the reasons of spontaneous phage lysis and detect the sources and transmission ways of phage contamination; b) to characterize isolated phage clones and determine relatedness among them according to their biological, morphological and serology features; c) to construct a mutant phage-resistant strain of *E. coli M17.*

Table 1

Comparative characterization of the phage clones isolated from different sources

PHAGE	HOST-STRAIN	MORPHOLOGY GROUP	NEUTRALIZATION INDEX	
			K min^{-1}	%
CB	*E. coli M17*	S_{II}	65	100
CB-17 F	*E. coli M17*	S_{II}	60	92.3
CB-M	*E. coli M17*	S_{II}	60	92.3
CB-T	*E. coli M17*	S_{II}	63	97
CB-P	*E. coli M17*	S_{II}	58	89
CB-1	*E. coli M17*	S_{II}	65	100
CB-2	*E. coli M17*	S_{II}	56	86
CB-3	*E. coli M17*	S_{II}	62	95.4
CB-4	*E. coli M17*	S_{II}	61	93.8
CB-5	*E. coli M17*	S_{II}	59	90.8

MATERIALS AND METHODS

Bacterial strains: *E. coli M17; E. coli K12: C600, KS 707, KS 720, B, C.* Newly isolated and standard strains of *E. coli, Sh. flexneri, Sh. sonnei, Sh. newcastle* (25 strains of each species).

Bacteriophage clones: *CB* series, isolated from: industrial samples of Colibacterin, industrial spaces, standard starter cultures obtained from different industrial plants (Gorkii, Perm, Moscow, Tbilisi), phage *PM17* (2, 3).

Media: Hottinger Agar, ENDO selective agar (*E. coli* strains give purple growth on it, *Shigella* - appears as white colonies).

Methods: Isolation and propagation of phages, study of their morphology, serology, host range, intracellular phage growth cycle, pro-phage induction and frequency of lysogenization have been studied according to well-known and/or modified methods (1, 4, 6, 8, 13, 16). Immunization of animals and preparation of anti-phage sera (APS) has been accomplished according to (9). Phage neutralization reaction due to application of specific APS was performed according to (1).

The index of antagonistic activity of *E. coli M17* and its derivative strains against *Sh. sonnei* and *Sh. flexneri* was accomplished in a mixed culture and estimated according to the following formula: A=K/K+F, where, A - index of antagonistic activity in per cent; K - number of the colonies of the *E. coli M17* on the plate with the selective media; F - number of the colonies of the test-bacteria on the plates with the selective media (5).

Isolation of the phage-resistant (P^R) bacterial mutants was carried out by indirect selection. Frequency of P^R mutant formation was estimated according to classical methods described in (12) and (14). The presence of phage DNA in bacterial cells was detected by Southern DNA-DNA hybridization method (16).

RESULTS AND DISCUSSION

Contaminating phages were detected in the probes obtained from industrial environment: desks, fermenters, sterile boxes, autoclaves, laboratory equipment located in different spaces. Morphological diversity of isolated phage clones families: Myoviridae, Styloviriadae, Podoviridae (16) indicated to different sources of phage contamination (7,10,18). At the same time one of the clones predominated, which was later on entitled as *CB*-phage. Proceeding from the above data we proposed that main contamination of the industrial plants and the end-products is caused by spontaneous lysis of a certain temperate phage, which is comprised in the host bacterial cell of *E. coli M17.*

At the first stage of our studies determination of the status *E. coli M17* bacterial cell (lysogeny or pseudolysogeny) has been done. For this purpose comparative studies of the *CB*-phages, isolated from different batches of

Colibacterin (production of Moscow, Perm, Tbilisi, Gorki bio-industries), have been performed. These phages were obtained due to spontaneous either UV inductions. Multiple passages through *E.coli M17* cells, cloning and concentration of isolated phage clones have been performed separately for each one. During these procedures the graduate changes of the morphology of the negative plaques (NP) and of the host range have been observed. In particular, initially turbid NP became completely transparent, while the host range got extremely narrow by time. Finally a *CB* clone, that specifically lysed a single host strain *E. coli M17* while remaining ineffective against other *E. coli* test-cultures, has been selected. We assume that this clone is a virulent mutant of an initial (wild) temperate phage. Rapid selection of the virulent mutants of phage during the concentration procedure has been observed by other investigators as well (1, 11).

The size of NP of the selected *CB* phage and peculiarities of its one-step growth cycle are in a strong dependence with environmental factors, especially temperature (Table 2). 28°C is temperature optimum for phage multiplication.

Table 2

Comparative studies of the parameters of the intracellular growth cycle of different phage clones at 28°C and 37°C

Phage	Adsorption rate onto the host-strain E. coli M17						Duration of the latent period at:		Progeny per cell at:	
		28°C			37°C		28°C	37°C	28°C	37°C
	time (min)	%	K (min^{-1})	time (min)	%	K (min^{-1})	time (min)		pfu/cell	
CB	10	84	$1.7x10^{-8}$	20	44	$1.2x10^{-8}$	24	47	128	95
CB-M17	10	82	$1.7x10^{-8}$	20	42	$1.2x10^{-8}$	24	45	125	92
CB-M	10	78	$1.6x10^{-8}$	20	38	$1.0x10^{-8}$	26	52	120	88
CB-T	10	75	$1.4x10^{-8}$	20	46	$1.3x10^{-8}$	22	48	118	75
CB-P	10	80	$1.6x10^{-8}$	20	40	$1.1x10^{-8}$	25	45	115	69

The progeny outcome of the phage per cell increases up to 115-120 plaque forming units (pfu)/cell, while at 37°C it remains comparatively low - 80-92 pfu/cell. Accordingly, at 28°C *CB* gives large NP (D = 5-7 mm), while at 37°C they are much smaller (D = 1-2mm). Application of APS revealed close relatedness among the different *CB*-phage clones (Table 2). Electron microscopy studies showed complete morphological identity of *CB*-phage with the phage FM17 previously isolated by other authors (5,6). All *CB*-phages were related to the family Styloviridae, type 11 (17) (Fig.1). Such morphological structure is usually characteristic for temperate phages (11). Thus, summarizing preliminary data we assumed that the starter strain of *E. coli M17* distributed from Moscow Control

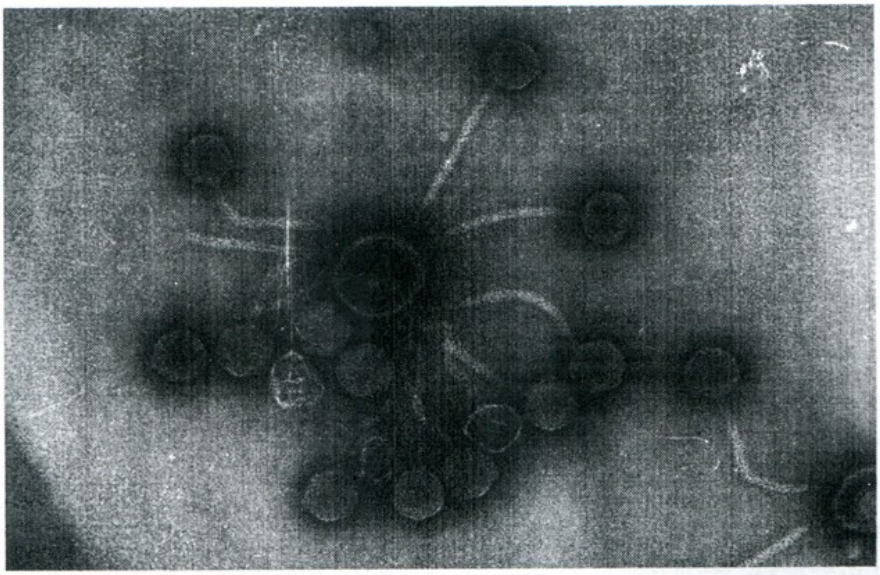

Fig.1. Phage *CB*, family Styloviridae, group I, type XI (SI-11) (16), magnification x 200 000, electron microscope JEOL EX 1200.

Institute to the bio-industrial plants all over the FSU has been lysogenized by *CB*-pro-phage. For approval of this assumption a series of additional experiments have been carried out that comprised induction of the pro-phage by application of physical and chemical agents (UV and Mitomicine C). Prior to application of these methods *E.coli M17* has been pretreated by specific CB-APS for neutralization of the free phage particles existing in inter-cellular areas. The UV-irradiated lysogenic cell produced twice as many phage particles than after MC-treatment of the same host. The phage clone obtained due to UV induction was entitled as *UV-CB,* morphologically and serologically it was completely identical to the rest of *CB* phages. The bacterial strain cured by consequent application of UV irradiation and APS treatment was named as *E. coli M17 UV-S.* The cured strain was unable to produce *CB* phages neither spontaneously nor due to UV induction. DNA heteroduplex analysis was performed between a) the initial host *E.coli M17* and *CB* phage and b) the cured derivative, particularly *E.coli M17 UV-S* and *CB* phage. DNA homology has been found in the case a) only. This fact indicates to lysogenic status of the initial host strain. We suppose that *CB* pro-phage may be still present even in the cured derivative, but in a defective (non-induced) status.

E.coli M17 UV-S strain was used for construction of the phage-resistant mutant of *E. coli M17 UV-S/CB* which was obtained through indirect selection method avoiding direct contact between the phage and its host. About 35 mutant bacterial cultures have been selected and thoroughly studied for their antagonistic activity and phage sensitivity. 12 cultures were selected for further studies. According to the results of the tests for growth efficiency and phage adsorption rate (Table 3) a mutant culture *E. coli M17/CB-7* has been selected.

Table 3

Comparative studies of the mutant and wild E.coli M17 strains according to their sensitivity towards phages

E.coli M17 Strains	PHAGES							
	CB-M		CB-T		CB--P		CB- M17 F	
	Adsorption %	Growth efficiency	Adsorption %	Growth efficiency	Adsorption %	Growth efficiency	Adsorption %	Growth efficiency
M17/CB 1	0	0	0	0	0	0	0	4 tv
M17/CB 3	0	0	0	10^{-8}	0	0	0	9tv
M17/CB 4	0	0	0	0	0	0	0	0
M17/CB 5	0	0	0	0	0	0	0	4tv
M17/CB 7	0	0	0	0	0	0	0	0
M17/CB 10	12	10^{-7}	7	3×10^{-8}	0	0	0	3×10^{-8}
M17	78	1,1	75	1,0	80	1,5	82	20

Phage-resistance of the mutant is based on the lack of the specific cellular receptors necessary for phage adsorption.

During the first 24 hours of incubation in a mixed culture the mutant strain shows comparatively lower antagonistic activity against *Shigella* strains, which is explained by its relatively slow growth rate. This fact is considered as a normal event for the majority of all bacterial mutants (12,13). Nevertheless, the antagonistic activity of this strain meets all requirements necessary for probiotic *E. coli* (Table 4). Reversion rate (RR = 4 x 10^{-8} cell/generation) of the phage-resistance mutation to the initial phage-sensitivity is slightly slower than the mutation rate (MR = 1 x 10^{-8} cell/generation). The mutant strain *E. coli M17/ CB-7* was tested in laboratory conditions for spontaneous phage induction. Pilot studies including 6 independent experiments have been performed in the industrial plant as well. None of 6 experimental batches revealed the presence of induced and/or contaminating bacteriophages. Low rates of phage induction (10^{-7} - 10^{-9} pfu/cell) and reversion of bacterial mutant to phage-sensitivity (1 x 10^{-8} cell/ generation) do not facilitate reproduction of the *CB* phage. Low probability of these two events practically excludes their coincidence and thus phage multiplication during the industrial process.

Thus, it was concluded that: 1) Spontaneous emergence of phages and subsequent lysis of the *E. coli M17* during the process of its industrial growth is caused by the lysogenic status of the starter strain; 2) Pro-phage is induced by a temperature shift occurring at the certain industrial stage in particular during cooling of the bacterial bio-mass from 37°C to 28°C which was accomplished

Table 4

Comparison of antagonistic activities of the standard and phage-resistant mutant strains

Test strain	Antagonistic activity (%)			
	E. coli M17-M		E. coli M17 CB-7	
	After 24 hours	After 48 hours	After 24 hours	After 48 hours
Sh. flexneri 16	84.7	97,2	79.6	97.1
Sh. flexneri 1a 1218	80	98	85.5	96.5
Sh. flexneri 2a 194	77	97,2	86	97.6
Sh. flexneri 2a 285	42	48	88	93.1
Sh. flexneri 3a 42	89	100	73.1	99.6
Sh. flexneri 3c 21	91	99	68.4	92.6
Sh. flexneri 3c 27	62	99,6	66.5	93.8
Sh. flexneri 4a 5241	69	100	89.8	100
Sh. flexneri 4a 1957	93	95	68.8	95.1
Sh. flexneri 4a 1956	90	98	39.7	96.4
Sh. sonnei 193	n/d	100	n/d	100
Sh. sonnei 307	n/d	98	n/d	95.5
Sh. sonnei 548	n/d	86	n/d	70.5
Sh. sonnei 550	n/d	95	n/d	84.6
Sh. sonnei 551	n/d	82	n/d	92.5
Sh. sonnei 568	n/d	78.8	n/d	86.6

Note: n/d = not detected.

according to the technology regulations. It was recommended to eliminate the cooling stage from the protocol; 3) Gene deficiency of the pro-phage caused by UV irradiation prevents its spontaneous induction during industrial processing; 4) For protecting of the industrial strain from the aggression of the virulent mutant of the *CB* phage a phage-resistant mutant culture *E. coli M17/CB-7* has been constructed. The new mutant culture repeats the same biological features of its precursor including antagonistic activity against intestinal pathogens. Therefore it was recommended to use the mutant as a starter strain for production of preparations Colibacterin and Bificol (the last is composed of bacteria *E. coli M17* and *Bifidobacterium bifidum*).Comparison of antagonistic activities of the standard and phage-resistant mutant strains.

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პრობიოტიკური პრეპარატის კოლიბაქტერინის ტექნოლოგიური პროცესის გაუმჯობესება: ფაგისაგან თავისუფალი ახალი სასტარტო შტამის კონსტრუირება

ნ.ჭანიშვილი, მ.თედიაშვილი, თ.ელიაშვილი, ქ.ფორჩხიძე, გ.ნატროშვილი, ნ.ზვიადაძე, დ.გიორხელიძე, ლ.კვაჭაძე, რ.ადამია, თ.ჭანიშვილი

საქართველოს მეცნიერებათა აკადემიის გ.ელიავას სახელობის ბაქტერიოფაგიის, მიკრობიოლოგიისა და ვირუსოლოგიის ინსტიტუტი, თბილისი

რ ე ზ ი უ მ ე

პრობიოტიკური პრეპარატი კოლიბაქტერინი ყოფილ სსრკ-ში 30-იანი წლებიდან ფართოდ გამოიყენება (5, 15, 19). პრეპარატის საფუძველია ბაქტერიული შტამი *E.coli* M17, რომელიც განაპირობებს მის ანტაგონისტურ თვისებებს კუჭ-ნაწლავის უმნიშვნელოვანესი ინფექციების მიმართ. 80-იანი წლების ბოლოს კოლიბაქტერინის წარმოებაში დიდი სირთულეები აღინიშნა, რაც დაკავშირებული იყო უცნობი ბაქტერიოფაგის გამოჩენასთან და ამ უკანასკნელის მიერ საბოლოო პროდუქტის დიდი მოცულობით სპონტანურ ლიზისთან. ამ მოვლენის მიზეზების გასარკვევად მრავალი სასტარტო კულტურისა და საწარმოო პროცესის მსვლელობის სხვადასხვა ეტაპზე მოპოვებული პროდუქტის ნიმუშების დეტალური ანალიზი ჩატარდა. გამოყოფილ და შესწავლილ იქნა ბაქტერიოფაგების კლონების რიგი (სახელწოდებით CB1, CB2, CB3...). ამ ფაგების ძირითადი ტაქსონომიური თვისებების შედარებით მათ შორის სრული იდენტურობა გამოვლინდა. დამტკიცდა *E.coli* M17 ლიზოგენური სტატუსი. აღმოჩნდა, რომ მასში არსებული ზომიერი *CB* ბაქტერიოფაგის ინდუქცია ტემპერატურის 37^0C-დან 28^0C-მდე დაწევით არის გამოწვეული. დადგინდა, რომ 280ჩ ოპტიმალურია *CB* ზომიერი ფაგის ვირულენტული მუტანტების სელექციისა და გამრავლებისათვის. ეს მონაცემები ხსნის საბოლოო პროდუქტის სპონტანური ლიზისის მიზეზებს. გენეტიკური მეთოდების გამოყენებით *E.coli M17/CB-7* სტაბილური ფაგო-რეზისტენტული მუტანტური შტამი იქნა კონსტრუირებული. ახალი შტამი, გაუნჯობესებული თვისებებით, დღეს წარმატებით გამოიყენება კოლიბაქტერინის წარმოებისათვის საქართველოში არსებული რამოდენიმე საწარმოს მიერ.

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ESTIMATION OF THE PHYSIOLOGIC CONDITIONS OF CERTAIN PARAMETERS OF THE DOPHAMINERGIC SYNAPTIC APPARATUS BY PLASMA LEVELS OF DOPAMINE AND HOMOVANILLIC ACID IN PARKINSONIAN PATIENTS

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Purpose of the present work was determining criteria for estimation of a physiologic condition of some parameters of the synaptic apparatus of dophaminergic system. Group of Parkinsonians, consisting of 92 patients was surveyed. Based on received data we elaborated the recommendations for setting up of the optimum plan of treatment for patients suffered from different forms of Parkinsonism.

Key words: *Parkinsonism, Dopamine reuptake, Receptor's threshold, Dopamine, Homovanillic acid.*

The Parkinsonism is one of the most widespread diseases of central nervous system. The average frequency of an incidence reaches from 1,0 up to 5,0 % of the population [5,], depending on age, that again confirms the high importance of medico-social aspect of this problem. Thus, the question of treatment of Parkinsonism represents the important problem both practical, and theoretical medicine.

The patients usually require contiguous and long treatment, the duration of which depends on many circumstances, but as a rule covers the rest of a life. The duly and adequate therapy enables not only break clinical manifestation of disease, but to protect ill from possible mental disturbances [6].

Despite plenty of research, devoted a problem of treatment of a Parkinsonism, the questions of efficiency of therapy remain a topical problem of a modern theoretical and practical neurology [4]. Above mentioned is supported also by that an arsenal of medications, used for treatment of a Parkinsonism, all time extends. The latter in many respects complicates the practical doctor in a choice of optimum tactics at realization mono- or polytherapy of a Parkinsonism. As is known, in treatment of Parkinsonism different combinations of L-dopa with medicines that are reducing and/or decreasing a threshold of postsinaptic receptors of the striatal dophaminergic neurons are used.

Hence, we were set by the purpose on the basis of the analysis of morning and

evening dynamics of patient's dopamine (DA) and homovanillic acid (HVA) blood levels to develop criteria for determination of physiologic condition of certain components of dophaminergic synaptic apparatus of Parkinsonian's.

MATERIALS AND METHODS

We spent research of plasma levels of dopamine (DA) and homovanillic acid (HVA) at 92 patients with Parkinsonism of different etiology.

Based on the analysis of received data, we divided patients on four groups:

In the first group have come 8 patients, from them in three cases there was rigid form and in five - tremor form of parkinsonism (division of patients under the clinical forms a little bit conditionally. In account took prevalence that or other symptom of disease).

In the second group have come 26 patients. From them, six suffered from trembling form, 10 from rigid and 10 from hypokinetic forms of Parkinsonism.

In third group have come 22 patients, from them, five with rigid form, 13 with tremor and 4 with hipokynetic form of disease.

The fourth group consisted of 37 patients. On clinical parameters, the fourth group consisted of nine patients with expressed rigidity, 7 patients with tremor and 21 patients with hipokynetic form of disease.

The researches of plasma parameters DA and HVA were carried out by High Performance Liquid Chromatography with electrochemical detector [2] on an equipment of "Millipore".

Estimation of clinical parameters we have done by a method offered Papavasiliou [3].

RESULTS AND DISCUSSION

In this group morning and evening dynamics of DA and HVA blood levels are resulted on table N°1.

Table 1

	Age	Dopamine (ng/ml)		HVA (ng/ml)	
		Time of investigation		Time of investigation	
		$9\text{-}9^{30}$	$18\text{-}18^{30}$	$9\text{-}9^{30}$	$18\text{-}18^{30}$
average	62.63	115.63	76.25	20.75	16.13

As it shown on table N°1, in morning hour's DA blood levels were around of normal values and the same parameters of HVA were decreased and by an evening were reduced much more. The analysis of preceding data shows, that consumption of DA was insufficient and, hence, it is possible to assume that in these cases we have an increased reuptake of DA.

Thus, in this case optimum treatment would be a combination L-dopa with preparations inhibiting DA reuptake [1].

In this group the DA level was lowered in both morning and evening hours, on the contrary - the HVA level was higher than norm (see table N°2).

Table 2

	Age	Dopamine (ng/ml)		HVA (ng/ml)	
		Time of investigation		Time of investigation	
		$9\text{-}9^{30}$	$18\text{-}18^{30}$	$9\text{-}9^{30}$	$18\text{-}18^{30}$
average	59.2	83.08	78.88	38.12	34.68

Received data shows that DA consumption preserved, but it is possible to suppose, that a threshold of dophaminergic receptors is raised. Hence, at such a coincidence of the above-described parameters it is desirable to carry out treatment by a combination L-dopa with DA agonists.

Third group from previous differs only by that DA levels both in morning and evening hours is in limits of norm, and HVA in the morning and in the evening is over the norm (see table N°3).

Table 3

	Age	Dopamine (ng/ml)		HVA (ng/ml)	
		Time of investigation		Time of investigation	
		$9\text{-}9^{30}$	$18\text{-}18^{30}$	$9\text{-}9^{30}$	$18\text{-}18^{30}$
average	69.11	111.84	113.37	47.26	46.84

In this case, it is possible to assume, that we had a significant increase of

dophaminergic receptors threshold. Hence was available to prescribe treatment by medications, which reduce a threshold of dophaminergic receptors.

Fourth group consisted of patients, which DA plasma parameters were very low (in the morning as well as in the evening hours), and the HVA levels very high (see table N°4).

Table 4

	Age	Dopamine (ng/ml)		HVA (ng/ml)	
		Time of investigation		Time of investigation	
		$9\text{-}9^{30}$	$18\text{-}18^{30}$	$9\text{-}9^{30}$	$18\text{-}18^{30}$
average	60.19	65.73	63.32	35.7	32.65

It is possible to assume that in this case we had a low DA level in central nervous system. Thus, in the fourth group optimum there could be the treatment by L-dopa.

Thus, the analysis of received data gives the basis on plasma parameters of a dopamine and homovanillic acid to judge physiologic condition of the dophaminergic synaptic apparatus, that in turn, makes possible in each separate case of disease to define (determine) optimum pathogenetic treatment.

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დოფამინერგული სინაფსური აპარატის გარკვეული პარამეტრების ფიზიოლოგიური მდგომარეობის შეფასება დოფამინისა და ჰომოვანილის მჟავის პლაზმური კონცენტრაციის მიხედვით პარკინსონიზმით დაავადებულთა შორის

შ.ჩხიკვიშვილი

ნევროლოგიისა და ფსიქიატრიის კათედრა, თბილისის სამედიცინო აკადემია

რეზიუმე

გამოკვლეული იყო პარკინსონიზმით დავადებული 92 ავადმყოფი, რომლებიც დოფამინისა (და) და ჰომოვანილის მჟავის (ჰვმ) პლაზმური პარამეტრების მიხედვით დავყავით 4 ჯგუფად.

და-ს და ჰვმ პლაზმური პარამეტრები ისაზღვრებოდა ილლიპორეის ქრომატოგრაფიული აპარატურით მაღალეფექტური სითხოვანი ქრომატოგრაფიის საშუალებით ამავე ფირმის ელექტროქიმიური დეტექტორის გამოყენებით.

კლინიკური გამოვლინებების შეფასება ხდებოდა აპავასილიოუს მიერ მოწოდებული მეთოდიკის საფუძველზე.

მიღებული მონაცემების ანალიზი საფუძველს გვაძლევს დოფამინისა და ჰვმ-ს პლაზმური პარამეტრების მიხედვით ვიმსჯელოთ დოფამინერგული სინაფსური აპარატის ფიზიოლოგიური მდგომარეობის შესახებ, რაც შესაძლებლობას იძლევა განისაზღვროს ყოველ ცალკეულ შემთხვევაში პათოგენეზური მკურნალობის ოპტიმალური ვარიანტი.

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THE INFLUENCE OF HIGH DOSES OF X-RAY IRRADIATION ON EXPERIMENTAL ANIMALS BONE MARROW AND PERIPHERAL BLOOD

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To study the influence of high doses, in particular of 3Gy, 4,5Gy, 6Gy and 8Gy X-ray irradiation on the bone marrow and peripheral blood investigation was carried out on 4 dogs. Morphological investigation of bone marrow and peripheral blood were conducted before and after irradiation on 2, 4, 5, 13 and 2, 4, 5, 6, 8, 10, 13 days, accordingly. In accordance with obtained data high dose irradiation was responsible for a deep myelokaryocytopenia, which especially was expressed after 24 hours from irradiation. At this time in all groups of experimental animals was revealed availability of multiple destructed cells, blast cells, gigantic myelo and metamyelocytes, pathological forms of mitosis and megaloblasts. In addition to significant increase of the number of rodnucleatic precursors, also plasmatic and reticular cells, was observed polysegmentation of neutrophils. After 72 hours above mentioned changes had a deepening tendency. Regardless of received doses, in all cases was mentioned a deepening leukopenia. It's noteworthy, that on 13 days from irradiation of 3Gy doses in bone marrow mononucleatic cells, was mentioned an appearance of addition micronucleuses, which presented the lethal structures. Obtained results indicate, that after high dose irradiation, myelodepression is extended in the bone marrow of experimental animals and relatively low doses of irradiation were responsible for alteration of bone marrow hypoplastic picture into myelodisplastic, on background of which development of pathological condition is possible in blood system.

Key wards: *Myelogramm, Influence of high doses of irradiation, Myelokaryocytopenia, Leukopenia, Dogs, Megaloblasts*

In recent years particular attention is paid on the influence of ionizing irradiation on organism. It is known, that hemopoietic system is one of the sensitive system to radiation. Early diagnostic and prognostic parameters are required in order to determine the severity of the damage and to make correct decisions regarding strategies for treatment. Nowadays, one of the such reliable parameter is leukocytes concentration in blood [2, 4]. It is known that, whole body irradiation leads to a deep leukopenia [1, 2, 4, 7, 12, 13].

Lymphocytes count during the first week after acute irradiation is significantly decreased [7] and as for leukocytes, having polymorphous compound couldn't exactly reflect the severity of the bone marrow injury, therefore the most short life span peripheral blood forms - the neutrophils and thrombocytes are used for this purpose [2, 10, 12], count of which after the irradiation influence is significantly decreased [1, 2, 3, 4, 7, 10]. The purpose of this study was to ascertain the regularity of the influence of high dose irradiation on hemopoetic organs (bone marrow) and peripheral blood. Investigations were carried out on dogs, which presented the convenient model for experimental study.

MATERIALS AND METHODS

Experiment was carried out on four dogs. Irradiation with high doses of X-rays, in particular with 3Gy, 4,5Gy, 6Gy and 8Gy was performed by means of equipment "Run - 17" at room temperature during 20, 35, 60 - 60 minutes accordingly. Voltage was 250 Kv, current power - 15 mA. Peripheral blood was taken before and after irradiation on 2, 4, 5, 6, 8, 10, 13, 18 and 26 days. Bone marrow taken from femur by means of "Kasirsky's needle" was investigated before and after irradiation exposure on 2, 4, 5 and 13 days. In peripheral blood the consistence of hemoglobin was determined and leukocytes, erythrocytes, thrombocytes were counted. Smears of peripheral blood and bone marrow were fixed with methanol and stained with "Giemza - Romanovsky stain" Leukocyte formula and myelogramm was counted. Investigations were carried out on light microscope. Graphs of neutrophil and lymphocyte amount were made up to prognosticate the severity of bone marrow syndrome after high dose irradiation exposure.

RESULTS AND DISCUSSION

Bone marrow morphological investigation has been shown that after 24 hours from dog's 3Gy irradiation a 5fold decrease of myelokaryocyte number was observed at the expense of myeloid branch. Myelosuppression mainly caused by erythroid element number significant decline (3fold) was responsible for erythrocyte amount decrease in peripheral blood. Furthermore, in red branch many destructed cells and appearance of megaloblasts were mentioned. In addition to, approximately a 2fold increase of neutrophilic myelocyte, rod and segmentnucleatic neutrophil amount, was observed. Regardless of a 4fold decline of neutrophilic myelocytes, in peripheral blood was mentioned neutrophiloses. At this time, large myelo-and metamyelocytes and polysegment neutrophils were revealed. In bone marrow Number of reticulocytes was raised to 3%, which is in accordance with data available in literature [1]. After 24 hours from irradiation in peripheral blood was observed lymphopenia and a 3fold decrease of eosynophil

and monocyte count.

After 72 hours in bone marrow a further 2fold decline of myelokaryocyte number was mentioned. In addition to, many destructed cells, naked nucleuses and gigantic neutrophil precursors were noticed. The total number of neutrophil precursors were dropped in 2fold at the expense of neutrophilic pro- and metamyelocytes which caused a decrease of number of circulating neutrophils in peripheral blood (Fig 1). In bone marrow at this time amount of eosynophilic and lymphoid precursors was decreased in 2-fold, however in peripheral blood their number was raised in 3- and 2-fold accordingly. As compared with this, a 3fold increase of monocytic precursors amount has been mentioned in consequence of which the number of circulating mature monocytes was raised. It's noteworthy that content of retyculocytes and plasmatic cells in bone marrow were remained high. After 72 hours of irradiation a significant changes were observed in red blood branch, in particular a 2-fold decrease of a number of megaloblastic forms and a 3-fold increase of amount of normoblasts. It must be emphasized, that at this time a tendency of increasing of pathological mitosis was also mentioned.

The total body irradiation after 96 hours have been induced a deep leukopenia - leukocyte count was constituted 3,4x10 ^{9}/, which is in agreement with the data reported in literature. [1, 2, 3, 4, 7, 10]. As compared to this, the number of erythrocytes was undergone an insignificant decrease.

By day 13 in bone marrow a deep changes have been observed in particular a tendency of further decrease of myelokaryocyte quantity was continued, blast forms up to 3% were revealed and the total amount of neutrophil precursors was againly increased at the expense of meylo- and metamyelocytes. The number of eosynophil precursors were undergone insignificant changes. By

contrast to this a 1.3 fold increase of lymphoid cell amount has been mentioned., which was responsible for a 1.6-fold increase of this mature forms in peripheral blood (Fig 2). By day 13 megablastic forms of erythroid lineage, also plasmatic cells and reticulocytes were disappeared. It's note worthy, that quantity of red blood branch cells has been reduced in 1.7-fold, consequence of which erythrocyte and hemoglobin content was decreased in peripheral blood.

A particular attention must be paid on an appearance of additional micronucleuses in lymphoid and blast cells on the 13th day after 3Gy dose total body irradiation. In recent years availability of such micronucleuses in lymphocytes were revealed as an indicator of radiation injury, frequency of which is depended on exposure dose in linear way. Nowadays, it was ascertained that above mentioned structures obviously, presented the lethal structures [5, 6, 8, 9, 11].

In the bone marrow of dogs exposed to 4.5Gy irradiation after 24 hours a deep myelosuppression have been mentioned. Cytological investigation of bone marrow has been showed: polysegmentation of neutrophil precursors availability of gigantic myelo- and metamyelocytes and insignficant decrease of the total quantity of neutrophil precursors, at the expense of a 5,3fold decrease of neutrophil myelocytes. Nevertheless 2.7-fold, 1.3-fold increase of rod nucleatic neutrophil

and eosynophil quantity respectively, also a 1.3-fold decrease of an amount of segmentnucleatic neutrophils were run parrallel with above mentioned changes (Fig 1). In regard to lymphoid precursors a 1.8-fold decline of their amount has been observed consequently of which quantity of lymphocytes in peripheral blood was reduced accordingly (Fig. 2). After 24 hours from irradiation, in bone marrow of experimental animals has mentioned a 2.5, 3.7- and 1.8fold increase of an amount of monocytic, reticular and plasmatic cells, respectively. At this time in bone marrow have been appeared megaloblastic forms, number of which was constituted 3%, also a great deal of destructed cells and pathological mitosis. In respect to red blood branch, their cells were undergone insignificant changes, in particular, their number was reduced at the expense of polychromatophilic normoblasts, resulted to slight number decrease of erythrocytes and hemoglobin content. Conversely, a deep leukopenia was observed in blood, quantity of white blood branch cells was declined from $14x10^9/l$, to $3,2x10^9/l$, which according to our point of view is caused by leukopoiesis inhibition.

After 72 hours from irradiation in bone marrow 4.8-fold raising of amount of megalo-macroblasts in red blood branch, also a lot of destructed cells, gigantic neutrophil cells and polysegmentation was revealed. Regardless of a high content of rodnuclear and segmentnuclear cells, neutrophil precursors total number was decreased in 1.2-fold at the expense of myelo- and metamyelocytes, which is in accordance with the data reported in literature [1, 2, 3, 4, 12]. A 1.8- and 1.7-fold decrease of eosynophilic and monocytic precursor and conversely, 1.2-fold increase of reticular cell amount was observed. No significant changes were mentioned with respect to plasmatic branch cells.

After 72 hours in bone marrow araised number of pathological mitosis of, as white, as well as red blood branch cells were revealed. In peripheral blood of experimental animals a deep lymphopenia was observed. In erythoid lineage normoblast amount was increased slightly.

By day 10 from irradiation a deep leukopenia ($3.0x10^9/l$) was maintained in peripheral blood. By day 18 a slight decrease of neutrophils, 4-fold decline of eosynophils and 1.5-fold increase of lymphocytes was mentioned (Fig 1, 2). By day 26 after irradiation a tendency of general increase of leukocyte total count was mentioned at the expense of neutrophils, eosynophils and monocytes (1.2, 3, and 1.5-fold raising respectively). Conversely, erythrocyte number was dropped to $2,7x10^9/l$.

In the bone marrow of experimental animals exposed to 6Gy irradiation, after 24 hours was mentioned myelosupression, polysegmentation of neutrophils, gigantic myelo- and metamyelocytes, 1.1-fold increase of neutrophil precursors at the expense of rod- and segmentnucleatic neutrophilic cells, resulted to raised neutrophil count in peripheral blood. Furthermore, number of myelo- and metamyelocytes was decreased in 6.3-fold and 3.3-fold accordingly. In regard to eosynophil and lymphoid precursors, their amount was decreased in 2.4-fold and 2-fold respectively. At this time monocytic, reticular and plasmatic cells were

revealed in high content (3.5%; 1.5% and 0.8% accordingly).

After 24 hours from irradiation of 6Gy in dog's bone marrow was mentioned appearance of megaloblastic forms (2%), 2fold decrease of the number of erythroid precursors and pathological mitosis of white (0.5%) as well as red (0.5%) blood.

After 72 hours from irradiation a 1.3-fold decline of total amount of neutrophils were observed at the expense of metamyelocytes and segmentnucleatic neutrophils (Fig 1). In respect of eosynophil precursors, they were undergone insignificant changes. Nevertheless, a 1.5-fold decrease of monocytic precursors and 1.6-fold raising of reticular cell number was mentioned. Increase of plasmatic and erythroid cell amount, appearance of pathological mitosis and megaloblastic cells were revealed for this time.

By day 8 in peripheral blood was observed anemia and a deep leukopenia ($3.0x10^9/l$), at the expense of, decrease of neutrophil, eosynophil and monocyte counts regardless of a 4-fold increase of lymphocyte number (Fig 1, 2).

Irradiation doses of 8Gy in experimental animals bone marrow has been caused a deep myelokaryocytopenia. After 24 hours from irradiation was mentioned neutrophil polysegmentation, appearance of gigantic myelo- and metamyelocytes, 2-fold increase of the number of rodnucleatic neutrophils and 2-fold decrease of eusynophilic cells. In regard to monocytic and lymphoid precursors also plasmatic, reticular cells, their amount was increased in 3-fold, 2-fold and 2% and 3% accordingly. At this time was revealed pathological mitosis of as white as well as red blood, and appearance of megaloblastic cells on a large amount. Furthermore, in the bone marrow a 1.4-fold decline of erythroid cells were mentioned. In peripheral blood leukocyte count was dropped to $9x10^9/l$

After 72 hours from irradiation deepening of myelokaryocytopenia was observed. Single blast cells were revealed in bone marrow. Polysegmentation of neutrophils and decline of their total amount at the expense of myelocytes were observed (Fig 1). However, content of rod- and segmentnucleatic cells, also reticular and plasmatic cells remained high. A 1.3-fold and 1.6-fold decrease of the number of lymphoid and monocytic precursors and conversely, 1.6-fold increase of erythroid normoblast amount was noticed. At this time many destructed cells were available in bone marrow.

By day 6 deepening of leukopenia was mentioned and leukocyte count constituted to $3.0x10^9/l$. In peripheral blood lymphopenia was run parallel with the increase of neutrophil amount (Fig 1, 2).

To conclude this paper, it must be emphasized, that in accordance with obtained data high dose irradiation was responsible for a deep myelokaryocytopenia, which especially was expressed after 24 hours from irradiation with X-rays. At this time in all groups of experimental animals were revealed availability of multiple destructed cells, blast cells, gigantic myelo- and metamyelocytes, pathological forms of mitosis, megaloblasts.

In addition to significant increase of the number of rodnucleatic neutrophil precursors, also plasmatic and reticular cells, was observed polysegmentation of neutrophils.

Fig. 1

The influence of high doses of X-ray irradiation (3 Gy, 4,5 Gy, 6 Gy and 8 Gy) on number of neutrophils x10⁹/l in peripheral blood

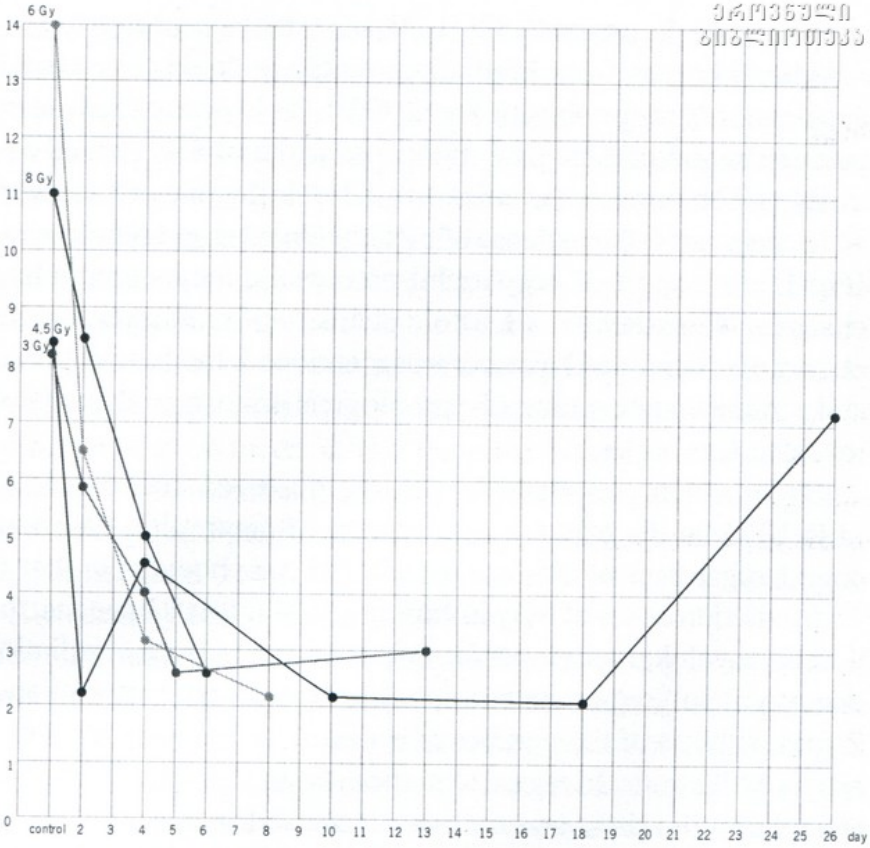
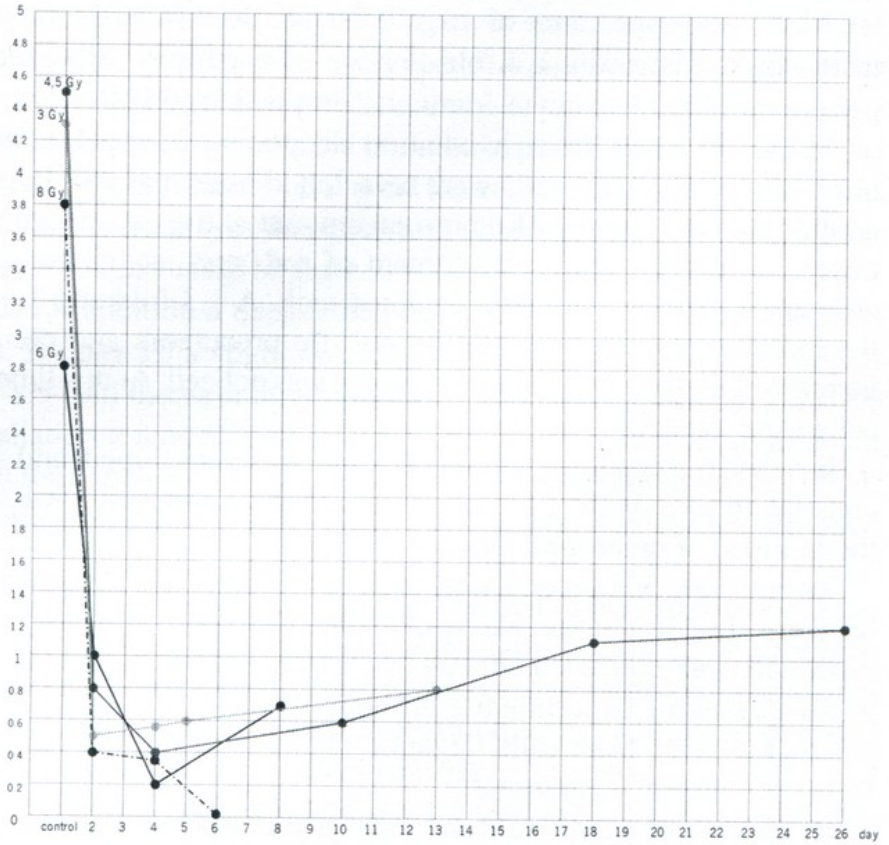


Fig. 2

The influence of high doses of X-ray irradiation (3 Gy, 4,5 Gy, 6 Gy and 8 Gy) on number of lymphocytes x10⁹/l in peripheral blood



After 72 hours from irradiation above mentioned changes were extended.

It's noteworthy, that regardless of the influence of different doses of irradiation on neutrophil precursors, in all cases was mentioned leukopenia, that is in accordance with data available in literature [1, 2, 4, 7, 12, 13].

After high doses of X-ray irradiation, the exposure of anemia in peripheral blood was mentioned somewhat later.

Especially attention from obtained results, must be paid on addition micronucleuses, available in bone marrow mononucleatic cells on 13 days from irradiation of 3Gy doses. These structures are presented the lethal structures in their nature.

To sum up our data, it must be mentioned that after high doses of irradiation myelodepression is extended in the bone marrow of experimental animals and the influence of relatively doses of irradiation the hypoplastic picture of the bone marrow is altered into myelodisplastic, on background of which development of pathological condition is possible in the blood system.

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რენტგენის სხივების მაღალი დოზის გავლენა ექსპერიმენტული ცხოველების ძვლის ტვინსა და პერიფერიულ სისხლზე

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ჰემატოლოგიისა და ტრანსფუზიოლოგიის ინსტიტუტი, თბილისი

რ ე ზ ი უ მ ე

ძაღლებზე ჩატარებული ექსპერიმენტებით, სხვადასხვა დოზის რენტგენული დასხივების პირობებში, შესწავლილია ძვლის ტვინი და პერიფერიული სისხლი. ძვლის ტვინისა და პერიფერიული სისხლის მორფოლოგიური გამოკვლევა ხდებოდა დასხივებამდე და დასხივების შემდეგ. მიღებული შედეგებით დადასტურდა, რომ მაღალი დოზით ირადიაცია იწვევდა ღრმა მიელოკარიოციტოპენიას, რომელიც განსაკუთრებით კარგად იყო გამოხატული დასხივებიდან 24 საათის შემდეგ. ამ დროისთვის, ცხოველთა ყველა ჯგუფში შეინიშნებოდა მრავლობითად დაზიანებული უჯრედები, ბლასტური უჯრედები, გიგანტური მიელო- და მეტა-მიელოციტები, მიტოზის პათოლოგიური ფორმები და მეგალობლასტები. მიღებული დოზის მიუხედავად, ყველა შემთხვევაში შეიმჩნეოდა ღრმა ლეიკოპენია.

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EMERGENCE OF DRUG DEPENDANT *M.TUBERCULOSIS* STRAINS AMONG PATIENTS WITH PULMONARY TUBERCULOSIS

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The object of our consideration is resistance to drugs of cultures isolated from the patients suffering from pulmonary tuberculosis. On the basis of material existing from 1998 to 1999, total of 415 strains isolated from the patients with pulmonary form were examined for resistance. 126 of them appeared to be multu-drug resistant, i.e. 30,4%. From the chronically ill patients with fibro-cavernous tuberculosis, M.tuberculosis were isolated which grew only in media containing antituberculosis drugs and do not grow in clean special nutrition medium without addition of tuberculosis drugs. To date, such isolates were detected in 9 among 137 patients wirh fibrous-cavernous TB. Thus not only drug resistant, but also drug dependent strains are available.

Key words: *Mycobacterium tuberculosis, Multi-drug resistance, Dependent strain.*

Tuberculosis in the world represents a growing public health problem [1]. One of the alarming factors of a recent increase in the incidence of Tuberculosis in certain parts of the world is the outbreak of multi-resistant forms of this disease. It should be noted that infection of Tuberculosis spreads readily and progresses rapidly [2, 4, 5].

The success of any antibiotic treatment regiment is largely dependent upon the susceptibility of target organism to the medicines used, TB not being an exception [3].

The recent economical and political upheavals had deteriorated TB infrastructure; because of shortages of drugs and extreme economic hardship even routine treatment ceased. Meanwhile, autotherapy and monotherapy were given a free hand. This promoted development of secondary tolerance to drugs and because of inefficient treatment and abundant sputum positivity, non-promising trends in prospects of TB control has been instated in Georgia: cases of advanced forms of sputum-positive tuberculosis and meningitis in children increased.

MATERIAL AND METHODS

In this condition the picture of drug resistance of M. tuberculosis in Georgia is of course worth attention. For comparison, following are data for 369 patients with AFB+ sputum, studied in 1987 in microbiological laboratory at the Institute of Pthisiology and Pulmonology, and, on the other hand, similar contingents in the years 1998 and 1999 up to this day. However, these groups were not statistically representative of the country, as our Institute gathers difficult patients and selection was therefore not random.

In 1987, of 369 studied cultures, 243 (65,8%) were new cases, and 126 (34,2 %) were retreatment cases. Of total 369 cultures, 65 (17,5%) proved to be MDR, 177 (47,5%) demonstrated other resistance, and 127 (35%)-susceptible to TB chemotherapy. As to 1998-1999, out of 415 cultures studied to date, 199 (48%) are new cases and 216 (52%)- retreatment. Resistance distributes in the following pattern: 150 (36,1%) sensitive, 126 (30,4%) - MDR, 139 (33,5%)- other resistance.

The structure of the resistance in those years was studied and yielded for 1987 of 243 new cases 122 (44,7%) cases were sensitive, 7 (2,6%) MDR, and 114 (52,7%) other resistant cases, while in 1998-1999, 199 new cases split into 116 (58,3%) sensitive, 16 (8%) MDR, and 67 (33,7%) - other resistant cases.

From 121 resistant new cases in 1987, resistance structure was composed of 78 (64,5%) cases resistant to one drug, 39 (32,1%)- resistant to two, 2 (1,7%)- to three, and 2 (1,7%)- resistant to four or more drugs. Meanwhile, in 1998-1999, 83 new resistant cases were comprised by 48 (57,8%) cases resistant to one, 16 (19,3%) –to two, 7 (8,4%)- to three, and 12 (14,5%)- to four or more drugs.

In 1987, frequency of resistance to specific drugs among 243 new cases was: Streptomycin-82 (33,7%), Isoniazid – 48 (19,8 %), Rifampicin – 16 (6,6%), Ethambutol – 0, Prothionamide- 3 (1,2%), Kanamicin – 9 (3,7%).The same frequencies for 199 new cases in 1998-1999 were Strptomycin – 49 (24,6%), Isoniazid – 34 (17%), Rifampicin – 27 (13,6%), Ethambutol – 4 (2%), Prothionamide- 12 (6%), Kanamicin – 11 (5,5%).

Reviewing of the structure of resistance among retreatment cases gives the following results:

Of 126 retreatment cases in 1987, 31 (24,6%) were multi-drug resistant, 86 (68,3%) cases showed other resistance, and 9 (7,1%) cases were sensitive. In 1998-1999, 216 retreatment cases, 34 (15,7%) are sensitive, 110 (50,9%)- MDR, and 72 (33,4%)- other resistant cases.Distribution of resistance drug-wise in 1987 was following: 117 resistant retreatment cases were structured into 9 (7,7%) cases resistant to one, 45 (38,5%)- to two, 30 (25,6%)- to three, and 33 (28,2%)-to four and more drugs. Among 182 retreatment resistant cases in 1998-1999, 23 (12,6%) were resistant to one, 37 (20, 3%)-to two, 44 (24,2) –to three, and 78 (42,9%)- to four and more TB drugs.

From 126 retreatment cases of 1987, 106 cases (83,9%) were resistant to Streptomycin, 104 (82,5%) –to Isoniazid, 47 (37,3%)-to Rifampicin, 8 (6,3%)- to Ethambutol, 21 (16,7%)-to Prothionamide, and 31 (24,6%)-to Kanamicin. To compare, 216 retreatment cases of 1998-1999, showed the following data : Strptomycin–142 (65,8%), Isoniazid–159 (73,6%), Rifampicin–117 (54,2%), Ethambutol–56 (25,9%), Prothionamide–47 (21,8%), and Kanamicin–70 cases (32,4%).

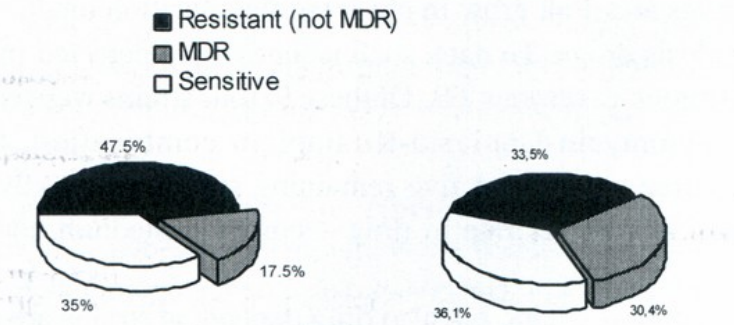
If susceptibility testing demonstrates resistance to two or more drugs (first line drugs are implied), this may be caused by the following reasons: either the true double, triple, or quadruple resistance is a feature of the strain, i.e., every bacillus is resistant to two, three, or four drugs simultaneously; or this is a false resistance, when, culture is constituted of different substrains, of which one is resistant to one drug, e.g., Streptomycin, and others - to other drugs (e.g., Isoniazid, Ethambutol, or Rifampicin). A truly double-, triple-, or quadruple- resistant culture will grow when seeded on the media containing combinations of drugs, while culture with false resistance will not proliferate on such media.

Our data on the true double-, triple-, or quadruple-resistant strain follow:

In 1987, the following data were obtained from 241 resistant cultures – true double resistance: 54 (22,3%) were resistant to Streptomycin + Isoniazid; 19 (7,8%) – to Isoniazid + Rifampicin.

In 1998-1999, 265 resistant cultures, 122 (46%) were resistant to Streptomycin + Isoniazid; 105 (39,6%) – to Isoniazid +Rifampicin; 59 (22,3%) – Streptomycin + Isoniazid + Rifampicin; 20 (7,6%) – Streptomycin + Isoniazid + Rifampicin + Ethambutol.

Drug-Resistant TB in Georgia
All Cases

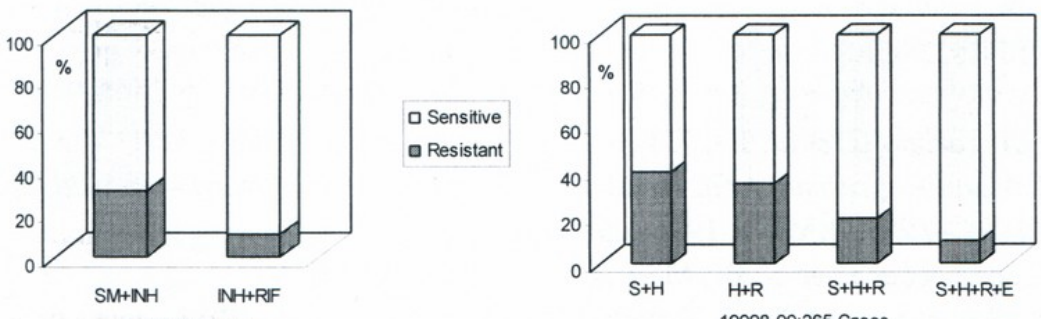


1987: 369 Cases

1998-99: 415 Cases

Source: Microbiological laboratory of the TB Institute

Drug–Resistant TB in Georgia
All Cases



1987:241 Cases

19998-99:265 Cases

Sourse:Microbiological Laboratori of the TB Institute

Review of the Results:By means of juxtaposition of old and recent evidence one can observe the following tendencies: the proportion of MDR strains increased from 17,5% in 1987 to 30,4% in 1998 - 1999; There are increases in M. Tuberculosis strains growing in Lowenstein-Jensen media containing double, triple and quadruple combinations of the first-line drugs, i.e. true resistance emerged. By resistance frequency, Isoniazid surpassed Streptomycin over the recent years, while Rifampicin resistance sharply increased and moved to the third place. Rise in resistance to drugs resulted in a reduction of treatment efficiency.

Based on the provided data, current difficulties in TB chemotherapy become obvious. Highly active tuberculostatic drugs of the first line are counteracted by high bacterial resistance rate, while the second line tuberculostatics are less active, excessively toxic, and expensive. This necessitates the involvement of yet more expensive general stimulants and pathogenetically active drugs and treatments along with highly expensive surgical treatment.

Under such circumstances the phenomenon observed by us certainly deserves much attention: from the chronically ill patients with fibro-cavernous tuberculosis, M.Tuberculosis were isolated which grew only in media containing antituberculosis drugs and dont grow in clean special nutrition medium without addition of tuberculosis drugs. To date, such isolates were detected in 9 among 137 patients with fibrous-cavernous TB. Of these 9, four strains were completely dependent on Streptomycin-Isoniazid-Rifampicin combination, failing to proloferate in drug-free media, and five remaining strains were only partially dependent, growing at a higher rate in drug – enriched medium compared to control medium.

Thus not only drug resistant, but also drug dependent strains are available. They were formed as a result of action of drugs and represent qualitatively a new stage of microbial variation.

Hence, answers should be provided to the following questions: what is the condition promoting their genesis; what is their occurrence; what is their stability, virulence, fermentative activity, the mechanism of their genesis; what is their clinical, epidemiological importance and how their genesis can be prevented.

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ტუბერკულოზის მიკობაქტერიის წამლებისადმი დამოკიდებული შტამების გამოვლენა ფილტვის ფორმით დაავადებულთა შორის

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საქართველოს ჯანდაცვის სამინისტროს ფთიზიატრიისა და პულმონოლოგიის სამეცნიერო-კვლევითი ინსტიტუტი, თბილისი

რ ე ზ ი უ მ ე

ტუბერკულოზის მიკობაქტერიის წამლებისადმი რეზისტენტობის პირდაპირი მეთოდით შესწავლისას ჩვენს მიერ იქნა დაფიქსირებული შემდეგი მოვლენა: სტრეპტომიცინის, იზონიაზიდის, რიფამპიცინის, ეტამბუტოლისა და მათი კომბინაციების შემცველ სინჯარებში მიღებული იყო ტუბერკულოზის მიკობაქტერიის საკმაოდ უხვი ზრდა, მაშინ როდესაც სუფთა ლევენშტეინ-იენსენის ნიადაგის შემცველ კონტროლის სინჯარებში ზრდა არ აღინიშნებოდა. ექსპერიმენტის ვადის გაგრძელებამ შედეგი არ მოგვცა. საქმე ეხებოდა ავადმყოფს ფიბრო-კავერნოზული ტუბერკულოზის ქრონიკული ფორმით, რომელიც ნამკურნალევი იყო ტუბსაწინააღმდეგო ქიმიოპრეპარატებით მრავალჯერადად.

დღეისთვის არსებობს ცხრა შემთხვევა გამოვლენილი 137 ანალოგიური კონტინგენტიდან. ოთხ მათგანში აღინიშნება მიკრობის სრული დამოკიდებულება ქიმიოპრეპარატებისადმი, ხოლო დანარჩენ ხუთ შემთხვევაში ნაწილობრივი - როდესაც პრეპარატების შემცველ ნიადაგში შეინიშნება საკმაოდ უხვი ზრდა კონტროლთან შედარებით.

ამრიგად, სახეზეა არა მხოლოდ წამლებისადმი გამძლე, არამედ წამლებზე დამოკიდებული შტამები, რომელიც წარმოიქმნა ქიმიოპრეპარატების მოქმედების შედეგად და წარმოადგენს მიკრობის ცვალებადობის თვისობრივად ახალ ეტაპს.

ეჭვს არ იწვევს აღნიშნული შტამების ყოველმხრივი შესწავლის საჭიროება. პასუხი უნდა გაეცეს შემდეგ კითხვებს: რა გარემოებები უწყობს ხელს მის წარმოშობას, რა სიხშირით გვხვდება იგი, როგორია მისი მდგრადობა, ვირულენტობა, ფერმენტული აქტიურობა, წარმოშობის მექანიზმი, როგორია მისი კლინიკური, ეპიდემიოლოგიური მნიშვნელობა და როგორ შეიძლება თავიდან ავიცილოთ მისი წარმოშობა.

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STUDY OF ANTITUBERCULOSIS ACTIVITY OF BEE HONEY

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In vitro experiments have shown that honey has a clear-cut tuberculostatic activity. In liquid nutrition medium this antibacterial activity of honey manifests itself at 1-16 dilutions. Furthermore, this activity is higher in the drug resistant culture. After 24-hr exposure of M.tuberculosis to natural honey, both laboratory and wild strains, lose ability of growth and development.

Key words: *Honey, Micobacteria tuberculosis, Antibacterial.*

Since the second half of this century by create and application of antituberculosis drugs a positive turning-point was reached in the cause of combat against tuberculosis. Unfortunately, shortly after this the undesirable affect has also become known. Application of every new drug was attended by microbe's development of resistance to it, mitigating its antituberculosis effect and eventually rendering it maleffective or of no good at all for the treatment. the phthisiotherapists were posed with a problem to overcome development of drug resistance by microbe and at the same time continue active search of new chemical drugs. In the next decades work was in full swing and successful in this respect and so was the fight against tuberculosis.

Since the nineties a wide spread of tuberculosis infection has dramatically altered the situation in the world. a grave picture has been fixed by leading phthisiotherapists as regards spread of the disease, its course, clinical forms and incidence of mortality [2-4].

Academician A. G. Khomenko is unequivocal in his statement that nowadays only chemiotherapy can not resolve the problem of treatment of tuberculosis, that apart from surgical intervention it is necessary what a variety of traditional and nontraditional means be used for the pathogenic treatment. these are: antiinflammatory, desensibilizing, immunomodulating, stimulating reparation processes, ect. [1].

In view of the foregoing, we have concentrated our attention to honey of natural origin. apart from the fact that honey is a very good food product, it is commonly employed in various fields of medicine for the treatment of gastric and duodenal ulcerations, hepatic and renal infections, upper respiratory and pul-

monary infections as well as for the treatment of heart and blood-forming organs, dermal and gynecological deseases, ophthalmonology, ect. [5].

It has been experimentally established that honey eliminates bactaria causing typhia, paratiphoid fever and disentery.

We determined to study the tuberculostatic activity of honey in order to judge by the results abaut the possibility of its use as an antituberculosis means.

MATERIALS AND METHODS

The present work is the first stage of in vitro experiment using dilution method. Dilution of honey was accomplished in half synthetic liquid nutrition medium offered by A.E. Shkolnikova for M. tuberculosis, to which 10% fresh human citrating plasma was added ex tempore.

Dilution from 1:2 to 1:512 was used. Four varieties of honey were the object of our study: 1-May honey (field flovers); 2-Linden; 3-locust; 4-Chestnut honey. Three strains of M.tubeculosis were used as test-microbes: 1. Human type standart /H37Rv/; 2. Human type wild, N520, sensityve to antituberculosis drugs of the first order; 3. Human type wild, N137,resistant to antituberculosis drugs of the first order.

From three-week cultures of the above to strains grown in levenshtein-Jensen medium 100-million suspension was introduced into the rows of test-tubes filled with predilute honey and additionally for the control, in order to evaluate the microbe growth, into the test-tubes with only liquid nutrition medium without honey.

The experiment was carried aut in centrifuge test-tubes in 2 ml volume. After they incubated in the thermostat at 37C for a fortnigth.

RESULTS AND DISCUSSION

Fter the lapse of incubation period from the precipitate of each tube smears were made wich was staned by method of Zill-Nilsen and finally studied microscopically. The intensity of microbe groth was estimated by the 4-score system.

The four variety of honey at 1:10 dilutions yield a full bacteriostatic affect on homan type M. tuberculosis /H37Rv/, while at 1:16 dilution there is a considarable imprairment of microbe growth.

In relation to wild strains (N137, N512) the bacteriostatic activity is still higher: honey of the 1st, 2nd and 3rd variety yield a full bacteriostatic effect at 1:16 dilution, only chestnut honey at the same dilution results in a considerable reductionof growth.

We have also used the exposure method for the detected the tuberkulostatic activity of honey. We took fluid honey of the same samples and 9.5 ml of them

were placed into 3 sterile tubes; respectively 0.5 ml suspension of test-microbe was poured into them; they were stirred up and after the exposure for a detonate time we took 0.5 ml of them, wich after being washed with sterile saline solution precipitated and centrifuged, were inoculated in 2 tubes containing special Levenshtein-Jensen nutrition medium. After they had been hermetically sealed, the cultures for the purpose of incubation were placed in the termostat at 37C. Together with them were placed the control cultures of the test-microbes prepared likewise the experimental ones only with no exposure to honey.

Periodically the cultures were surveyed to establish the growth time and intensity. We used a wide scope of exposure times: from 5 min. to 48 hr.

Our studies enable us to conclude that 24 hr contact of M. tuberculosis strains with natural honey makes them lose the ability of growth and development.

In summary, in vitro experiments, using dilution and exposure methods, it has been established that honey shows rather high tuberculostatic activity, the more so against its wild strains.

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თაფლის აქტივობის შესწავლა ტუბერკულოზის მიკობაქტერიის მიმართ

ლ. დანელიშვილი

საქართველოს ჯანდაცვის სამინისტროს ფთიზიატრიისა და პულმონოლოგიის სამეცნიერო-კვლევითი ინსტიტუტი, თბილისი

რ ე ზ ი უ მ ე

ტუბერკულოზის პრობლემა ერთერთი აქტუალური პრობლემაა თანამედროვე მედიცინაში. დღეს მხოლოდ ქიმიოთერაპია ვერ გადაწყვეტს ტუბერკულოზის მკურნალობის პრობლემას. ქირურგიული ოპერაციების გარდა აუცილებელია მრავალი მედიკამენტოზული და

არამედიკამენტოზური საშუალებების მოშველიება პათოგენეზური მკურნალობის ჩასატარებლად. ასეთებია: ანთების საწინააღმდეგო, მადესენსიბილიზებელი, იმუნომამოდულირებელი, რეპარაციული პროცესების მასტიმულირებელი და სხვ.

ჩვენ შევისწავლეთ თაფლის ტუბერკულოსტატიკური მოქმედება განზავებისა და ექსპოზიციის მეთოდებით, რათა მიღებული შედეგებით გვემსჯელა თაფლის ტუბერკულოზის საწინააღმდეგო მედიკამენტად გამოყენების შესაძლებლობაზე.

გამოკვლევებმა გვიჩვენეს, რომ თაფლის სხვადასხვა სახეობა 1:10 განზავებაში იძლევა სრულ ბაქტერიოსტატიკურ ეფექტს, ხოლო 1:16 განზავებაში მიკრობის ზრდის მნიშვნელოვან დაქვეითებას, ველური შტამების მიმართ ბაქტერიოსტატიკური აქტივობა უფრო მაღალია.

ექსპოზიციის მეთოდით დადგინდა, რომ ნატურალურ თაფლში 24-საათიანი კონტაქტი ტუბერკულოზის მიკობაქტერიებს, როგორც ლაბორატორიულ, ასევე ველურ შტამებს, უკარგავს ზრდა-განვითარების უნარს.

ამრიგად, ჩვენს მიერ ინ ვიტრო ჩატარებული ცდებით დადგინდა თაფლის საკმაოდ მაღალი ბაქტერიოსტატიკური აქტივობა ტუბერკულოზის მიკობაქტერიების და უფრო მეტად მისი ველური შტამების მიმართ.

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NON - SPECIFIC ADAPTATION RESPONSE (NAR) UNDER PHYSICAL LOADS OF DIFFERENT LENGTH AGAINST THE BACKGROUND OF RETICULAR FORMATION FUNCTIONAL STATE CHANGES

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On the background of functional alteration of the reticular formation the non-specific adaptive reactions were experimentally investigated in the rats, during physical work of various duration. It was found that suppression of the rostral adrenergic substrate in the reticular formation results in elevation of physical load-induced stress. It is suggested that above substrate plays an important role in adaptive abilities of the organism.

Key words: *Reticular formation, Physical loads, Adrenergic substrate, Adaptation, Rats*

The studies of the functional state of sportsmen are closely interrelated with a general biological problem of organism adaptation to physical activity factors. It is quite regular that success in great sport very much depends upon the organism optimal adaptation to stress factors such as extremal competitive and training loads.

In case of long and intensive physical loads, when organism brings adaptive mechanisms into action for the stress effect realisation, some significant changes of homeostasis take place and a special state of organism is developed, one of its features being the reduction of immuno-biological resistibility. Some findings may be given here in favour of the above assumption indicating a direct relation between mechanisms of the stress response development and those of immune system.

The closiest anatomic and physiologic relations between the central controller of homeo-stasis-hypothalamus and the brain stem non - specific activating system, i.e. reticular formation (RF) give good reasons to assume that reticular structures may be essential for the development of a stress response as well as for that of organism's immune - biological reactions.

Therefore, the evalution of NAR level under muscular activity against the background of modulation of RF functional state is of major theoretical and practical interest.

As far as it is known the lyimphocite to segmented neutrophile ratio (LSNR) may serve as one of the characteristics of NAR development. According to the conception suggested, NAR gradation is established within the following values: activation response (LSNR=0.51-0.69); trainy response (LSNR=0.34-0.50) and stress response (LSNR=0.33 and below).

Based upon this assumption the dependance of NAR level has been studied under conditions of the functional state activation of adrenergic substrate of rostral reticular formation (ASRF).

The studies have been performed on a group of 140 white male rats of mixed population weighing 184±14 g. Physical loads were given according to the methods being in common use (swimming in water, temperature 30 - 32 °C, load - 6% of the body mass).

To estimate dynamics of the parameter changes under study, the laboratory animals were divided into 9 groups. Physical load lasted 15 minutes in the first group, in the second group - 2 hours, in the third - 6 hours. In subsequent 6 groups physical load of the same duration was given to the rats with preliminary activation or depression of the functional state of ASRF through intramuscular injection of 0.04 mg/kg adrenaline or 2 mg/kg chlorpromasine. There were 3 control groups of animals: intact rats (n=8) and the rats in which the activation (n=12) and depression (n=12) of the ASRF was performed in the resting state (i.e. no physical load).

The comparison of findings of all the groups with control groups animal indices was done.

The reliability of the results was evaluated by Student's *t*-criterion.

The background value of LSNR coefficient appeard to be 0.51±0.12. The coefficient in question was 0,45±0,09 when adrenaline intramuscular injecting and - 0.50±0.04 when chlorpromasine administration at physiological rest. Such LSNR values are considered to be a favourable background for revealing a high level efficiency and they correspond to a training respouse zone [1].

A 15 min. physical load in unusual conditions caused the reduction of non-specific adaptation respouse level, and the value of LSNR after its complection was equal to 0.42±0.08 ($P<0.05$). Physical load of the similar lengh with preliminary activation of ASRF reduced the coefficient of LSNR as well and its value comprised 0.39±0.078 ($P<0.05$), but with depression of ASRF the value was 0.44±0.037 ($P<0.001$).

After completion of 2 hour physical load the reduction of LSNR coefficient was revealed in all series under study. In groups where physical loads were given to animals without preliminary injection, the level of NAR approached the lower limit of the training response zone. In series where 2 hour physical load was given with functional changes of ASRF, the value of LSNR coefficient was within the stress response zone. However, reliable data differences while comparing the groups where not revealed.

Excessive physical load in unusual conditions reduced NAR level and the value of LSNR coefficient after the excessive physical load completion was equal to 0.24±0.002 ($P<0.001$).

After the excessive physical load was completed in animals with preleminary

activation of ASRF, the value of LSNR coefficient comprised 0.22 ±0.03 ($P<0.001$; $P<0.05$). The excessive physical load under conditions of ASRF depression caused the reduction of LSNR coefficient up to the value 0.15±0.02 ($P<0.001$; $P<0.001$).

As far as it has been seen from Table 1, the value of LSNR coefficiet was reduced in all series of our studies irrespective of the length of physical load. This proves the hypothesis that physical loads in unusual conditions serve as a stress stimulus. The level of NAR organism is within the stress response zone after 2-hour physical load completion against the background of the ASRF functional state changes. The excessive physical load was defined to reduce significally the level of NAR in animals of all series. However, LSNR coefficient reduction has been evidently shown in the series where animals were subjected to physical load against the background of preliminary chlorpromasine injection.

Thus, the functional state depression of ASRF increases the stress effect of physical load upon organism. The conclusion has been made that ASRF is one of components, which controls the adaptation abilities of organism under condition of physical loads.

Table 1

LSNR and NAR of Rats After Excessive Physical Load in Condition of Functional State Changes (for abbreviation see text)

The groups of animal	LSNR (avarage values+S.E. of the mean)		
	NAR Response Zones		
	Activation	Training	Stress
Intact (n=8)	0.51±0.12	—	—
Intramuscular injection of adrenaline (n=12)	0	0.45±0.091	—
Intramuscular injection of chlorpromasine (n=12)	—	0.50±0.033	—
15-min physical load(n=12)	—	0.42±0.080	—
2-hour physical load (n=12)	—	0.35±0.060	—
6-hours physical load (n=12)	—	—	0.24±0.020
15-min physical load with adrenaline injection (n=12)	—	0.39±0.078	—
2-hour physical load with adrenaline injection (n=12)	—	—	0.33±0.057
6-hour physical load with adrenaline injection (n=12)	—	—	0.22±0.022
15-min physical load with i.m. chlorpromasine injection (n=12)	—	0.44±0.037	
2-hour physical load with i.m. chlorpromasine injection (n=12)	—	—	—
6-hour physical load with i.m. chlorpromasine injection (n=12)	—	—	0.31±0.034
			0.15±0.024

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ფიზიკური დატვირთვის გავლენა ორგანიზმის არასპეციფიკური ადაპტაციური რეაქციის დონეზე რეტიკულური ფორმაციის ფუნქციური მდგომარეობის მოდულირების პირობებში

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საქართველოს ფიზიკური კულტურის სამეცნიერო კვლევითი ინსტიტუტი, თბილისი

რ ე ზ ი უ მ ე

თეთრ ვირთაგვებზე ჩატარებული ექსპერიმენტით გამოკვლეული იქნა რეტიკულური ფორმაციის (რფ) ფუნქციური მდგომარეობის ცვლილების ფონზე ორგანიზმის არასპეციფიკური ადაპტაციური რეაქციის (აარ) სხვადასხვა ხანგრძლიობის კუნთური მუშაობისას.

რეტიკულური ფორმაციის როსტალური ნაწილის ადრენერგული სუბსტრატის (ასრფ) ფუნქციური მდგომარეობის დათრგუნვის პირობებში იზრდება ორგანიზმზე ფიზიკური დატვირთვების სტრესულობა.

გაკეთდა დასკვნა, რომ ფიზიკური დატვირთვების ფონზე ორგანიზმის ადაპტაციური შესაძლებლობების მარეგულირებელ ერთ-ერთ რგოლს წარმოადგენს ასრფ.

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SIGNIFICANCE OF AUTONOMIC NERVOUS SYSTEM IN MECHANISMS OF THE INFLUENCE OF EXCESSIVE PHYSICAL LOADS ON THE ORGANISM'S NON-SPECIFIC ADAPTATION RESPONSE LEVEL

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The organism's non-specific adaptation reaction was determined in the albino rats on the background of the peripheral autonomous nervous system's functional state modulation during maximal physical loads. It was shown that paripheral part of sympathetic and parasympathetic autonomous nervous system plays a key role in the organism's adaptive regulatory complex system, in conditions of physical loads.

Key words: *Autonomous nervous system, Adaptation, Physical loads, Stress, Rats*

There is no doubt today that physical loads serve as stress factors. The central nervous system, as the main system controlling organism, is considered to be prominent in adaptation of organism to stress effects. However, the final pulse realisation originating from central structures to effectory links of adaptation functional system is accomplished mainly by Autonomic Nervous System (ANS).

On this basis, it is of great theoretical and practical interest to evaluate the level of non-specific adaptation response (NAR) under the effect of excessive physical loads upon organism against the background of the functional state modulation of the ANS.

As far as it is known, one of the signs of NAR development is the coefficient of lymphocyte to segmented neutrophyle ratio (LSNR). According to the conception proposed, NAR gradation is established as follows: activation response (LSNR = 0.51 – 0.69), training response (0.34 – 0.50) and stress response (0.33 and below) [1].

Taking into account the above values, it has been studied the dependence of dynamics of organism's NAR level under an excessive physical load against the background of inhibition of sympathetic and parasympathetic innervation.

Studies were carried out in 68 adult albino rats of mixed population (males) weighing 194 ± 12 gr. standardized by a feeding factor. Physical load was given to the rats " up to overflowing", comprising 5.57 ± 0.13 hours by methods being in common use (swimming in water under 30 –32°C with load amounted to 6% of the body mass). They were divided into 3 groups. In the first group NAR level was estimated under a momentaneous excessive physical load, in the second and third groups - under a momentaneous excessive physical load giving against the background of depressed sympathetic and parasymphatetic innervation. The functional state change of peripheral autonomic channels was performed by intramuscular injection of 1 mg atropine or 1 mg ergotamine.

In control groups the level of NAR was defined in intact animals as well as in animals subjected to atropine and ergotamine injection at physiological rest. Reliability of the results obtained was evaluated according to the Student's *t*-criterion.

Findings of all above mentioned groups were compared with indices of intact animals. A data comparative analysis of animals was done additionally after completion of excessive physical load and of those subjected to excessive physical load against the background of the functional state changes of sympathetic (group 2) and parasympathetic (group 3) parts of ANS. Summarised results of the studies are given in Table 1.

Table 1

The average values (mean ±S.E.) of NAR under different experimental Conditions (see text for details)

Response Zones		Intact animals	Atropine	Ergotamine	Physical load	Atropine – physical load	Ergotamine –physical load
NAR	activation	0.51±0.12	—	—	—	—	—
	training	—	0.49±0.16	0.48±0.07	—	—	—
	stress	—	—	—	0.24±0.02	0.23±0.054	0.16±0.024

A background value of LSNR coefficient in intact animals has appeared to be 0.51±0.12. The coefficient in question was equal to 0.49±0.16 (P>0.05) when intramuscular injecting of atropine at physiological rest. Ergotamine intramuscular injection showed the coefficient to be 0.48±0.07 (P>0.05). Such values are considered to be a favorable background for manifesting a high-level working capacity and they correspond to the training response zone.

An excessive physical load in unusual conditions caused the level of NAR to be reduced and after its completion the value of LSNR coefficient was equal to 0.24±0.002 (P<0.001). It came up to 0.23±0.54 after the excessive physical load being completed against the background of preliminary depression of parasympathetic part of ANS.

When depressing the sympathetic innervation the excessive physical load caused LSNR coefficient to be decreased to 0.16±0.024 (P<0.001).

As far as Table 1 has shown, the value of LSNR coefficient was reduced under excessive physical load in all series of the investigation. This indicates that NAR level of organism was in the zone of a stress response. However, it appeared that NAR level reduction upon depression of sympathetic innervation by ergotamine intramuscular injection was expressed more significantly, and LSNR coefficient in animals of this series was reliably different from that of the first and second groups.

On the basis of the given studies the conclusion has been made that the excessive physical load in unusual conditions (being a stress stimulus) causes NAR level reduction and its identifying value can be found within the stress response zone. The same was revealed after completion of the excessive physical load in unusual conditions against the background of preliminary changes of the functional state of ANS.

In general the results allow to conclude that sympathetic and parasympathetic parts of ANS are the links of a complex system, which controls the adaptation capabilities of organism under physical load.

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საქართველოს ფიზიკური კულტურის სამეცნიერო კვლევითი ინსტიტუტი, თბილისი

რ ე ზ ი უ მ ე

თეთრ ვირთაგვებზე ჩატარებული ექსპერიმენტით ვეგეტატური ნერვული სისტემის ფუნქციური მდგომარეობის მოდულირების ფონზე მაქსიმალური ფიზიკური დატვირთვის პირობებში განსაზღვრულ იქნა

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მაქსიმალური ფიზიკური დატვირთვა წარმოადგენს სტრესულ გამღიზიანებელს, რომელიც იწვევს ორგანიზმის აარ-ის დაქვეითებას, რომლის განმსაზღვრელი კოეფიციენტი მდებარეობს სტრესის რეაქციის ზონის ფარგლებში. ფიზიკური დატვირთვების სტრესულობა უფრო მეტად ვლინდება სიმპათიკური ინერვაციის გამოთიშვის პირობებში.

დადგინდა, რომ ფიზიკური დატვირთვების პირობებში ორგანიზმის ადაპტაციის მარეგულირებელი კომპლექსური სისტემის ერთ-ერთ ძირითად რგოლს წარმოადგენს სიმპათიკური და პარასიმპათიკური ნერვული სისტემის პერიფერიული ნაწილები.

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NON-SPECIFIC ADAPTATION RESPONSES (NAR) OF ORGANISM AT DIFFERENT LEVELS OF PHYSICAL LOAD

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Experiments carried out in sportsmen have shown that there is a reliable correlation between decrease of adaptive abilities of the organism and intensity and duration of physical loads (stress).

Key words: *Sportsmen, Adaptation, Physical load, Lymphocytes, Stress*

The volume of competition and training loads at the current stage of sports development essentially approaches the level of utmost possibilities of a human organism. In this connection the functional state of a sportsman at its so-called of "peak of athletic fitness" may, apparently, be considered as a variety of a stress state.

There are a lot of facts at present indicating that with the growth of training extent the sensitivity of a sportsman's organism to various pathogenic effects increases [1,4,5]. This may reasonably assume that a high training extent is a particular state, one of characterics of that is reduction in immunobiological resistance of organism [1,4,5].

The urgency of the given investigation is estimated by the fact that there is no integral index available in sports medicine, which is able to reflect dynamics of a sportsman's functional state during training processes. If non-specific adaptation response is considered to prevent pathology development (i.e. to keep health in its wide sense), one may assume that the values expressing different stages of a general adaptation syndrome (GAS) may be used (at the first approach) as index sought for.

As far as it known, the factor of lymphocytes-segmented neutrophiles ratio (LSNR) may serve as one of the features of non-specific adaptation response [2]. According to the conception proposed, the gradation of non-specific adaptation response is established within the following values: activation response (LSNR=0.51-0.69); training response (LSNR=0.34-0.50) and stress response (LSNR=0,33 and lower) [2].

Based upon these data, in the given work the level of NAR during physical loading of different length in unusual conditions was studied.

12 male athletes of the average skill (age 17.3±0.4) being in the transitional period of training, took part in the studies. Physical load with a critical power "to overflowing" was given to the sportsmen using a cycle-ergometer of "Monarch" Company. A mean working time under load was 4.8±1.1 min.

In parallels, tests were carried out on 36 puberty white rats of mixed population with mass 191±11 grams, standardized by a feeding factor. Physical loads were given to the rats by the method of swimming in water under 30-32C with load amounted to 6 % of the body weight. For estimating the dynamics of the ratio changes under study, the animals were divided into 3 groups: physical load was given during 15 minutes in the first group; in the second group the length of physical load comprised 2 hours, in the third group - "up to overflowing" which averaged 5 h.50 min.±18 min. In the later group the factor of LSNR was estimated in a recovery period (passive relaxation) as well in 1,2,3 - 6 hours after completion of maximum physical load.

The obtained initial values of LSNR in athletes comprised 0.58±0.16, in laboratory animals - 0.51±1.0. Such values are considered to be a favourable background for manifestation of a high - level physical efficiency and they correspond to an activation response zone [2, 3] (Table 1).

Table 1

Dynamics of LSNR factor changes at different levels of physical load.

	Zones of NAR	**Background**	**Physical load**			
			Critical Power	15 min.	2 hours	6 hours
Sportsmen (n=12)	Activation Training Stress	0.58±0.16	0.44±0.04			
Laboratory animals (n=36)	Activation Training Stress	0.51±0.10		0.42±0.08	0.35±0.07	0.24±0.02

A momentaneous physical load "up to over flowing" given to the sportsmen caused reduction in LSNR factor by 24.1%. The same was recognised in rats after completion of 15 min. physical loading in unusual conditions; reduction in LSNR factor came up to 17.6%. In both cases the value of LSNR showed the organism to be in a training response zone.

After completion of 2 hour physical load in unusual conditions reduction in LSNR factor amounted to 31.3% and corresponded to the lower limit of the training response zone, i.e. prestressed state of organism.

After completion of single maximum physical load given to the animals, the value of LSNR was reduced by 52.9% which was specific to a stress response according to data [2] and was estimated as a pathologic state.

Thus, the studies carried out have established a reliable ($P<0.05$) correlation between reduction of adaptation possibilities of organism and intensity and length of

physical loads in unusual conditions. It should be noted that even after completion of 6 hour recovery period under conditions of passive relaxation, LSNR factor didn't tend to increase, i.e. a stress response continued even without a stress stimulus.

However, in our opinion, a low index of LSNR after completion of maximal physical load can't be considered as a pathological symptom, but rather it should be estimated as a sign of reduction in immune reactivity of organism which may be resulted in development of pathologic processes. The results obtained enable us to set a problem concerning the expediency of developing and carrying out some special measures to strengthen the immune system of sportsmen being in the state of high - level training.

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ორგანიზმის არასპეციფიკური ადაპტაციური რეაქცია (აარ) სხვადასხვა ფიზიკური დატვირთვების დროს.

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საქართველოს ფიზიკური აღზრდისა და სპორტის აკადემია, საქართველოს მეცნიერებათა აკადემიის ი. ს. ბერიტაშვილის სახელობის ფიზიოლოგიის ინსტიტუტი, საქართველოს ფიზიკური კულტურის სამეცნიერო კვლევითი ინსტიტუტი

რ ე ზ ი უ მ ე

არასპეციფიური ადაპტაციური რეაქციის (აარ) დონე განიხილება როგორც ორგანიზმის იმუნური სისტემის ფუნქციური მდგომარეობის მაჩვენებელი.

ექსპერიმენტული გამოკვლევების საფუძველზე გამოვლინდა

სარწმუნო კორელაცია ორგანიზმის ადაპტაციური შესაძლებლობების დაქვეითებასა და ფიზიკური დატვირთვების ინტენსიობასა და ხანგრძლივობას შორის (სტრესი).

მაქსიმალური ფიზიკური დატვირთვის შემდეგ ლიმფოციტების სეგმენტბირთვიან ნეიტროფილებთან შეფარდების კოეფიციენტის (ლსნშ) დაბალი მაჩვენებელი მიუთითებს არა პათოლოგიის არსებობაზე, არამედ განიხილება როგორც ორგანიზმის იმუნური რეაქტიულობის დაქვეითება, რის გამოც იზრდება მგრძნობელობა სხვადასხვა პათოგენურ ზემოქმედებაზე.

ხოლო ლსნშ კოეფიციენტი შეიძლება მიჩნეულ იქნას ინტეგრალურ მაჩვენებლად, რომელიც ასახავს სპორტსმენის ფუნქციური მდგომარეობის დინამიკას.

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STUDY OF PSYCHOSOCIAL FACTORS AND LIPID METABOLISM IN THE WOMEN EARLY MENOPAUSE

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Our investigations confirmed that the women with early menopause have dyslipidemia at young age, which is a high risk factor for coronary heart disease. Psychosocial factors, personality peculiarities and psychoadaptation play certain role in development of early menopause.

Key words: *Psycho-social factors, Lipid metabolism, Menopause.*

The problems, concerned with menopause in women, are encountered in many countries of the world [3, 4, 8, 9, 11, 27, 35, 37, 40].

Menopause might be defined as a developmental process in a life cycle, when women are becoming adjusted to the biological, social and psychological changes, which accompany ovarian failure and cessation of menses.

As tendency to early menopause became more frequent in women of our country, we aimed to study: 1.The influence of psychosocial factors and the importance of personality peculiarities in early menopause formation; 2.Lipid metabolism indices in women with early menopause: – serum cholesterol as a risk factor for coronary heart disease and high – density lipoprotein cholesterol as an antiatherosclerotic factor.

For this purpose it was carried out a complex psychological study and blood lipids characteristic among 52 female with early menopause, aged 30-43 (I group), and 30 female of the same age without menopause (II group – control).

The method of Fixed Set by D.Uznadze was used in our psychological study. In his scientific works Uznadze had studied those aspects of set, which allow understanding and explaining the nature of an individual's mental activity. D.Uznadze had created the general psychological theory of set, which changes unilateral views about human psyche. According to this theory set is an integral psychophysical state of an individual [18, 20, 25, 29].

In our complex psychological investigation MMPI (Minnesota Multiphasic Personality Inventory), which is one the popular methods of psychometry, was used as well.

In our study special attention was paid to filling of a psychosocial questionnaire, which gives detailed information about an individual's development, family and interpersonal relations, psychic trauma history and premorbid characteristics.

The analysis of the data received by Set method study has shown that among women with early menopause prevailed static (40,9 %) and variable (36,4 %) set types, and only 22,7 % was dynamic set type, while most women without menopause were of dynamic set type (65,7 %) and only 14,3 % of such women had static set type and 20 % had variable set type.

According to the MMPI, most women without menopause had almost all scale indices in norm, while most women (65,9 %) with early menopause, had significantly higher scores on depression and hypochondria scales.

Thus, summarizing the received data, we can conclude, that most women with early menopause are characterized by difficulties in adaptation to the environment, deep and heavy internal conflicts, negative emotions, egocentrism, latent aggression, sensitiveness, autoaggression, pessimism, autistic emotions, apathy and hypochondria, which are limiting interpersonal relations and individual's activities.

Psychosocial investigations revealed, that most women with early menopause had histories of stresses in childhood: death or illness of a loved person, divorce of parents, conflicts in the families, oppressive influence of their parents on them. A single parent (mostly mothers) often raised such women. Hence, psychic status of the women with early menopause is somehow related to the type of care for them in childhood, casual emotional stresses, emotional atmosphere in the family, and parents' characters.

Women with early menopause often mentioned, that before the beginning of menopause they had psychic stresses: conflicts with relatives – 20,4 %; death or serious illness of a loved person – 22,7 %; conflicts at a job – 15,9 %; 31,8 % of these women were experiencing unconscious psychic traumas; in particular, dissatisfaction with family relationships, job, profession.

Thus, disharmony of personality structure may play a certain role in early menopause formation. It seems that women with psychoadaptation disorders are predisposed to early menopause, especially in case of psychic trauma at the mentioned age.

It is well known that the age difference (7-15 year) between men and women for CHD is due to cardioprotective action of female hormones [4, 5, 10, 16, 22].

Many studies have shown, that estrogens affect lipid metabolism, blood coagulation and arterial vessels [6, 7, 15, 23, 26, 30, 34, 38]. Besides estrogens act as antioxidant, Ca-antagonist, (2-inhibitors and can lessen insulin resistance [17, 24, 31, 32, 36].

Many studies have reported that menopause is accompanied by changes of lipid profile, in particular, the increase of total cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides and lipoprotein (a), and decrease of high-density lipoprotein (HDL) cholesterol [7, 15, 23, 28, 30, 39].

In blood taken from vein of a women 12 hours of fasting, lipids were measured according to the international standards, using spectrophotometer "LOMO - SF 46" and BIOLABO (France) reagents.

The data obtained show that total cholesterol in the first group was 6.26 and in the control group 4.77, HDL cholesterol I group was 0.88 and in the control group 1.91.

As the above data show, women with early menopause have dyslipidemia, which is a high risk factor for CHD. If we review these two studies, conducted in different directions, we can conclude, that psychosocial factors play a certain role in development of early menopause, and deficit of female hormones results in lipid metabolism changes.

It is very interesting how many psychosocial factors and personality peculiarities influence lipid metabolism. Importance of an individual's psychological profile in pathogenesis of CHD and hypertension is subject of active discussion. Many authors think, risk of these diseases increases in case of certain behavioral and characteristic of peculiarities of an individual [2, 12-14, 19, 21, 33]. The mechanisms are not perfect and studied enough. It requires further investigations. Until more precise data is available, we recommend to identify high-risk groups of women and to take preventive measures.

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ფსიქოსოციალური ფაქტორების და ლიპიდური მეტაბოლიზმის შესწავლა მენოპაუზურ პერიოდში მყოფ ქალებში

მ.ჯებაშვილი

საქართველოს ჯანდაცვის სამინისტროს ექსპერიმენტული და კლინიკური თერაპიის ინსტიტუტი, თბილისი

რ ე ზ ი უ მ ე

შრომის მიზანს წარმოადგენდა: 1) ფსიქოსოციალური ფაქტორების და პიროვნულ თავისებურებათა, და 2) ლიპიდური ცვლის შესწავლა პოსტმენოპაუზურ პერიოდში მყოფ ქალებში.

ამ მიზნით კომპლექსური ფსიქოლოგიური კვლევა და ლიპიდური ცვლის მაჩვენებლების გამოკვლევა ჩაუტარდა 30-დან 43 წლამდე ასაკის 52 პოსტმენოპაუზურ პერიოდში მყოფ ქალს და ამავე ასაკის 30 ქალს, რომელთაც მენოპაუზური პერიოდი არ დაწყებიათ.

კომპლექსური ფსიქოლოგიური კვლევისათვის გამოყენებული იყო დ. უზნაძის ფიქსირებული განწყობის მეთოდი, I-ის მეთოდი და ფსიქოსოციალური ანკეტა.

ლიპიდური ცვლის მაჩვენებლებიდან ვიკვლევდით ქოლესტერინს, – როგორც გულის იშემიური დაავადების რისკ-ფაქტორს და მაღალი სიმკვრივის ლიპოპროტეინების ქოლესტერინს, როგორც ანტი-ათეროსკლეროზულ ფაქტორს.

გამოკვლევის შედეგების ანალიზმა გვიჩვენა, რომ პიროვნების სტრუქტურის თავისებურებანი, ემოციურ სტრესთან ერთობლიობაში, მნიშვნელოვან როლს თამაშობს ნაადრევი მენოპაუზის ფორმირებაში. მენოპაუზას კი თან ახლავს არასასიამოვნო ცვლილებები ლიპიდურ პროფილში, რაც ახალგაზრდა ასაკში ქმნის გულის იშემიური დაავადების განვითარებას რისკს.

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INFLUENCES OF PLAFERON-LB, P6 AND METADOXYL ON THE AMINO ACID CONTENT, NITROGENE OXIDE BIOSYNTHESIS, AND INTENSITY OF THE EPR SIGNALS IN THE BRAIN OF ALCOHOLIZED RATS

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Experimentally was shown that in the brains of the alcoholized (for 60 days) rats the rise of arginine and aspartic acid volume is evident; Decrease of the glutamate-stimulated nitrogene oxide synthesis was found as well; Simmultaneously with these processes increase of the Mo^{5+} EPR signal was observed, which indicates an activation of xanthine oxidase. In a course of the withdrawal period the volume of the excitatory amino acids decreased, whereas production of the glutamate-stimulated NO increased. Plaferon-LB, its active peptide fraction P6, and hepato-protective substance Metadoxyl, determined a correction of the amino acids metabolism; This was followed by normalization of nitrogene oxide metabolism and decrease of xanthine oxidase activity. Insofar all the tree above preparations exert an inhibitory influence on the NMDA-glutamate receptors, suggestion was made that the NMDA antagonists may be used in pharmacological treatment of the abstinence syndrome.

Key words: *Alcoholism, NMDA-glutamate receptors, NMDA antagonists, Abstinence, Nitrogene oxide, Pharmacology*

Although alcoholism is considered as one of the commonest pathologies in the modern humankind, neurobiologic mechanisms of its clinical manifestations still remain obscure [12]. Ethanol acts on several neurotransmitters' receptor systems of the brain, among which the most important are considered to be the g-aminobutyric acid (GABA)- and glutamic acid receptors [8]. Ethyl alcohol determines inhibition of the NMDA-glutamate receptors, which results in hampering of the glutamatergic neurotransmission and ensuing slowing down of excitatory neurotransmission in the certain brain regions [6]. Long-lasting action of

ethanol produces oversensitization and up-regulation of the above receptors and their number in synaptic membranes increases many-fold [4]. It is suggested that the above alterations determine development of physical dependence towards ethanol, euphoria, decrease of cognitive function of the brain, formation of the Wernike-Korsakoff's syndrome, cerebellar degeneration, cortical atrophy and formation of susceptibility to alcohol in the fetus [2].

Pharmacological intervention in the alcoholism treatment is based mainly on the modulation of the GABA-receptors [5]. In the last years successful clinical evaluation of the NMDA-glutamate receptor antagonists is carried out. These substances constitute the structural analogues of glutamic acid [13]. They significantly decrease relaxation, prolong the abstinence period and relieve its clinical manifestations [11].

The pilot studies have shown that medicinal preparation Plaferon-LB (Product of Institute of Biotechnology, Georgian Academy of Sciences, Tbilisi) has an antagonistic properties towards the NMDA-glutamate receptors. Proceeding from this finding the active peptide fraction with molecular weight of 6000-8000 Da (P6) was isolated and purified from the above preparation. With an aim to obtain the fraction a primary liquid mass of Plaferon was passed on the 10 kDa-catching ultrafilters, filtrate was then applied on the G-10 Sephadex gel column to desalinate as well as to partially purify the substance. Farther the fraction obtained was acidified (pH-2.7) and impurities were separated from the mass with the Sep-pak C_{18} cartridges. The liquid output mass was lyophilized and thus made ready for the farther investigations. Standardization of the preparation was performed in a simple isokratic regime by the high-pressure liquid chromatography on the Nova-pak C_{18} column with methanol (60%)hexanesulfonate (0.005%), in a specially designed conditions.

It was established earlier that Plaferon has a well-expressed biological activity in the cases of various functional disorders of the brain. Plaferon's neuroprotective action was shown in the model of photo-chemically induced brain infarction [7], when, as compared with control, it dramatically decreased the area of locally induced cortical lesion and promoted normalization of the capillary system and blood-flow in the same area. Investigation carried out in treatment of the neurosurgical patients has shown that the whole preparation exerts strong neurotropic influence and determines an optimizing effect [10]. The above effects are characterized with antitoxic, antiinflammatory action and with sharp redistribution of the neurohormonal content of the brain, producing thus an obvious improvement in the heavy neuro-resuscitation conditions.

Considering partly all the above-mentioned data, objective of the present investigation was influence of Plaferon-LB and its antiglutamate fraction on the alcohol-loaded and abstinent rats' organism and comparison of these influences with action of the hepato-protective antialcohol preparation - Metadoxyl, which is a chemical analogue of glutamic acid.

MATERIAL AND METHODS

Experiments were carried out in the adult albino rats. One group of animals (Group I) for 60 days instead of water were given 20% ethanol solution (ALC-rats). Following 1-3 days after the alcohol withdrawal they developed an abstinence syndrome, which was tested according to the typical autonomous disorders (ABST-rats).In the rats of groups II, III and IV alcoholization was performed, respectively, on the background of Plaferon-LB (20 mg), p6 peptide (10 mg), and Metadoxyl (2 mg) daily administration. The rats were sacrificed via quick decapitation. The brain, liver and the blood were kept frozen in liquid nitrogen until the following analysis.

The amino acids analysis was performed after the PITC-derivatization on the Pico-Tag Analyzer (Waters, USA). Volume of the nitrogen oxide was determined after incubation of the synaptic membranes with arginine. The incubation medium contained 2 mM/l arginine; 50 mM/l Tris-HCl, pH-7.4; 2 mM/l NADPH (Basal formation of the nitrogen oxide). In the respective experiments into the incubation medium were added 1.5 mM/l glutamic acid and 1.5 mM/l glycine (NMDA-receptor-stimulated biosynthesis of the nitrogen oxide). Following incubation (20 min, 25°C), 10% trichloroacetic acid was added into the samples. After centrifugation the nitrogen oxide was determined in the supernatant following the staining with the Gris reagent [3]. Recording of the electroparamagnetic signal was made on the EPR-spectrophotometer (EP-6, Russia). Volume of the blood glucose and g-glutamyltransferase was evaluated with the EPA test-kits.

RESULTS AND DISCUSSION

Experiments have shown that the volume of glutamate in the ALC-rats brain did not change tangibly, while in the ABST-rats it decreased significantly (Table 1). In the rats, which were given Plaferon-LB, decrease of the glutamate volume was found in the ALC-rats too, whereas during abstinence the concentration of this amino acid was even increased. The same effect was induced by the P6 fraction - its action on the alcohol-loaded rats induced decrease of glutamate and in the ABST-rats - increase of the amino acid.

Volume of the other excitatory neurotransmitter - aspartate - in the ALC-rats' brain increased against the controls, while in the ABST-rats it decreased. On the background of Plaferon-LB and P6 fraction administration aspartate volume in the ALC-rats decreased, while in the alcohol withdrawal period (especially after the P6 action) - it increased (Table 1). Similar of Plaferon-LB and peptide preparations' effects were observed in a case of chronic administration of Metadoxyl as well. This preparation in the ALC-rats elicited decrease of the both excitatory neurotransmitters and in the ABST-rats it induced their normalization.

The foregoing data certify that Metadoxyl, Plaferon-LB and P6 peptide act

as typical NMDA-receptor blockators, effects of which are directed towards enhancement of ethanol influences. Because ethyl alcohol by itself is a non-competitive inhibitor of the NMDA-receptors, it could be supposed that chronic administration of the above substances enhance effect of ethanol and, as a result, volume of the excitatory neurotransmitters decreases in a compensatory mode, while sensitivity of the NMDA-receptors increases (up-regulation). So far as the above shifts are the major etiologic factors of the abstinence syndrome formation [12], we suggest that clinical application of Plaferon-LB, its active peptide, and Metadoxyl, on the background of the alcohol action, is not recommended. However, because these preparations increase the volume of excitatory neurotransmitters during the abstinence period, which inevitably entails the NMDA-receptors' normalization (down-regulation), one can suggest that their efficiency may be revealed in relieving of clinical manifestations of the abstinence syndrome.

In the ALC-rats, as compared to the controls, content of arginine was increased, while in the withdrawal period it was decreased (Table 1). Unlike

Table 1

Quantitative alterations of amino acids in the rat brain during alcohol loading and alcohol withdrawal, in a course of action of Metadoxyl, Plaferon-LB, and P6 fraction.

	Animal group								
Amino acids	ALC	ALC+ MET	ALC+ PL-LB	ALC+ P6	ABST	ABST+ MET	ABST+ PL-LB	ABST+ P6	NORM
ASP	0.75±0.15	0.14±0.03	0.39±0.08	0.45±0.09	0.42±0.08	0.67±0.05	0.28±0.04	1.24±0.25	0.28±0.05
GLU	0.66±0.08	0.11±0.02	0.08±0.01	0.27±0.06	0.02±0.003	0.02±0.03	0.25±0.05	1.06±0.29	0.78±0.09
ARG	2.71±0.73	0.26±0.04	0.99±0.18	0.54±0.07	0.69±0.12	1.69±0.07	1.21±0.32	5.90±1.10	1.15±0.23

The figures presented are mean±S.E.M. of the tree experiments, nM/g of the tissue.

Metadoxyl and P6, which decreased amount of arginine in the ALC-rats, Plaferon-LB did not change amount of this amino acid in the brain. On the other hand each of the three preparations elicited an increase of arginine in the ABST-rats, which shows that they induce decrease of the arginine metabolism. If one bears in mind that the main metabolite of arginine is the nitrogen oxide (NO), it could be suggested that Metadoxyl, Plaferon-LB and P6 induce an elevation of the nitrogen oxide production in the alcohol-loaded rat's brain, while in the withdrawal period they decrease its production. Because the nitrogen oxide is an adaptive-compensatory retro-messenger, it should not be excluded that on the background of the

above pharmacological preparations, in withdrawal period, the brain metabolic maintenance is improved, while during the alcohol intake, on the contrary, the deterioration is the case.

Considering that the NO-synthase is the NMDA-glutamate receptor-bound enzyme [3], in the next series of experiments activity of this enzyme was evaluated in the rat brain during its loading with alcohol and during development of the abstinence syndrome. It was found that whereas in the control (NOR-rats) a 10-fold increase of the glutamate-stimulating NO synthesis is observed, in the ALC-rats such an activity was not recorded at all, while in the withdrawal period the normalization of the process is evident (Table 2). On the background of

Table 2

The NO biosynthesis intensity in the rats' brain synaptic membranes, during alcohol loading and alcohol withdrawal,in a course of action of Metadoxyl, Plaferon-LB, and P6 fraction.

	Velocity of NO production (μM/mg/15 min)	
Animal group	-GLU	+GLU
NOR	0.050±0.12	0.520±0.162*
ALC	0.047±0.030	0.052±0.077
ALC+PL-LB	0.420±0.095*	1.530±0.185*
ALC+P6	0.053±0.014	0.055±0.015
ALC+MET	0.940±0.145*	0.950±0.180*
ABST	0.052±0.014	0.470±0.125*
ABST+PL-LB	0.070±0.027	0.075±0.040
ABST+P6	0.650±0.160*	0.675±0.240*
ABST+MET	0.975±0.275*	0.988±0.260*

The figures presented are mean±S.E.M. of the tree experiments; *P<0.05.

Plaferon-LB chronic administration in the ALC-rats restoration of the glutamate-stimulating NO-synthase activity was observed (the basal production of NO was increased in such a case, as well), while in the ABST-rats this activity decreased. Chronic administration of P6 elicited inhibition of the glutamate-stimulating NO synthesis in the ALC-rats (desensitization of the NMDA-receptors), while in a withdrawal period intensive synthesis of basal NO only was observed. Metadoxyl, in the both cases, abolished the glutamate-stimulating activity altogether, but increased the basal NO synthesis.

The data obtained show that in a course of alcoholization a desensitization of the NMDA-receptors does occur, which is certified by decrease of the glutamate-stimulating NO biosynthesis. It should be noted as well that during the abstinence an ethanol intake produces restoration of the glutamate-stimulating activity, which points at

resensitization of the NMDA-receptors. Treatment of the rats with Plaferon determines an up-regulation of the above receptors in the ALC-rats, and their desensitization in a withdrawal process, which, once again, certifies for the preparation potency in relieving clinical manifestations of the abstinence syndrome. Unlike Plaferon-LB, Metadoxyl and P6 fraction in all the cases induce a desensitization of the NMDA-receptors, which speaks in favor of their high pharmacological potency in both alcoholization and withdrawal period. It should be noted as well that both of these preparations increase basal NO synthesis, which indicates engagement of the brain adaptive-compensatory mechanisms and elevation of the metabolic maintenance of the brain.

Therefore, in the process of alcoholization Plaferon-LB decreases amount of the brain main excitatory neurotransmitters - glutamate and aspartate, does not affect the basal metabolism of arginine, but determines increase of glutamate-stimulated nitrogen oxide amount. At the stage of the abstinence syndrome development Plaferon-LB elicits decrease of glutamate, increase of arginine, and reduction of the glutamate-stimulated NO biosynthesis. These changes, in a course of withdrawal process, indicate a desensitization of the NMDA-glutamate receptors, which points at the antiabstinence potency of the preparation.

The P6 preparation elicited decrease of the excitatory amino acids and arginine in the ALC-rats, as a result of which the sensitivity of the NMDA-receptors to glutamate increased and, hence, the small amounts of the nitrogen oxide were produced. In the withdrawal period P6 elicited increase of glutamate and arginine amount and, as in the case with Plaferon-LB, determined the NMDA-glutamate receptors' desensitization.

Metadoxyl elicited increase of the excitatory amino acids and arginine concentration in the ALC-rats, as a result of which a desensitization of the NMDA-glutamate receptors is probable. In a presence of this preparation in the ABST-rats the normalization of glutamate and aspartate does occur, although, according to the glutamate-stimulated NO biosynthesis, the NMDA-receptors' activity remains decreased.

In the next series of experiments alterations of the paramagnetic centers in the rat's brain and liver were investigated, in the periods of alcoholization and withdrawal syndrome development. Firstly it was found that alcohol administration and alcohol withdrawal induced decrease of the mitochondrial Fe-S signal in the brain, which points at deceleration of electrons' transport at the NADH:ubiquinone-oxydoreductase site. Interestingly, none of the investigated preparations induced the respiration chain restoration at this site during alcoholization; However, in the withdrawal period Metadoxyl, Plaferon-LB, and its peptide fraction determined the NADH:ubiquinone-oxydoreductase signal correction, which points at normalization of the respiration chain (Table 3 and Table 4). Unlike the brain, in the liver all the three pharmacological agents restored the electron transport system even in the alcoholization process, which speaks in favor of their hepato-protective property.

As a result of alcoholization and development of the abstinence syndrome,

also increase of the paramagnetic signal of the Mn^{2+}-containing complexes was observed in both liver and nervous tissue (Table 3 and Table 4). Such a signal points at the lesion of the membrane integrity, which may be due to the A2 phospholipase activation and initiation of the lipoperoxidation processes. Influence of Metadoxyl, Plaferon-LB, and P6 on the correction of this signal in the brain in a course of alcoholization is weakly displayed, but they significantly improve these indices in the withdrawal period. It is important to note as well that in the liver all these preparations show membrane-protective property only and decrease an intensity of the Mn^{2+}-containing complexes'

Table 3

Alterations of the EPR-spectra in the rats' brain during alcohol loading and alcohol withdrawal, in a course of action of Metadoxyl, Plaferon-LB, and P6 fraction

Animal group	Electric paramagnetic resonance spectra					
	Free radical	FeS		Mn^{2+}-complex	Mo^{5+}-complex	
	g=2.00	g=1.94		g=2.032	g=2.14	g=1.97
	I	ΔH(Hs)				
NOR	7.2±0.5	12.8±1.3	17.0±1.8	-	6.5±0.5	1.5±0.5
ALC	10.8±1.3*	8.2±0.8*	6.8±1.4*	10.2±1.8*	11.7±1.8*	9.0±1.9*
ALC+PL-LB	16.5±1.5*	11.3±1.2	10.4±1.9	6.6±0.4*	9.3±2.1*	-
ALC+P6	12.5±1.2*	11.8±1.1	10.2±1.6*	4.1±1.1*	8.3±1.1	-
ALC+MET	12.5±1.6*	9.1±1.4	7.4±1.2*	4.2±0.6*	10.2±1.9*	3.2±0.8
ABST	9.6±0.9	9.2±1.2	6.3±1.1*	6.7±1.2*	12.2±2.4*	6.1±1.6*
ABST+PL-	23.7±3.8*	12.4±1.8	15.3±1.7	6.5±1.1	6.3±0.9	-
LB	15.2±2.4*	12.3±1.9	12.8±2.1	2.3±0.7	5.2±0.7	-
ABST+P6	10.5±2.1	11.0±2.2	8.1±1.1*	4.5±0.5	8.1±0.9	-
ABST+MET						

Figures represent mean 1 mm/mg±S.E.M., out of the three experiments; *P<0.05

paramagnetic signal during both alcoholization and withdrawal periods.

Investigations have confirmed that one of the sources of destructive free radical is xanthine oxidase. It was found that in the brains of the ALC- and ABST-rats the Mo^{5+} paramagnetic signal did not increase noticeably, while under the influence of pharmacological preparations, especially Plaferon-LB and P6, its intensity decreased dramatically. Because xanthine oxidase is the main generator of the superoxide radical, activation of which determines an initiation of the neurodegenerative processes [9], decrease of the Mo^{5+} signal speaks in favor of the neuroprotective properties of the

preparations under study.

Emerging of the g=2.032 signal in the ALC- and ABST-rats should be noted as well, which testifies to the inactivated superoxide dismutase production. Plaferon-LB, its peptide fraction, and Metadoxyl induced decrease of the signal intensity in the liver (Table 4), which once more corroborates a hepato-protective property of these substances.

Finally, our experiments have shown that the blood glucose concentration does not increase in the withdrawal period. Metadoxyl, Plaferon-LB, and P6 induce elevation of the glucose concentration in the blood plasma in both withdrawal and alcoholization periods (Table 5). It should be noted also that, unlike the ABST-rats, in the ALC-rats' blood plasma the g-glutamyltransferase activity was increased as well. Activity of the above indicative enzyme decreases significantly after the animals' treatment with Metadoxyl, but does not change under the Plaferon-LB and P6 influence (Table 5). In the withdrawal period all the three preparations induced much faster decrease of the g-glutamyltransferase activity, which suggests that their action intensifies the reparation processes in the liver.

Table 4

Alterations of intensity of the g-spectra in the rats' liver during alcohol loading and alcohol withdrawal, in a course of action of Metadoxyl, Plaferon-LB, and P6 fraction

Animal group	Electric paramagnetic resonance spectra					
	Free radical FeS			Mn^{2+}-complex	Mo^{5+}-complex	
	g=2.00		g=1.94	g=2.032	g=2.14	g=1.97
	I	ΔH(Hs)				
NOR	28.2±3.5	12.8±1.3	27.0±3.8	-	5.5±0.5	1.9±0.8
ALC	30.8±3.3	9.5±0.8*	11.4±1.4*	2.2±1.8*	8.1±0.8*	6.0±1.9*
ALC+PL-LB	22.3±1.5*	10.3±1.2	30.4±4.9	2.4±0.4*	5.3±1.1	6.1±1.1*
ALC+P6	20.4±1.2*	13.0±1.1	24.2±1.6	3.0±0.7*	5.3±1.1	4.3±1.5
ALC+MET	30.1±2.6	11.8±1.4	25.1±1.2*	6.0±1.6*	5.5±1.9	4.5±0.8*
ABST	15.2±1.9*	9.2±1.2*	9.0±1.1*	2.1±1.2*	12.9±2.4*	6.5±1.6*
ABST+PL-	41.7±6.8*	12.4±1.8	27.3±1.7	5.0±1.1*	8.3±1.9*	4.5±1.4*
LB	18.2±1.4*	12.3±1.9	20.8±2.1*	3.9±0.7*	3.2±0.7*	1.6±0.6
ABST+P6	13.5±2.3*	11.9±2.2	26.5±2.1	3.6±0.5*	3.1±0.6*	4.0±0.9*
ABST+MET						

Figures represent mean 1 mm/mg±S.E.M., out of the three experiments; *P<0.05

Table 5

Volume of glucose and g-glutamyltransferase (GGT) in the rats' blood plasma during alcohol loading and alcohol withdrawal, in a course of action of Metadoxyl, Plaferon-LB, and P6 fraction

Animal group	Glucose, mg%	GGT, unit/l
NOR	6.73±0.95	4±1
ALC	8.40±1.80**	34±7*
ALC+PL-LB	9.94±1.75**	39±7*
ALC+P6	9.37±1.70**	38±9*
ALC+MET	9.62±2.10**	19±4*
ABST	6.67±1.20	23±5*
ABST+PL-LB	7.35±1.45	11±3*
ABST+P6	9.17±1.78**	9±2*
ABST+MET	8.32±1.67**	5±1

Figures represent mean±S.E.M., out of the three experiments; *P<0.05; **P>0.05.

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პლაფერონ-ლბ-ს, P6 პეპტიდის და მეტადოქსილის გავლენა ალკოჰოლიზირებული ვირთაგვების თავის ტვინში ამინმჟავების რაოდენობაზე, აზოტის ოქსიდის ბიოსინთეზზე და ელექტროპარამაგნიტური სიგნალების ინტენსიურობაზე

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საქართველოს ჯანდაცვის სამინისტროს ნარკოლოგიის ინსტიტუტი, თბილისი
საქართველოს მეცნიერებათა აკადემიის სამედიცინო ბიოტექნოლოგიის ინსტიტუტი, თბილისი
საქართველოს მეცნიერებათა აკადემიის ი.ბერიტაშვილის სახელობის ფიზიოლოგიის ინსტიტუტი, თბილისი
საქართველოს სახელმწიფო სამედიცინო უნივერსიტეტის კლინიკური და ექსპერიმენტული მედიცინის ინსტიტუტი, თბილისი

რ ე ზ ი უ მ ე

ექსპერიმენტულად ნაჩვენებია, რომ ვირთაგვების ხანგრძლივი (60 დღე) ალკოჰოლიზაციის შედეგად თავის ტვინში ადგილი აქვს არგინინის და ასპარაგინის მჟავას რაოდენობის ზრდას, აგრეთვე გლუტამატ-სტიმულირებული აზოტის ოქსიდის სინთეზის დაქვეითებას; პარალელურად იზრდება Mo^{5+}-ის ელექტროპარამაგნიტური სიგნალი, რაც ქსანტინოქსიდაზის გააქტიურებაზე მიუთითებს. აბსტინენციის დროს ამაგზნებელი ამინმჟავების რაოდენობა მცირდება, ხოლო გლუტამატ-სტიმულირებული NO-ს პროდუქცია - მატულობს. როგორც პლაფერონი-ლბ, ასევე ამ პრეპარატიდან მიღებული აქტიური პეპტიდური ფრაქცია P6 და ჰეპატოპროტექტული ნაერთი-მეტადოქსილი– განაპირობებს ამინმჟავათა ცვლის კორექციას, რის შედეგადაც წესრიგდება აზოტის ოქსიდის მეტაბოლიზმი და ქვეითდება ქსანტინოქსიდაზის აქტიურობა. ვინაიდან სამივე გამოყენებული პრეპარატი ამჟღავნებს NMDA-გლუტამატის რეცეპტორის მაინჰიბირებელ თვისებებს, გამოთქმულია მოსაზრება, რომ NMDA-ანტაგონისტები შეიძლება ეფექტური აღმოჩნდეს აბსტინენციის სინდრომის ფარმაკოლოგიურ თერაპიაში.

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ALTERATION OF THE LYMPHOCYTES' PROLIFERATIVE ACTIVITY *IN VITRO,* UNDER INFLUENCE OF PLAFERON-LB FRACTIONS

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Immunologically active fractions of Plaferon-LB and their influence on the functional activity of the mononuclear cells (MNC) in the lymphocytes' blast-transformation reactions were investigated. Lymphocyte proliferation rate was defined by tetrazolium-based colorimetric MTT assay. It was shown that Fraction III of Plaferon-LB, as the whole preparation, inhibited proliferative activity of the stimulated mononuclear cells. Plaferon-LB contains the other fraction (Fraction II) with different stimulating effect on the cell proliferation. Thus, Fractions II and III contain immunologically active substances, which determine Plaferon-LB's immunomudulatory effect.

Key words: *Placenta, Plaferon-LB, Cell proliferation*

Plaferon-LB is a peptide preparation obtained from the amniotic membranes of placenta. Biologically active endogenic substances of Plaferon-LB are responsible for various physiological effects of the preparation [3, 5, 6, 7]. Plaferon-LB's immunomodulatory activity was shown in *in vitro* and *in vivo* studies. It was shown to increase the total amount of the lymphocytes and improve impaired balance of CD4+ and CD8+ phenotype of cells in the cases of immunodefficiency [2, 4]. An immunomodulatory activity is also characteristic of Plaferon - precursor preparation of Plaferon-LB [1, 8]. It was shown that Plaferon and Plaferon-LB inhibit functional activity of the MNC and proteinkinase C activity in these cells. This confirms a 76 supposition that both of these preparations contain the same immunologically active substances [1].

The aim of our work was isolation of immunologically active substances from Plaferon-LB. Immunologically active fractions of Plaferon-LB and their influence on the functional activity of the MNC in the lymphocytes' blast-transformation reactions were investigated.

MATERIAL AND METHODS

Fractionation of Plaferon-LB was executed by means of Sephadex G-10 gel chromatography. Distilled water was used as an eluent. The flow rate of eluent was 1 mm/min. Optical density was detected at 220 nm wave length by Waters-484.

The lymphocyte proliferation rate was assessed by tetrazolium-based colorimetric MTT assay. The suspension of the healthy donors' MNC at concentration of 10^6/ml was poured into the microplate wells, 200 ml per well. For lymphocyte stimulation a nonspecific mitogen phytohemaglutinin (PHA) (Bacto) was used at 50 μl per well (PHA dilution - 1:100). Plaferon or any of its fractions was added simultaneously with the mitogen to final concentration of 320 mg/ml. On the third day of incubation MTT was added to final concentration of 0.25 mg/ml. Live cells convert MTT into formazan crystals, which were dissolved by SDS. Optical density in the wells was measured at 540-570 nm in the microplate reader. Cell proliferation was evaluated by the stimulation index:

$$SI = \frac{\text{optical density in experimental well}}{\text{optical density in control well}}$$

RESULTS AND DISCUSSION

Plaferon-LB was divided into four fractions - Fraction I, high molecular protein fraction; Fractions II and III, with molecular weight of 3-8 kDa; Fraction IV, mixture of low molecular substances (salts, etc.) (Fig. 1)

The analysis of results has shown that in a case of MNC activation by the mitogen, stimulation index of lymphocytes was 2.35, after addition of Plaferon-LB, it was 1.00, and in a case of addition of the fractions into this reaction, SI was: I - 1.91; II - 3.89; III - 0.98; IV - 2.43. It was shown that Fraction III (Fig. 2) of Plaferon-LB, as the whole preparation, inhibited proliferative activity of the stimulated MNC. Interestingly, Plaferon-LB contains the fraction (Fraction II) with different stimulating effect on the cell proliferation.

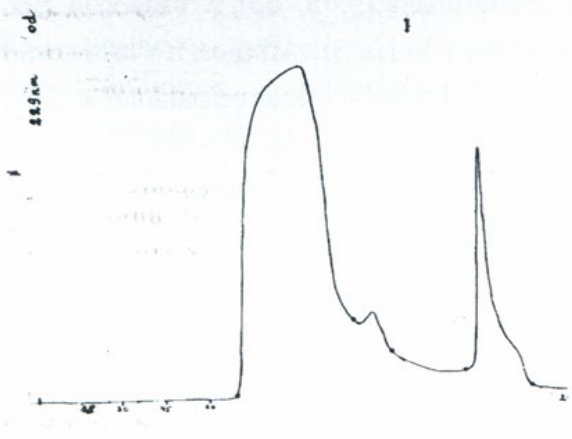

Fig. 1. Chromatographic profile of Plaferon-LB on Sephadex G-10 column of 1.6 x 100 cm; Eluent H_2O; Flow rate 1 ml/min.

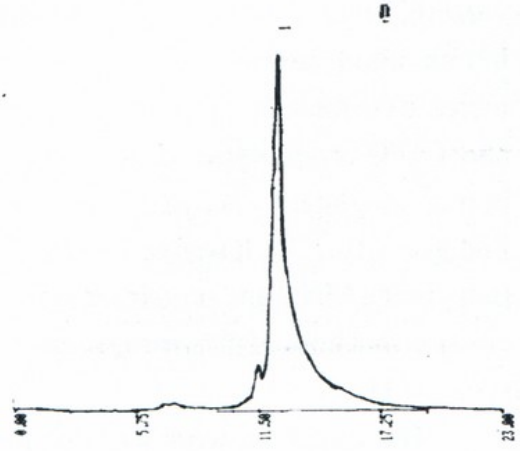

Fig. 2. Analysis of Fraction III by HPLC. Prot. Pak SW60 2 column; 0.1 M KBS pH 5; Flow rate 1.5 ml/min.

Thus, the Fraction III only has a similar effect with Plaferon-LB. We suggest that the immunomodulatory effect of the whole preparation of Plaferon-LB is determined by immunologically active substance contained in the Fractions II and III.

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In vitro ლიმფოციტთა პროლიფერაციული აქტიურობის ცვლილება პლაფერონ-ლბ-ს ფრაქციებით

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საქართველოს მეცნიერებათა აკადემიის სამედიცინო ბიოტექნოლოგიის ინსტიტუტი, თბილისი

რეზიუმე

პლაფერონ-ლბ პლაცენტის ამნიონური გარსიდან მიღებული ცილოვან-პეპტიდური ბენების პრეპარატია, მასში შემავალი ბიოლოგიურად აქტიური ნივთიერებები პრეპარატის მრავალმხრივ ფიზიოლოგიურ მოქმედებას განაპირობებს. in vitro და in vivo გამოკვლევებით პლაფერონი-ლბ იმუნომამოდულირებელ მოქმედებას ავლენს. სხვადასხვა იმუნოდეფოციტის პირობებში იგი ზრდის T-ლიმფოციტების საერთო რაიდენობას და ასწორებს CD4+ და CD8+ ფენოტიპის უჯრედების დარღვეულ ბალანსს. იმუნომა-

მოდულირებელი მოქმედება ახასიათებს ლბ-ს წინამორბედ პრეპარატ პლაფერონსაც. დადგენილია, რომ პლაფერონი და პლაფერონი-ლბ ერთნაირად მოქმედებენ მონონუკლეურ უჯრედთა აქტიურობაზე და ამ უჯრედებში პროტეინკინაზა-C-ს აქტიურობაზე, რაც ამ ორ პრეპარატში ერთი და იგივე იმუნოლოგიური აქტიური საწყისის არსებობაზე მიუთითებს.

წინამდებარე შრომის მიზანს წარმოადგენდა პლაფერონი-ლბ-დან იმუნო-ლოგიურად აქტიური საწყისის გამოყოფა. პრეპარატის ფრაქციონირება ხდებოდა პრეპარატული გელ-ქრომატოგრაფიის საშუალებით სეფადექს G-10 სვეტზე. ქრომატოგრაფიული სურათი დაიყო 4 პირობით ფრაქციად. I - მაღალ-მოლეკულური ცილოვანი ფრაქცია; II და III - 3-8 კდ მოლეკულური მასის; IV - დაბალმოლეკულური ნაერთების ნარევი (მარილები და სხვ.).

იმუნოლოგიურად აქტიური ფრაქციის გამოსავლენად შესწავლილია მათი გავლენა მონონუკლეური უჯრედების ფუნქციურ აქტიურობაზე ლიმფოციტების ბლასტტრანსფორმაციის რეაქციებში. შედეგების ანალიზმა ცხადჰყო, რომ თუ მიტოგენით გააქტიურების შემთხვევაში ლიმფოციტების სტიმულაციის ინდექსი 2,35-ს შეადგენდა, პლაფერონ-ლბ-ს დამატების შემდეგ ინდექსი 1,0 იყო. რაც შეეხება ფრაქციების გავლენას ლიმფოციტების ფუნქციურ აქტიურობაზე, ფრაქციის დრის ის 1,91; -3,89; -0,98; და -2,43 იყო.

ამგვარად, III ფრაქციამ იგივე ეფექტი გამოიწვია, როგორც მთლიანმა პრე-პარატმა - ის აინჰიბირებს სტიმულირებული ლიმფოციტების პროლიფერაციას. II ფრაქცია საპირისპიროდ მოქმედებს. სავარაუდოა, რომ II და III ფრაქციებში შემავალი ნივთიერებები განაპირობებს სწორედ პლაფერონ-ლბ-ს იმუნომა-მოდულირებელ მოქმედებას.

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OXIDATION PROCESSES IN B- AND C-HEPATITIS

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The work has shown that during chronic forms of hepatitis B and C there occurs decrease of antioxidant properties of the blood, erythrocyte hemolysis, generation of nitric oxide and xanthyn-oxidase. These, in their turn, promote hyperoxidation processes and destruction of the cells.

Key word: *oxidation, hepatitis*

It is difficult to overestimate the importance of viral hepatitis (HBV and HCV), which is one of the most pressing problems in current medicine, due to their wide spreading, severity of outcomes, and intensity. Therefore studying the HV-pathogenesis is very actual, especially on cellular and subcellular levels. We have studied oxidation processes and changes of cellular membranes during HBV and HVC. The blood paramagnetic centers have been observed by Electronic Paramagnetic Resonance (EPR) methods in persons affected with B- and C- hepatitis at acute, recovery and chronic stages of disease.

The blood EPR spectra were defined with the PE -1307-Radiospectrometer at the liquid nitrogen temperature. The results are shown in the Table below. The blood EPR specter in healthy men consists of oxidized ceruloplasmin (g=2,050), Fe^{3+}, transferrin (g=4,2) and free radical EPR signal of lesser intensity.

It was established that in the blood spectrum of the patients with acute HBV, EPR signal intensity of oxidized ceruloplasmin increased by 52% and the signal intensity of Fe^{3+} transferrin decreased by 12% in comparison with the control indices.

In HCV the signal of oxidized ceruloplasmin increases by 100% and signal intensity of Fe^{3+} transferrin decreased by 40%.

During acute HBV and HCV hepatitis in the blood EPR spectrum the appearance of intensive EPR signals of methemoglobin (g=6,0), Mn^{2+}- Mo^{5+}-containing and nitrogen oxide complexes with non-hemic iron takes place.

Moreover, there was revealed as well (g=2,01) EPR signal, which characterizes the inactive state of adrenoreceptors.

At HCV in the blood EPR specter the free Fe^{2+} EPR signal was registered as well.

Ceruloplasmin is the enzyme for carrying of multifunctional copper. It is

characterized with antioxidant, peroxidizing and aminoxidizing properties [1].

Increasing of signal intensity of its oxidized form in the blood EPR specter indicates the activation of lipids' peroxidation and reduction of antioxidant ability.

Ceruloplasmin causes iron oxidation, joining it to apotransferrin and to outflux of iron ions - which is powerful generator of lipid peroxidation from the blood, thereby contributes to apotransferin's antioxidant properties.

In its turn Fe^{3+} transferrin - iron-transferring protein is an active co-participant of hemo- and erithropoiesis.

So, reducing of Fe^{3+} transferrin EPR signal intensity in the blood specter during acute HBV and, especially in HCV, is the hallmark of inhibition of the blood antioxidant properties and hemopoiesis.

It is possible that sharp reduction of Fe^{3+} transferrin EPR signal intensity (40% in comparison with control indices) partially causes accumulation of free iron ions and occurrence of Fe^{2+} ions EPR signals in the EPR blood specter of patients with acute hepatitis.

The source of iron ions would be also hemolyzed erythrocytes and ferritin, discharged from distracted hepatocyes.

As it is known, Fe^{2+} is an initiator of free radical forms of oxygen creation and therefore generator of the lipids' peroxidation.

Activation of lipid peroxidation is followed by damage of cellular, and among them red cell membrane structures, revealed by appearance of intensive EPR signal (g=6,0) of methemoglobin in the blood EPR specter during B- and C-hepatitis in our study. Production of methemoglobin is a hallmark of cell hemolysis.

Occurrence of methemoglobin in the blood indicates reduction of the functional hemoglobin, which in its turn causes development of hypoxia through erithropoiesis suppression.

Existence of the EPR signals of Mo^{5+}-containing complexes in the blood EPR specter is the reason of intensified catabolic processes, activation of their enzymes (xanthinoxidase, sulfidoxidase) and their outflux from necrosed liver tissues to blood [3].

As it is shown in the Table, the value of signal intensity in HCV is especially high.

The appearance of EPR signal of nitrogen oxide complexes with non-hemic iron (FeS-No, g=2,03) in the blood specter indicates intensification of nitrogen oxide creation.

According to some authors, nitrogen oxide is inductor of immunoreactions [2]. Intensification of its generation has compensative character and is directed to the correction of disturbed methabolism. The long ischemic process, followed by hemodinamic disturbances and injury of hepatic tissue in hepatitis causes significant increasing of nitrogen oxide, which in its turn causes induction of free-radical peroxidation reactions, damages of cells and destruction of tissue, during the existence of superoxidradicals, derived after xantinoxidase reaction of free radical compounds.

The activation of nitrogen oxide and creation of xantinoxidasa are carrying on with participation of Ca2+ ions, that gives possibility to admit, that cytozolic Ca2+ ions accumulation in cells is the result of ischemic processes development and decreased energetic supply.

The existence of EPR signal contenting Mn2+ in blood specter indicates to the dearrangment of membrane structures integrity and destruction of cells. At the same time the increasing of Mn2+ ions would indicate to be free from transporting proteins, albumin.

Table 1

Changes of electric-paramagnetic centers in the blood during B- and C-hepatitis

	g=2.01	MetHb g=6.0	Fe^{3+} Transferrin g=4.2	Ceruloplas - min g=2.056	Mn^{2+} g=2.14	FeS-NO g=2.03	Mo^{5+} g=1.97
I Control (n=10)	-	-	38.0+2.5	21.0+1.5	40.0+2.0	-	-
II Acute B (n=36)	2.6+0.4	21.0+1.3	33.3+2.43	32.3+1.7	10.5+1.0	3.5+0.5	5.0+0.7
III Rec.B (n=20)	1.6+0.2	10.0+2.5	41.6+2.3	21.0+1.0	7.3+1.5	3.0+0.5	3.0+1.0
IV Chr.B (n=21)	1.2+0.1	11.0+2.0	33.0+2.0	26.0+1.0	7.5+1.2	3.0+0.5	3.0+1.2
V Acute B (n=13)	2.3+0.1	22.5+0.5	22.5+0.5	42.0+5.0	11.0+0.1	11.5+0.5	8.5+0.7
VI Chr.C (n=21)	1.6+0.4	16.5+1.5	37.5+2.5	37.5+2.5	10.5+0.5	8.25+0.75	5.5+0.5

g=2.01: II-III - P<0.1; II-IV - P<0.001; II-V - P<0.001; IV-V - P< 0.001;
MetHb (g=6.0): II-III - P<0.05; II-IV - P<0.001; II-V - P<0.001; IV-VI - P< 0.001;
Fe^{3+} (g=4.2): II-III - P<0.001; II-IV - P<0.001; II-V - P<0.001; IV-VI - P< 0.001;
Ceruloplasmin (g=2.056): I-II - P>0.05; I-III - P<0.01; I-V - P<0.001; I-IV - P<0.01: II-III - P<0.001; II-IV - P<0.001; IV - VI - p<0.001;
Mn^{2+} (g=2.14): I-II - P>0.05; I-III - P<0.01; I-V - P<0.001; I-IV - P< 0.01; II-III -P<0.001; II-IV - P<0.001; IV-VI - P<0.001;
FeS-NO (g=2.03): II-III - P<0.001; II-V - P>0.1; II-V - P>0.1; IV-VI - P< 0.001;
Mo^{5+} (g=1.97): II-III - P<0.001; II-IV - P<0.001; II-V - P<0.001; IV-VI - P< 0.001;

So, analyzing the indices we can conclude that during B- and C-hepatitis the following events take place:

–The activation of generators of oxygen free radical forms - nitrogen oxide, xantinoxidase, and Fe^{2+} creation, intensification of peroxidation and decreasing of the blood antioxidant ability;

–Hemolysis of erythrocytes, depression of hemopoiesis processes and development of hypoxia and ischemia;

–Inactivation of adrenoreactive structures;

–Destruction of membrane structures;

–Decreasing of albumin transporting function.

As it is shown, the indices, which are given in the Table correspond to the

period of B-hepatitis recovalescence.

Though oxidized ceruloplasmin and Fe^{3+} transferrin signals approach the control indices, but free-radical oxygen generators consistence of (nitrogen oxide and xantioxidasa) remains high yet, which indicates to high level of peroxidative processes.

It is proved by high intensity of EPR signals of methemoglobin and Mn^{2+}-containing complexes in the blood and this, in its turn, causes deficiency of functional hemoglobin, failure of albumin transporting function and facilitates the development of hypoxia and ischemia and creation of new generators of lipid peroxidation, so the circle is closed.

During the chronical forms of B- and C-hepatitis depression of the blood antioxidant abilities, red cells hemolysis, development of hypoxia and ischemia in organism, the creation of oxygen free-radical forms generators - nitrogen oxide and xantioxidase take place, facilitating the development of peroxidation processes and cell destruction.

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ჟანგვითი პროცესები **B-** და **C-**ჰეპატიტის დროს

ნ.ყიფიანი

თბილისი სახელმწიფო სამედიცინო უნივერსიტეტი

რ ე ზ ი უ მ ე

ჩვენს მიერ შესწავლილია ჟანგვითი პროცესები B- და C-ვირუსული ჰეპატიტების დროს. ელექტრონული პარამაგნიტური რეზონანსის (ეპრ) მეთოდით გამოკვლეულია B- და C-ჰეპატიტით დაავადებულთა სისხლის პარამაგნიტური ცენტრები დაავადების მწვავე, რეკოვალესცენციის და ქრონიკულ სტადიებზე. სისხლის ეპრ სპექტრებს ვსაზღვრავდით რადიოსპექტრომეტრზე РЭ 1307 თხევადი აზოტის ტემპერატურაზე.

მიღებული მონაცემების ანალიზის საფუძველზე შეიძლება დავასკვნათ, რომ B და C მწვავე ჰეპატიტის დროს ადგილი აქვს: ჟანგბადის თავისუფალრადიკალური ფორმების გენერატორების -

აზოტის ოქსიდის, ქსანტინოქსიდაზას, e^{2+} წარმოქმნის აქტივაციას, ლიპიდების ზეჟანგური ჟანგვის ინტენსიფიკაციას და სისხლის ანტიოქსიდანტური უნარის დაქვეითებას; ერითროციტებუს ჰემოლიზს, ჰემოპოეზის პროცესების დათრგუნვას და ორგანიზმში ჰიპოქსიის და იშემიის განვითარებას; ადრენორეაქტიული სტრუქტურების ინაქტივაციას; მემბრანული სტრუქტურების რღვევას; ალბუმინების სატრანსპორტო ფუნქციის დაქვეითებას.

B- და C-ჰეპატიტის ქრონიკული ფორმის დროს ადგილი აქვს სისხლის ანტიოქსიდანტური უნარის დაქვეითებას, ერითროციტების ჰემოლიზს, ორგანიზმში ჰიპოქსიის და იშემიის განვითარებას ჟანგბადის თავისუფალრადიკალური ფორმების გენერატორების– აზოტის ოქსიდის და ქსანტინოქსიდაზას – წარმოქმნას, რაც, თავის მხრივ, ხელს უწყობს პეროქსიდაციული პროცესების განვითარებას და უჯრედის დესტრუქციას.

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THE ROLE OF WINE BASIC COMPONENTS IN LYSINE BIOSYNTHESIS

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Using labeled compounds, the wine basic organic- and amino acids participating in lysine biosynthesis were detected. It has been established that during secondary alcoholic fermentation carbon atoms of α-ketoglutaric, acetic, oxaloacetic, glutamic acids and lysine are involved in the formation of lysine carbon skeleton. The lysine formed as a result of biotransformation of these compounds were identified in the fractions of yeast proteins, free amino acids, and wine amino acids.

Key words: *Lysine, secondary alcoholic fermentation, organic acids, amino acids.*

The pathways of essential amino acids biosynthesis in microorganisms has been a subject of intensive study. On the basis of experimental data obtained for the past four decades, the metabolic peculiarities of yeast, which are related to the specific enzyme conversions at separate stages of biosynthesis of essential amino acids, were revealed. These peculiarities were principally confirmed by studies of lysine biosynthesis in *Saccharomyces* yeast.

In this context it is of interest to study the possibilities of formation of essential amino acids in such extreme conditions as is the case during secondary alcoholic fermentation; high pressure of carbon dioxide, complex content of alcoholic medium restricted anaerobic conditions alongside with other factors exert an essential influence on the direction and intensity of cellular metabolism [5]. It is also noteworthy that under these conditions the biotransformation products of various sources of carbons available in the medium during wine champagnization to a large extent determine the quality and stability of ready production.

The present work was aimed at revealing the carbon sources, which more or less can play a definite role in lysine accumulation and can present more accurately the regularities of its biosynthesis during secondary alcoholic fermentation under extreme conditions.

MATERIAL AND METHODS

Saccharomyces vini-39 – an industrial strain of wine yeast – was used as a fermentation agent for bottle champagnization. Major products of alcoholic fermentation (ethanol, carbon dioxide), secondary products (glycerol, acetic acid), and organic acids of the Krebs cycle C -C were introduced into the fermentation medium. Wine basic amino acids were also used. Radioactivity of each ^{14}C-compound being introduced in the medium amounted 23.1 MBq per litre of wine material. Fermentation proceeded at 14-16°C. Assessment of the yeast and wine components was made as soon as the primary fermentation was over, using chemical, chromatographic, and autoradiographic methods [3, 9]. Radioactivity of the identified compounds was measured on the LKB type scintillation spectrometer Rackbeta.

RESULTS AND DISCUSSION

Our findings indicate that during secondary alcoholic fermentation not a single carbon of ethanol and glycerol takes part in lysine biosynthesis. But in the fermentation medium especially favorable conditions are created for CO refixation, as a consequence of which carbon atom of labeled CO is found in a lysine molecule of both yeast and wine amino acids. In this respect the specific role of organic acids is more important (Table 1). The table indicates that carbon atoms of the organic acids introduced into the medium are involved with different intensity in lysine biosynthesis. Major part of them remains in the yeast biomass during champagnization.

Table 1

Lysine biosynthesis from wine organic acids

Compound	% radioactivity of lysine in overall activity of amino acids identified in yeast	% radioactivity of lysine in overall activity of amino acids identified in wine
1^{14}C-acetic acid	14.2	11.3
2^{14}C-acetic acid	25.5	15.2
1^{14}C-pyroracemic acid	7.1	6.8
1^{14}C- α-ketoglutaric acid	32.3	18.8
1^{14}C-oxaloacetic acid	12.7	9.3
1,4^{14}C-succinic acid	6.6	5.7
2,3^{14}C-succinic acid	7.8	8.2
1^{14}C-malic acid	7.3	9.7

Especially high radioactivity of lysine is noted when 2^{14}C-acetic acid is introduced into the medium. Similar results were obtained when other yeast strains were employed [4]. It has been shown that the entire carbon skeleton of acetate participates in lysine biosynthesis. Although specific peculiarities of conversion is suggested by diverse intensity of incorporation of various carbon atoms of acetic acid in lysine mol-

ecule during champagnization.

Rather a stable source of lysine biosynthesis appear to be ketoacids of the Krebs cycle. Part of lysine formed by participation of their carboxylic carbons transfers into wine. Among the examined ketoacids $1^{14}C$ α-ketoglutaric acid is distinguished by its exceptional ability of lysine biosynthesis. In terms of the available evidence, kysine biosynthesis in yeast occurs in amino-adipinic way [2, 8], in which it is α-ketoglutarate that is one of the principal sources of lysine carbon skeleton.

Nowadays lysine-dependent auxotropic mutants have been obtained and assessed an d the stages of biosynthesis by relevant genes have been established [1].

For the lysine to be accumulated in wine conversion of amino acids appears to be of no less importance (Table 2). Various amino acids belonging in the group of triose, pentose, ketoacids were used in the experiments. The results of distribution of radioactivity indicate that as a result of conversion of each of them yeast forms a variable amount of lysine. Although the compounds examined by us during secondary alcoholic fermentation are actively involved in amino acid intermediate exchange, it is evident that the role of individual amino acid in lysine biosynthesis is rather diverse and is bound with specific conversions occurring during alcoholic fermentation in anaerobic conditions [6].

Table 2

Lysine biosynthesis from wine amino acids

Compound	% radioactivity of lysine in overall activity of amino acids identified in yeast	% radioactivity of lysine in overall activity of amino acids identified in wine
$1^{14}C$-glycine	4.9	4.4
$2^{14}C$-glycine	3.3	1.0
$3^{14}C$-serine	9.2	5.1
$1^{14}C$-alanine	14.1	6.1
$5^{14}C$-glutamic acid	24.0	11.3
$2^{14}C$-leucine	4.1	6.0
$1^{14}C$-phenylalanine	8.7	9.2
$1^{14}C$-proline	18.5	10.8

Determination of lysine both in the yeast and wine has shown that radioactivity is higher than the activity of all identified amino acids when $5^{14}C$-glutamic acid is introduced into the medium. It seems that in our conditions too is functioning the indicated amino-adipinic way of lysine biosynthesis, glutaminic acid just being one of its intermediates. In this process its further conversion occurs due to amino-adipatamino-transferase, which is present in *Saccharomyces cerevisiae* cells in cytoplasmic and mitochondrial for, [7].

A high radioactivity of lysine both in yeast and wine is noted also at the introduction of $1^{14}C$-proline into fermentation medium. It is clear that the direct matabolic link existing between proline and glutamic acid manifests itself during secondary alcoholic fermentation as well.

Participation of the compounds of quite diverse nature, available in the fermentation medium, in lysine synthesis clearly indicates the metabolic potential of the yeast used. This latter manifests itself in such a complex isolated ecological system as is created by bottle champagnization during secondary alcoholic fermentation in extreme conditions.

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ღვინის ძირითადი კომპონენტების როლი ლიზინის ბიოსინთეზში მეორეული სპირტული დუღილის დროს

ე.კირთაძე, ვ.აპლაკოვი

საქართველოს მეცნიერებათა აკადემიის ს.დურმიშიძის სახელობის მცენარეთა ბიოქიმიის ინსტიტუტი, თბილისი

რეზიუმე

შესწავლილია ეთანოლის, ნახშირორჟანგის, გლიცერინის, კრებსის ციკლის მჟავებისა და ღვინის ძირითადი ამინმჟავების როლი ლიზინის ბიოსინთეზში. დადგენილია, რომ ეთანოლისა და გლიცერინის ნახშირბადატომების გამოყენება ლიზინის ბიოსინთეზში მეორეული სპირტული დუღილის დროს არ ხდება. შესწავლილი ორგანული მჟავებისა და ამინმჟავების სხვადასხვა ნახშირბადატომები განსხვავებული ინტენსიურობით მონაწილეობს ლიზინის ბიოსინთეზში. წარმოქმნილი ლიზინის ნაწილი საფუვრის ბიომასაში რჩება, ნაწილი კი დუღილის პროცესში ღვინოში გადადის. ლიზინის ბიოსინთეზში ძირითად როლს გლუტამინის მჟავა და კრებსის ციკლის კეტომჟავები ასრულებენ.

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INVESTIGATION OF THE BLOOD PHYSICAL-CHEMICAL PROPERTIES AND HORMONAL STATUS IN THE PATIENTS WITH BENIGN PROSTATIC HYPERPLASIA AND PROSTATE ADENOCARCINOMA

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The quantitative alterations of the steroid hormones in the blood serum of the male patients with benign prostate hyperplasia and prostate adenocarcinoma was studied. The cholesterol concentration and the blood lipid volume were studied as well in the above patients. It was determined that in the both diseases there occurs decrease of the anioxidant properties of the blood, MetHb-complexes' formation, activation of the lipids' peroxidation, and decrease of the hemopiesis. Increase of the steroid hormones was found as well, however, the mechanisms of their elevation or decrease must be different. Increase of the cholesterol volume was observed in the both cases, although in a case of the malignant tumor the cholesterol volume was increased most sharply. It is suggested that on the background of increased cholesterol, the whole shift of the hormones promotes an origination and development of the disease.

Key-words: *Oncology, Prostate tumors, Blood serum, Cholesterol, Human, Male patients.*

The solving of oncological problems requires special investigation of molecular aspects of the tumor growth and development, because the basis of oncological diseases are the changes proceeding on the molecular level.

The prostate tumors, in general, maintain 3rd-4th places in the oncological disease incidence [6], while in the male oncological diseases they keep the first place [3].

An incessant growth of the prostate tumors and the lack of reliable non-invasive diagnostics methods set a number of the problems of the disease exposure. Therefore, treatment of the disease, and, first of all, establishing the mechanisms of its origin and development, revealing the prostate tumors' incidence at the early stages of the dis-

ease, and development of the new diagnostics means, all are the most pressing current problems.

It is noteworthy as well that the malignant tumors at the early stages of their occurrence and the number of alterations set up at the further stages, elicit disorders of the organism's regular activity, which are mostly displayed in the blood circulation system. That is why an investigation of the physical-chemical changes of the blood during the tumor growth processes should be considered an important means for solving of the above problems.

The prostate gland development, growth, and function are determined by the androgenes - testosterone (T) and dihydrotestosterone (DHT). Obviously the male prostate cancer elicitation and probably its development are also due to the androgenes [4]. It should be noted that the above androgenes belong with the steroid hormones. The steroid hormones include progesterone (P) and estradiole (E_2) as well.

SUBJECTS AND METHODS

The aim of the present study was investigation of the blood physical-chemical properties and the changes of the hormonal status in the patients with the malignant and benign prostate tumors. Insofar cholesterol is the precursor of the number of the steroid hormones [5], and its volume in the blood plasma correlates with the steroids' volume, indicating the degree of their synthesis, it was considered expedient to evaluate cholesterol volume in the blood plasma of the healthy donors and the patients with BPH and PAC.

The blood of 15 healthy patients (controls) and the same number of the patients with BPH and PAC (total of 15 patients each) was investigated. An average age of the patients was 60-75 years. The patients had a primary manifestations of the disease. The diagnosis and clinical stages were established by the rectal, histological, and echographic examinations. The cholesterol concentration was assessed by the test method (Cholesterol CHOD - PAP). The hormonal studies were carried our by the radioimmunological method.

RESULTS AND DISCUSSION

The experiments have shown that in the blood plasma of the PAC patients cholesterol content was increased about 3-times, while in the patients with BPH - about twice (Table 1). Increase of the cholesterol content in a course of the tumor growth period may be determined by the quantitative changes in the high density lipoproteins [6]. It was established that the latter promote "liberation" of cholesterol from the blood-vessel walls and, respectively, play a protective role against the free cholesterol volume growth. Besides, regulation of the cholesterol synthesis in the organism is carried out by the endocrine and humoral factors (hormones, vitamins, metal ions) [1]. Because the

prostate tumor is a process originating on the background of the hormonal disorders, the latter may activate the cholesterol biosynthesis, which probably determines its excessive volume and *vice versa*. The rise of the cholesterol volume in the tumor growth is viewed by some authors as a stress reaction of the organism against the developed tumor [2].

Table 1

Alterations of the Lipids' Volume in the Blood Plasma

Substance	Healthy donors	BPH	PAC
Plasma cholesterol, mmol/l	0.99±0.8	2.7±0.26	3.36±0.3
Amine-containing (Phosphatidylethanolamine, Phosphatidylserine) phospolipids, mg/1 mg lipid	0.09±0.02	0.12±0.06	0.18±0.06
Choline-containing (Phosphatidylcholine, Sphingomyelin) phospholipids, mg/mg lipid	0.14±0.04	0.17±0.04	0.28±0.1
Total of phospholipids	0.28±0.07	0.32±0.1	0.48±0.12
Lipids' peroxide oxidation	$0.0435 \cdot 10^{-5} \pm 0.02$	$0.077 \cdot 10^{-5} \pm 0.02$	$0.117 \cdot 10^{-5} \pm 0.04$

As was noted above, cholesterol is a primary source of the steroid hormones in the organism. In the regular physiological conditions transformation of cholesterol into the steroid hormones goes in the following way [4] (Fig. 1):

Our investigations have shown that all the three hormones (T, P, E_2) are significantly increased in the blood of the patients with both BPH and PAC, as compared to the blood of healthy donors. Notably, the above increase is less pronounced in the PAC patients than in the BPH cases (Table 2).

It is known that in the steroid hormones' bio-synthesis the main stage is pregnenolone enzymatic production from cholesterol. These enzymes firstly oxidize cholesterol and then eliminate its side-chain. On the next stage cytochrome P-450 catalyses cholesterol transformation into pregnenolone [5]. This stage is a determinant of the steroids' bio-synthesis velocity. Pregnenolone is a direct precursor of progesterone. Admitting that in both diseases of the male organism cholesterol transformation pathway (Δ^5-way) to pregnenolone and then to dihydropiandrosterone is increased because the cholesterol excess (Table 2), then it is understandable why increase of testosterone may go as in the one way: dihydropiandrosterone - androstadiene - testosterone (conditional way I), so in the other: dihydropiandrosterone - testosterone (way II). As to the lesser increase of testosterone volume in PAC, as compared to BPH, it may

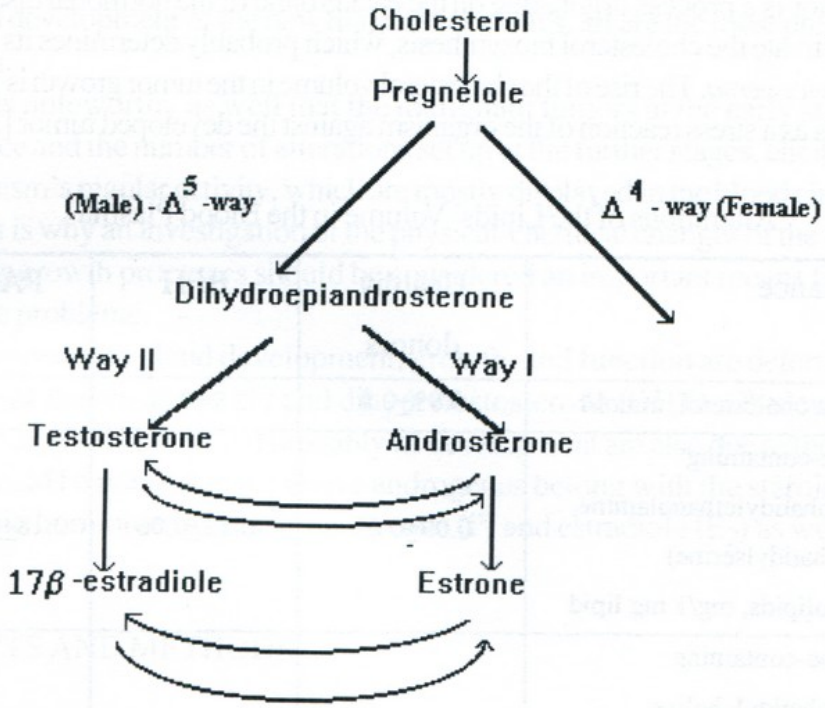

Fig. 1. Cholesterol-to-steroid hormones transformation pathways

be considered that in this case testosterone production from androstadiene (way III) is suppressed and the main load is on the dihydropiandrosterone - testosterone direction. Respectively, the volume of testosterone is decreased as well as the volume of estradiole in the blood of PAC patients, as compared to the blood of BPH patients (Table 2).

Table 2

The Quantitative Changes of Steroid Hormones (Progesterone, Testosterone, and Estradiole) in the Blood Serum (nmol/l)

Disease	Age	Number of patients	P nmol/l	T mg/ml	E_2 pg/ml
The donor blood The donor blood*	60-70 20-40	10	0.53±0.01 0.05±3.2	7.17±0.2 8-32	16.7±1.8 <55
BPH	60-75	15	13.1±2.0	16.14±1.9	51.41±3.2
PAC	60-70	15	4.6±0.9	13.3±1.2	36.5±2.1

* - The blood of the men 20-40 years of age (reproductive age) does not conform with the material of investigation, which is clearly shown by the healthy donors' blood.

The volume of estradiole, as compared to the blood of healthy patients, was increased in the both cases as follows: the blood of healthy donors, PAC, BPH. The

literary data testify that testosterone undergoes transformation in the two ways. The first is an activation way, during which testosterone is transformed into dihydrotestosterone, and the second way is an aromatization one, which results in production of estradiole [1]. We suggest that in a course of testosterone increase in the patients with BPH, its transformation by the aromatization way increases as well (Table 2). It is known also that concomitant administration into the organism of progesterone and estradiole, the former decreases the effect of the latter [5], i.e. the antiestrogene action of progesterone is determined by the long-lasting blockage of the hormone's receptors and, respectively, by accumulation of the free estradiole. Therefore, in a case of BPH, as compared to PAC, the volume of progesterone is increased. Accordingly, the estradiole volume should be increased as well, which was found in our experiments (Table 2). All the afore-mentioned allow to put forward one more suggestion on the estradiole increase. As to the patients with PAC, in such a case the process of testosterone transformation into dihydrotestosterone, or an increase of the activation way, could be presumed. It should be mentioned as well that in the blood of the PAC patients increased accumulation of dihydrotestosterone is the case [4, 6]. Decrease of estradiole in the blood of the PAC patients, as compared to the BPH patients, may be due to depression of testosterone by the aromatization way, or to decrease of the progesterone volume in the PAC patients, which, in its turn, induces decrease of the free estradiole.

In the next series of our work the other lipids' - phospholipids' - quantitative changes in the blood during above diseases were evaluated. Investigations have shown that both amine-containing and choline-containing phospholipids as well as the total volume of phospholipids do increase (Table 1). It should be noted here that in the blood plasma of the PAC patients activation of the LPO was found as compared to the healthy donors' blood, while in the BPH patients' blood the LPO changes were negligible (Table 1).

Organism has an inborn antioxidant systems, which impede the LPO. As a corroboration of this fact some segments of EPR-spectra of the metabolic paramagnetic centers from the BPH- and PAC patients' blood are presented in Fig. 2.

One of the organisms' antioxidant chains is presented by transferrin, which is an iron transporting protein, and an active participant of the erythro- and hemopoiesis [7]. The apotransferrin ability to bind the iron determines its antioxidant properties.

Investigations have shown decrease of the Fe^{3+}-transferrin EPR-signal (Fig. 2) in the PAC patients' blood as compared to the helthy donors'- and BPH patients' blood. This fact points at reduced antioxidant properties of the blood [8], activation of the LPO, and decrease of the hemopoiesis. The latter is well in concordance with the hemoglobin volume in the blood of the PAC patients - in such a case the hemoglobin volume is relatively decreased (Table 3).

Thus, it could be suggested that development of the human PAC elicits sharp activation of the LPO, which, probably, manifests certain reactions of the pathology and is reflected in decrease of the blood antioxidant properties (decrease of Fe^{3+}-transferrin EPR-sygnal intensity), and methemoglobin (MetHb, g=6.0) production. The EPR-sygnal characteristic of the MetHb never occurs in the healthy donors' blood, while in the PAC the sygnal is increased as compared to the BPH.

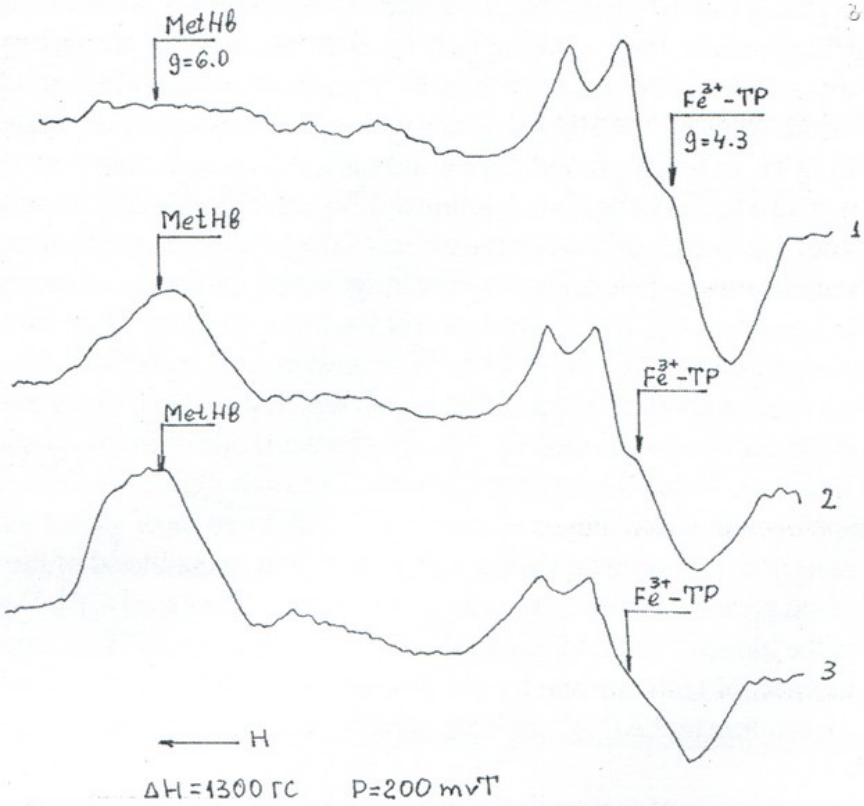

Table 3

The Hemoglobin and Erythrocyte Quantitative Changes in the Blood of the BPH, and PAC Patients

Item	Healthy donors	BPH		PAC	
		-	$PING_1$	$G_{(1-5)}$	$G_{(5-10)}$
Hemoglobin	130-160	134.4±7.8	123.8±70	120.6±3.4	115.1±5.1
Erythrocyte count	4.0-5.0	4.36±0.8	4.36±0.9	4.35±0.5	3.9±0.5

Our investigations have shown that in the blood of the BPH- and PAC patients the rise of steroid hormones does occur, although the mechanisms of their increase or decrease are different. In the both cases the volume of cholesterol - the primary source of the steroid hormones - is increased but in a case of the malignant tumor the cholesterol volume is risen sharply. It is suggested that on the background of increased volume of cholesterol the entire hormonal system's shifts promote a disease origination and development. Thus, the certain steps were made to elucidate which factors are most important in the above pathologies and which determine the first shove in the tumor elicitation.

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პროსტატის კეთილთვისებიანი ჰიპერპლაზიით და ადენოკარცინომით დაავადებული მამაკაცების სისხლის ფიზიკურ-ქიმიური თვისებებისა და ჰორმონული სტატისის ცვლილებების შესწავლა

ნ.კოტრიკაძე, მ.ჭელიძე, მ.ალიბეგაშვილი, ლ.მანაგაძე, თ.ჩიგოგიძე, დ.კივილაძე, ა.ხომასურიძე, ნ.ტაბატაძე, ნ.კვაშილავა, ბ.ლომსაძე, მ.ცარციძე

ი.ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტის ბიოლოგიის და მედიცინის ფაკულტეტი, ბიოფიზიკის კათედრა და კანცეროგენეზის მოლეკულური მექანიზმების სამეცნიერო-პრობლემური ლაბორატორია, საქართველოს ჯანდაცვის სამინისტროს თბილისის ა.წულუკიძის სახელობის უროლოგიის სამეცნიერო-კვლევითი ინსტიტუტი

რ ე ზ ი უ მ ე

შესწავლილია პროსტატის კეთილთვისებიანი ჰიპერპლაზიითა და პროსტატის ადენოკარცინომით დაავადებული მამაკაცების სისხლის შრატში სტეროიდული ჰორმონების რაოდენობრივი ცვლილებები. აღნიშნული დაავადებების შემთხვევაში აღნიშნულია ქოლესტერინის კონცენტრაციისა და სისხლის ლიპიდების რაოდენობრივი ცვლილებები.

აღმოჩნდა, რომ პროსტატის კეთილთვისებიანი ჰიპერპლაზიით და ადენოკარცინომით დაავადებული მამაკაცების სისხლში ადგილი აქვს სისხლის ანტიოქსიდანტური თვისებების დაქვეითებას, მეტჰემოგლობინის კომპლექსის წარმოქმნას, ლიპიდების ზეჟანგური ჟანგვის აქტივაციას და ჰემოპოეზის დაქვეითებას. გარდა ამისა, ადგილი აქვს სტეროიდული ჰორმონების რაოდენობის მატებას, იმ განსხვავებით, რომ მატების ან კლების მექანიზმი სხვადასხვაა. ადგილი აქვს ქოლესტერინის, როგორც სტეროიდული ჰორმონების პირველწყაროს, მატებას ორივე შემთხვევაში, იმ განსხვავებით, რომ ავთვისებიანი სიმსივნის დროს ქოლესტერინის რაოდენობა მკვეთრად გაზრდილია. ვვარაუდობთ, რომ ქოლესტერინის გაზრდის ფონზე ჰორმონული ძვრების ერთობლიობა აძლიერებს დაავადების წარმოქმნას და განვითარებას.

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THE FOUNDATIONS OF BIOMECHANIC OF RIDING THERAPY AT DISPLASTIVE SCOLIOSIS

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Riding Therapy differs from all existing methods of medical physical culture because of the treatment is made in such necessary merging that called "patient-horse." model. I.e. ill is represented in role of rider, and one uniform biological system named "horse-patient" is meant as the rider. In interaction of these two live creatures both the form and contents of a procedure is expressed. Proceeding from it, movements and actions of a horse and "patient-horse" present interdependent and uniform process, based on biological feedback.

Key words: *Riding Therapy, Displastive Scoliosis, antigravity system, training, static load, trunk, correction.*

The purpose of the work is to determine the effects of Riding Therapy (RT) at Displastive Scoliosis (DS).

During riding a trunk of a child has balanced. Its general center of gravity at correct landing has projected on center of gravity of a horse. Not only whole body is in balance, but also other its chains. In area of curvature of the spine, as well as in other its parts, the action of weight of a body is counterbalanced by efforts of muscles and chords. The resultant force of all these moments on each of levels of a backbone passes through a pulpous nucleus of an interbone discus. In time of riding the functions of tuning movements are focused, on the other hand, to approach of a trunk gravity center to longitudinal axis and to of horse gravity center, and on the other hand - to creation of optimum conditions for work of muscles, compensating occurrence of non-uniform distribution of weights under the scoliosis. Both these moments have adapting character, they are focuses on the expenditures of energy, necessary for stabilization of a vertical standing of a trunk, a support of which is a deformed backbone.

In each cycle of riding (training and working lynx, gallop) muscles work in overcoming and in making a concession mode, which for muscles of a trunk is main, as in this mode muscles generate the highest activity [4].

During riding the part of a load is perceived by thorax and abdominal cavities, filled by air and liquid. A role of these cavities, as at this time due to work of stomach

muscles the rigidity of walls of cavities is increased and pressure in thorax and abdominal cavities grows. At action of the gear of pressurization a load on backbone in its breath and lumbar parts is reduced.

At riding the form of a backbone varies, basically, because of its lumbar part. This part represents original system, which, on the one hand, permits to make movements necessary for riding, and, on the other hand, opportunity provides because of the top part of a backbone to save vertical standing.

In time of riding exercises not only dynamic character are executed, but also static character, at which muscles work in the isometric mode. The amortization of the vertical loads on backbone is carried out by interbone discus. During deformation of a backbone the pulpous nucleus is displaced in convex party of curvature on top of primary curvature. At riding the training lynx a tendency occurs to moving of decentralized pulpous nucleuses to center and, hence, active moving of "stacking" bones as promotes correction of curvature.

The equilibrium standing of a trunk is supported not only efforts of muscles, but also other forces - chords. Yellow chords are opposite to the chords of backbones' bodies. They functionally unload disks, preventing to their excessive compression. Taking into account fact of a preliminary tension of chords, there is possible to approve, that they brake lightly even the smallest mutual moving of backbones.

At standby position the ligament apparatus DS ills spread in greater degree, than at health [1, 2, 5]. In time of riding lower extremities of rider "are switched off" in maintenance of a trunk. Under scoliosis in time of standby or walking the displacement of segments of a trunk, caused by deformation of a backbone, is completely compensated, the general center of gravity saves a former situation with the help of the whole body (trunk, basin, lower extremities, etc.) while at riding all this occurs with the help of a trunk, i.e. the rider's center of gravity passing basin and lower extremities "reaches the balance", that undoubtedly facilitates process of self-correction of a backbone. The direct contact (through saddle) rider's trunk with horse enables a rider's trunk to feel and "to realize" nuances of movement of a horse and gives the correct motor response to these movements. Achievement of asynchronous movement of child of horse, i.e. the creation of uniform biological system is really a basis of self-correction of a curvature of the spine. The achievement of that condition is necessary to consider as the beginning of correct medical effect of riding to ill child.

MATERIALS AND METHODS

Objects of supervision were 517 ills of DS of different degree and localization in the age of 7 to 15. From them the number of the children with breast Displastive Scoliosis of 1st degree is 116, of 2nd degree is 58 and the number of the children with breast-lumbar Displastive Scoliosis of 1st degree is 117 and of 2nd degree is 71. The number of children with lumbar Displastive Scoliosis of 1st degree is 92 and of 2nd degree is 43.

We have been carrying out the following clinicofunctional researches: some

anthropometric researches; determination of strength and static endurance of the stomach muscles and body; functional probation of cardio-vascular system; PWC_{170} and roentgenopraphy of the spine (once a year).

The purpose of the first preparatory period was antigravitational reorganization of organism of ill from standing position to the situation of sitting on horse, i.e. riding training, development of balance in saddle, achievement of correct easy landing on all types of horse pace (step, lynx, gallop) [3].

The first period takes approximately 20-30 sessions.

The purpose of the second period of RT is to correction of curvature of the spine, creation of the muscle belt around of it. The sessions are carried out on specially selected horses (having into the account the amplitude of horse back's movement), basically on working saddle.

RESULTS AND DISCUSSION

In the results of conducted treatment was observed: reduction of a curvature arch for a functional deviation of a backbone. Treating 326 patients with the use of RT we observed the following: reduction of a curvature arch for a functional deviation of a spine in 178 patients; there hasn't been any change in 144 cases, there has been progress in four cases.

Treating 197 patients with the use of therapeutic physical training. There hasn't been any change in 105 cases. There has been progress in 9 cases.

Taking it all into consideration, the cases of improved of functional difference of curvature turned out to be much more ($p<0.001$) in the main group and cases of progress ($p<0.001$) in the control group (Table 1); increase of static endurance of straightening muscles of a trunk and stomach ($p<0.001$); increase of general physical serviceability (from 214.51 ± 2.57 kgm per minute up 510.65 ± 3.29 kgm per minute ($p<0.001$); improvement of the phsychoneurologic status.

At RT procedure there is mainly the training straightening muscles of a trunk, the load on which is given both as symmetric and asymmetric as well. To increase a load during the correction of curvature various gymnastic tools (stick, ball, etc.) are applied. During the correction of breast-lumbar and lumber scoliosis important significance there is the training of breath and lumbar muscle. During facilitated lynx there is the active training indicated muscle as at five-minute average lynx it is reduced and weekend 400-500 times [3, 5]. By change of stirrup levels symmetric and asymmetric training of breath and lumbar muscle is possible that is significant the correcting factor.

CONCLUSION

Thus, the Displastive Scoliosis treatment, as well as other diseases, is effective in the event that is pathogenic. From this point of view the scoliosis treatment consists of consecutive effect first of all on decentralized pulpous nucleus of a interbone discus, and then - on linking and muscle system [1, 5].

All that can be achieved with the help of corrective mechanisms of Riding Therapy that will determine high-efficiency of treatment of Displastive Scoliosis (1st and 2nd degree).

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რაიტთერაპიის ბიომექანიკის საფუძვლები დისპლაზიური სკოლიოზის დროს

მ.ლორია

თბილისის დიპლომისშემდგომი განათლების სამედიცინო აკადემია

რ ე ზ ი უ მ ე

ნაშრომში მოცემულია რაიტთერაპიის, ანუ სამკურნალო ცხენოსნობის ბიომექანიკის არსი, რამაც შექმნა საფუძველი იმისათვის, რომ იგი გამოყენებული ყოფილიყო დისპლაზიური სკოლიოზების (I და II ხარისხი) დროს.

დისპლაზიური სკოლიოზით დაავადებული ბავშვის ვერტიკალური მდგომარეობისათვის დამახასიათებელია პარავერტებრალური კუნთების ასიმეტრიული მუშაობა. დგომის დროს მყესოვანი აპარატი სკოლიოზით დაავადებულებს მეტად აქვთ დაჭიმული, ვიდრე ჯანმრთელებს. ამხედრება ამსუბუქებს გრავიტაციული ძალების მოქმედებას, ვინაიდან ადამიანის ქვედა კიდურები შეცვლილია ცხენის კიდურებით და წონასწორობის, ანუ ვერტიკალური მდგომარეობის შენარჩუნება მთლიანად განპირობებულია ხერხემლის უშუალო კონტაქტით (უნაგირის საშუალებით) ცხენის ზურგთან. ანტიგრავიტაციული სისტემიდან ქვედა კიდურების გამორთვა ერთის მხრივ, იწვევს

ხერხემალზე დატვირთვის შემცირებას, ხოლო მეორეს მხრივ, ხერხემლის გამშლელი და მუცლის პრესის კუნთების გააქტიურებას. მუცლის პრესის გამაგრება განაპირობებს გულმკერდისა და მუცლის ღრუში წნევის ზრდას, ამიტომ გაბერვის მექანიზმის მოქმედებით დატვირთვა ხერხემლის გულმკერდისა და წელის ნაწილში მცირდება.

მხედრის სტატუსის მიღწევისათვის აუცილებელია ცხენის ნებისმიერი ალურის (ნაბიჯი, ჩორთი, ნავარდი) დროს მხედრისა და ცხენის სიმძიმის ცენტრების თანხვედრა, რაც გულისხმობს იმას, რომ ბავშვმა ცხენის ზურგზე წარმოშობილი სულ უმნიშვნელო რხევებიც (მოძრაობები) კი შეიგრძნოს თავისი ტორსით და მისცეს მასზე სწორი მოტორული პასუხი. ასეთი მდგომარეობის მიღწევას ქვეცნობიერად ახლავს თვითგაჭიმვის პროცესი, რაც განპირობებულია აღნიშნული კუნთების უპირატესად სტატიკური დატვირთვით. ბავშვისა და ცხენის სინქრონული მოძრაობების მიღწევა უნდა ჩაითვალოს სწორი სამკურნალო ზემოქმედების დასაწყისად.

ყოველივე აღნიშნულის გათვალისწინებით, შემუშავებულ იქნა რაიტთერაპიის ორიგინალური მეთოდიკა, რომელიც შესდგება ორი პერიოდისაგან. პირველი პერიოდის მიზანია ბავშვის ანტიგრავიტაციული სისტემის გადაწყობა ფეხზე დგომიდან ცხენზე ჯდომარე, ანუ მხედრის სტატუსის მიღწევა. მეორე პერიოდის მიზანს შეადგენს ხერხემლის გამრუდების კორეგირება მის გარშემო ძლიერი კუნთური კორსეტის შექმნის გზით.

კლინიკურ-ფუნქციური და რენტგენოლოგიური მონაცემებით დადგენილია რაიტთერაპიის ეფექტიანობა დისპლაზიური სკოლიოზის დროს, რაც გამოიხატა ფუნქციური კომპონენტის ხარჯზე გამრუდების პირველადი რკალის შემცირებაში (ზბ0ძ01), ხერხემლის გამშლელი მუცლის პრესის კუნთების სტატიკური გამძლეობის ზრდაში(ზბ0ძ01), ორგანიზმის ზოგადი შრომისუნარიანობის გაუმჯობესებაში (ზბ0ძ01) და სხვა.

ამგვარად დადგენილ იქნა, რომ რაიტთერაპია დისპლაზიური სკოლიოზის (I და II ხარისხი) ეფექტიანი, პათოგენეზური მკურნალობის მეთოდია, რომელიც ხელს უწყობს დეცენტრირებული პულპოზური ბირთვის ცენტრირებას ან ტენდენციას ცენტრირებისაკენ, ხერხემლის გარშემო ძლიერი კუნთური კორსეტის შექმნას და შესაბამისად გამრუდების კორეგირებას.

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THE STEM HEIGHT-DEPENDENT PHYSIOLOGICAL PECULIARITIES OF THE VINE AND THEIR ASSOCIATION WITH FROST-RESISTANCE

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Experimentally obtained results have shown that the relative frost-resistance in the high-stem (200 cm) vine is higher than in the low-stem (50 cm) one, which is due to the higher physiological activity of the high-stem vines during the vegetation period. It was found as well that the high-stem vine is characterized with the deeper resting phase.

Key words: *Photosynthesis, Respiration, Transpiration, Field observations, Delayed fluorescence, Phosphoroscopy, Vine.*

It is known that in practical viticulture, with an aim to increase the vine frost-resistance, such an agricultural means as increasing of the vine stem height are used. The high-stem forms of the vines suffer less negative impact of the frosts than the low-stem vines.

The goal of our investigation was the study of those physiological processes, which occur in the high-stem vine and determine its frost-resistance.

MATERIAL AND METHODS

The two varieties of the vine - Chinuri and Goruli Mtsvane - were investigated. Photosynthesis and respiration intensity were evaluated in a field conditions, in a specially designed chambers. Intensity of the delayed fluorescence (DF) in the vine leaf and one-year shoots' felloderm was investigated by means of phosphoroscopy [3].

RESULTS AND DISCUSSION

First of all we evaluated a relative frost-resistance in the vines with the different height-stems as follows: the shoots, in the laboratory conditions, were placed at various low temperatures (-20^0C, -22^0C), and the number of the damaged buds was calculated. As one can see in the Table 1, the high-stem (200 cm) vine shoots are characterized with relatively high frost-resistance, as compared to the low-stem (50 cm) vine. At the same time, the highest relative frost-resistance was found in the Chinuri variety (Table 1).

Table 1

The frost-resistance of the vine varieties - Chinuri and Goruli Mtsvane

Vine variety	Stem height, cm	Incubation temperature	Damaged buds, %	
			December	January
Chinuri	50	-20⁰C	45	28
Chinuri	200	-20⁰C	36	23
Chinuri	50	-22⁰C	100	86
Chinuri	200	-22⁰C	96	79
Goruli Mtsvane	50	-20⁰C	50	34
Goruli Mtsvane	200	-20⁰C	42	30
Goruli Mtsvane	50	-22⁰C	100	91
Goruli Mtsvane	200	-22⁰C	100	83

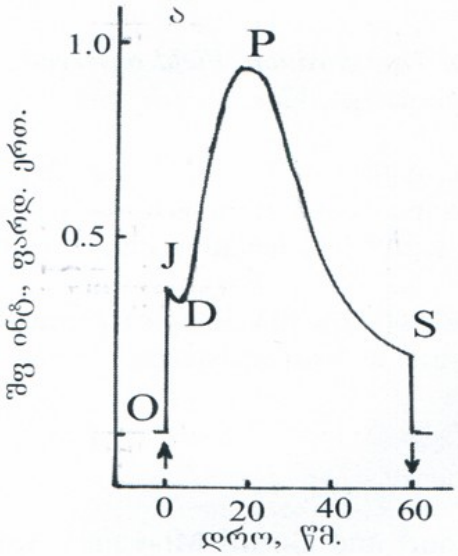


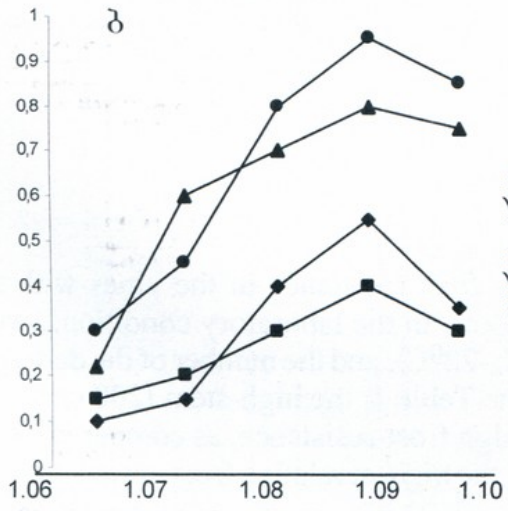
Fig. 1. Intensity of delayed fluorescence in the photosynthetic apparatus in the leaf (a, b) and felloderm of the Goruli Mtsvane vine, measured by seasons.

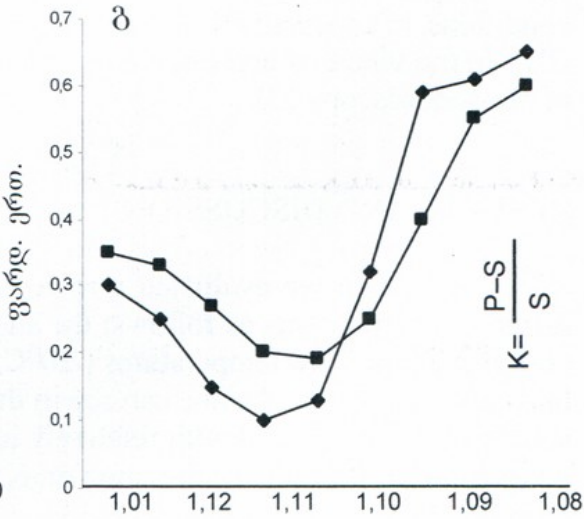
a - Inductive point (a a - light on, and light off, respectively);

b - D-P phase (1, 1¹), P-S phase (2, 2¹);

g - coefficiet K=P-S/S;

(1, 2 - 200 cm-high vine, and 1¹, 2¹ - 50 cm-high vine)





With an aim to explain the results obtained, the primary processes of the vine leaf photosynthetic apparatus were investigated by the DF method, according to the ontogenetic phases. The Fig.1[a] shows the vine leaf DF induction point. It is known [2] that the D-P phase of induction points is in a direct connection with the photophosphorillation process and correlates with the DpH gradient value apposited onto the photomembrane. The P-S phase characterizes the CO_2 fixation. The higher the difference between P and S intensities, the higher is the CO_2 fixation velocity. As reported in literature [1], a single light-impulse-induced DF may serve as an argument on the quality of oxygen-releasing system's functioning: the ratio of the DF intensity elicited by third light-impulse (J_3) and the intensity obtained after the first impulse (J_1), is in a direct connection with the oxygen-releasing system. The Fig.1[b] shows that during the whole ontogeny the photophosphorillation - (D-P) and the CO_2 fixation are most effective in the high-stem vine. Examination of the J_3/J_1 ratio has shown that in the high-stem vine the oxygen-releasing system works with the highest loading as well.

The analogous results were obtained during investigation of the leaf photosynthesis and respiration intensities in a course of ontogeny (Table 2). The variety-bound differences were revealed - the Chinuri variety is characterized with the highest intensity of photosynthesis and traspiration in both florescence and technical ripeness, while in respiration intensity no significant differences were found. These results indicate the high viability of the Chinuri variety in the given micro-zone. As to the stem height, relatively high intensities of photosynthesis and respiration were found in the high-stem (200 cm) vines, in the both varieties and in a whole ontogenetic period. Similarly high level of transpiration was determined as well. Therefore, according to the data obtained, it could be concluded that the 200 cm-stem vines, at all stages of ontogeny, are characterized with high physiological activity, as compared to the 50 cm-stem vines.

Table 2

Intensity of the vine (Chinuri and Goruli Mtsvane varieties) leaf photosynthesis, respiration, and transpiration, by the ontogenesis phases

The literary data [5] indicate that the plant frost-resistance is closely related to

Variants	Photosynthesis intensity, CO_2 (mg)		Respiration intensity, CO2 (mg)		Transpiration intensity, (g)	
	Florescence	Technical ripeness	Florescence	Technical ripeness	Florescence	Technical ripeness
Goruli Mtsvane, 200 cm	5.7	2.6	1.65	1.06	3.47	1.88
Goruli Mtsvane, 50 cm	4.3	2.7	2.02	1.50	2.71	1.74
Chinuri, 200 cm	6.1	3.2	1.31	1.20	4.22	2.18
Chinuri, 50 cm	4.5	3.6	2.17	1.49	2.77	2.69

the phase of organic rest, during which those organic alterations do occur, which determine its ensuing frost-resistance. In our earlier investigation we proposed an original method for evaluation of the vine organic rest phase dynamics. It was found that in felloderm of a one-year shoot of the vine an alteration index of the photo-membrane K=P-S/S (where: P-S is a DF inductive maximum, and S - stationary value of DF) is in a direct relation with the resting phase. The Fig.1^{c} shows that into the resting phase the low-stem (50 cm) vine enters first, and than, almost two weeks later, - the high-stem (200 cm) one. However, the high-stem vine is characterized with the much deeper resting phase. Our investigation shows that just this very factor determines the high frost-resistance of the plant [4].

The data obtained provide a proof for conclusion that the high-stem vine, as compared to the low-stem one, is characterized with higher frost-resistance. That is why, in a field conditions, in the frosty zones, in a case of the low temperatures the high-stem vines suffer less. Obviously, in such a case certain role playes a difference between the temperatures on the high- and low-stem vines' levels (e.g., at 50 cm and 200 cm heights the temperature difference in winter may be 0.5^{0}C), but the leading part in the frost resistance belongs to those physiological processes, which occur in a course of ontogenesis. Our results show that in the high-stem plant the primary photosynthetic processes are executed most effectively, e.g. photophosphorillation, oxygen-releasing system, the CO_2 fixation; more intensive are the photosynthesis, respiration, and transpiration systems. According to the above-mentioned it could be suggested that the high-stem vines are better prepared for the winter. This is supported by the fact that the high-stem vine has a deeper resting phase, which is a premise for a relatively high frost-resistance.

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ვაზის მცენარეობის ინსტიტუტის

ფიზიოლოგიური თავისებურებები და მათი კავშირი ყინვაგამძლეობასთან

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შესწავლილია სხვადასხვა შტამბის სიმაღლის მქონე ვაზის ყინვაგამძლეობა. საცდელად აღებული იყო ცხვადასხვა შტამბის მქონე (50 - 200 სმ) ორი ჯიშის ვაზი - ჩინური და გორული მწვანე. საველე პირობებში განსაზღვრულია სუნთქვის, ფოტოსინთეზის და ტრასპირაციის ინტენსიურობა. ფოტომემბრანის მუშაობის ინტენსიურობა შესწავლილია შენელებული ფლუორესცენციის მეთოდით. აღმოჩნდა, რომ მაღალშტამბიანი (200 სმ) ვაზის ფარდობითი ყინვაგამძლეობა მეტია დაბალშტამბიან (50 სმ) ვაზთან შედარებით. აღნიშნული შედეგები გამოწვეულია იმით, რომ მთელი ვეგეტაციის პერიოდში მაღალშტამბიან ვაზში ფოტოსინთეზის პირველადი პროცესები - ფოტოფოსფორილირება, ნახშირორჟანგის ფიქსაცია და ჟანგბადგამომყოფი სისტემის მუშაობა - უფრო ეფექტურად მიმდინარეობს. ასევე მაღალი აქტიურობით მიმდინარეობს მაღალშტამბიან ვაზში ისეთი ფიზიოლოგიური პროცესები, როგორიცაა ტრანსპირაცია, ფოტოსინთეზი და სუნთქვა. სწორედ ეს განაპირობებს ვაზის ყინვაგამძლეობის ზრდას.

შენელებული ფლუორესცენციის მეთოდით შესწავლილია მოსვენების ფაზაში ვაზის შესვლის დინამიკა. აღმოჩნდა, რომ მოსვენების ფაზაში პირველად შედის დაბალშტამბიანი (50 სმ) ვაზი, მაგრამ მაღალშტამბიანი (200 სმ) ვაზისათვის დამახასიათებელია გაცილებით "ღრმა" მოსვენების ფაზა. სწორედ ესაა ერთ-ერთი ფაქტორი, რომელიც განაპირობებს მაღალშტამბიანი ვაზის მეტ ყინვაგამძლეობას. დადგენილია, რომ ჯიში ჩინური უფრო ყინვაგამძლეა, ვიდრე გორული მწვანე.

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INCREASE OF QUALITY AND BIOLOGICAL VALUE OF THE BREAD BY ADDING THE PROTEIN CONCENTRATE FROM GRAPE POMACE

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With an aim to increase nutritional and biological value of the bread the protein concentrate from grape pomace was used. By chemical (chemical score) and biological (experiments in rats) methods we studied the biological value of the bread, which contained the protein concentrate from grape pomace. It turned out that addition of 1.2 and 5% of the protein comcentrate to the bread significantly increases its biological value.

Key words: *Food additives, Bread, Nutritional value, Grape pomace, Proteins.*

The bread plays an important role in the diet of people, satisfying about 1/3 of their need in calories. In this regard it was considered to increase those qualities of the bread, which satisfy the needs of the rational diet.

Protein of food grains is poor in some amino acids (lysine, threonine), which determines their low biological value [7]. In the works of many investigators it is recognized reasonable to increase the biological value of the bread with products, which are good sources of the proteins [1, 6, 9].

The most rational way of usage additional resources of the proteins for nutrition, on the basis of mutual enrichment, is a study of compositions of a protein. This effect should be based on theoretical preconditions of amino acid balance and possibilities of its practical realization.

The process of production of food products with balanced protein compositions and increased biological value consists of the three stages: amino acid balancing; material implementation of calculation; medical-biological value of a product [10-11].

One of the most important indicators while production of enriched bread for general use is a low cost of the protein enrichers. In this regard the most favourable for the baking industry will be usage of the proteins of vegetable origin, particularly protein concentrate from grape pomace. Mentioned concentrate is obtained from the remains of industrial processing of a grape and meets all above mentioned requirements.

In the present study we offer the results of usage of the protein concentrate from grape pomace for increase the nutritional and biological value of the product.

In the study participated G. Karchava, G. Grigorashvili, N. Maglaperidze

MATERIAL AND METHODS

For achievement the goal we determined the amino acid content of a protein concentrate from grape pomace, protein of a flour of 1st grade and control and experimental samples of the bread by automatic amino acid analyzer [5]. Optimal correlation of the two proteins, when the protein mixture had the highest biological value, was determined by mathematical method [4].

For studying the efficiency of a protein composition after adding the protein concentrate into the bread we carried out the experiment in 40 rats (4 groups, 10 rats in each group) with initial body weight of 55 g. Animals of experimental (I, II, III) groups received the bread containing 0.8; 1.6 and 4.0 g of the protein concentrate (6.25; 12.5 and 31.25% according to protein). Animals of control group received the bread without additives.

Biological value of the bread protein we determined by combined method [8]. Duration of the experiment was 28 days. During last 5 days we carried out balance investigations [2]. Balance of nitrogen was calculated by generally accepted method [3].

RESULTS AND DISCUSSION

Amino acid content and chemical score of flour and grape pomace protein are presented in the Table 1.

Table 1

Amino acid content and chemical score of grape pomace protein concentrate and grain protein

Amino acid	ideal protein for adults		grape pomace protein concentrate		grain protein	
			In correlation with ideal protein			
	a	s	a	s	a	s
isoleucin	4,0	100	4,1	103	3,5	88
leucin	7.0	100	7.1	101	7	101
leusin	5.5	100	7.0	127	3.0	54*
methionin+cistin	3.5	100	2.4	70*	4.3	123
phenylalanin+ thirosin	6.0	100	8.5	141	8.1	135
threonin	4.0	100	4.4	110	3.1	78*
triptophan	1.0	100	0.8	80*	1.2	120
valin	5.0	100	5.6	112	4.7	94
chemical score, %	–	100	–	70.0	–	54.0

Note: a - content of amino acid on g/100gr protein;
5 - chemical score; *,*,* - accordingly first and second limiting amino acid.

As shown in the Table, grape pomace protein concentrate is limited by the content of sulphur-containing amino acids (methionine and cysteine), and triptophane, while content of these amino acids in grain protein is higher than their content in ideal protein. Grain proteins are limited with the content of lysine and threonine, while content of

these amino acids in protein concentrate is higher than in ideal protein. Hence, the real mutual enrichment of these proteins can be achieved after their combination.

Determination of optimal correlation of grain protein and grape pomace protein concentrate was carried out by mathematical method. According to the data obtained from the computer-aided calculation, a bit higher (in comparison with requirement of adult people in amino acids) content of sulphur-containing amino acids, triptophane, lysine, threonine in protein preparations, makes possible to add the protein concentrate by ratio of 44:56. Besides, increase of a chemical score by lysine, makes possible to consider this combination as promising (Table 2).

As shown in the Table, in case of grain protein and grape pomace protein concentrate 54:44 ratio, biological value of summarized proteins in combined products approximates to the same index in ideal protein.

Table 2

Chemical score of combined product (grain protein and grape pomace protein concentrate; 56:44)

amino acid	ideal protein for adults		combined product	
	a	s	a	s
isoleucin	4.0	100	3.8	95
leucin	7.0	100	7.1	101
leusin	5.5	100	4.8	87
methionin+cistin	3.5	100	3.5	100
phenylalanin+ thirosin	6.0	100	8.3	138
threonin	4.0	100	3.7	93
triptophan	1.0	100	1.0	100
valin	5.0	100	5.1	102

Note: a - content of amino acids, in g/100gr protein;
s - chemical score, %.

Thus, for obtaining this correlation, flour (protein content 12%) and grape pomace protein concentrate (protein content 80,0 %) ratio, while producing the bread, should be 90:10. But the method of chemical score does not allow to discuss the efficiency of enrichment, as in this case possible technological effects and physical and biological peculiarities of real utilization of mixtures are not considered. That is why carrying out of studies during different correlations of protein concentrate and the bread protein allowed us to obtain data about the utilization of protein composition. As it was mentioned above, experimental groups (I, II, III) received the bread containing 0.8; 1.6 and 4.0 g grape pomace protein concentrate per 1 00 g flour, which corresponded to the inclusion of grape pomace protein by 1.2 and 5%. Content of a protein in experimental samples of the bread ~x5,7), according to the amount of the included protein concentrate made up 7.2; 7.4 and 8.0%. Control samples contained 7% of protein and were given to the animals of control (IV) group. Results of the experiment indicate the increased efficiency in case of enrichment of the bread with protein concentrate in comparison with control group (not enriched bread) (Table 3).

Table 3

Biologocal value of bread containing protein concentrate from grape pomace

group	biological value	utilization of protein, %	consumption, %
I	72,4 + 2 3	63.6 +3.6	86.6 +2.1
II	76,1 ±1,4	68.5 +0.8	90.1 +3.1
III	88.7 ±1.9	79.8 +1.0	89.1 +1.5
IV	71.2 +2.5	64.3 +2.7	90.3 +2.1

By the balance method we determined the highest level of nitrogen utilization in III group, which received the bread with highest content of protein concentreate (5% according to protein). Accordingly we determined the best utilization of proteins in such bread. during the calculation of indices of protein application efficiency (biological value, utilization of protein) the same requlority was fixed. Adding of protein concentrate in experimental bread increased its biological value by 1.2 -17.5%, which indicated an improvement of amino acid balance of the bread proteins.

Table 4 shows amino acid content of experimental and control bread samples. As one can see, adding *5%* of protein concentrate into the bread increases amount of lysine by 37%, threonine - by 17.0%, sulphur-containing amino acids by 3.0%, triptophan - by 7%. In comparison with experimental bread sample, chemical score of given bread sample increases by 18%.

Table 4

Amino acid content of bread sampl

amino acid	content of amino acid in mg on 100 gr product		correlations of amino acid content in experimental and control samples of bread
	control sample of bread	experimental sample of bread	
isoleucin	277,0	296,0	107
leucin	465,0	501,0	108
leusin	221.0	302,0	137
methionin+cistin	271,0	278,0	103
phenylalanin+ thirosin	605,0	688,0	114
treonin	241,0	281,0	117
triptophan	88,0	94,0	107
valin	339,0	384,0	113
Total protein (Nx5.7),%	7,0	8,0	increased by 14%
chemical score, %	57,0	75,0	increased by 18%

Thus, adding of the protein concentrate into the bread significantly increases its biological value.

Protein concentrate obtained from the remains of wine-making industry may be added into such traditional food as the bread for increase of its biological and nutritional value. Besides, the bread acquires the qualities, which satisfies the requirements of rational diet.

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პურის ხარისხის და ბიოლოგიური ღირებულების გაზრდა ყურძნის ჭაჭას ცილის კონცენტრატის დამატებით

ი.ფაღავა

საქართველოს ჯანმრთელობის დაცვის სამინისტროს გ.ნათაძის სახელობის სანიტარიისა და ჰიგიენის ინსტიტუტი, თბილისი

რეზიუმე

პურის საკვები და ბიოლოგიური ღირებულების გაზრდის მიზნით გამოყენებულ იქნა ჭაჭას ცილის კონცენტრატი. კვლევის ქიმიური და ბიოლოგიური მეთოდებოთ შესწავლილ იქნა პურის ბიოლოგიური ღირებულება, რომელიც ჭაჭას ცილის კონცენტრატს შეიცავდა. აღმოჩნდა, რომ პურში ცილის კონცენტრატის 1,2 და 5% (ცილაზე გაანგარიშებით) დამატება არსებითად (4,9-17,5%-ით) ზრდის პურის ბიოლოგიურ ღირებულებას. პურის საცდელი ნიმუშის ბიოლოგიური ღირებულების მაქსიმალური მნიშვნელობები, საკონტროლოს მიმართ, მიღებული იყო 5% ცილის პრეპარატის დამატებისას. ჭაჭას ცილის კონცენტრატი შეიძლება გამოვიყენოთ პურის წარმოებაში მისი ბიოლოგიური და საკვები ღირებულების გაზრდის მიზნით.

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STUDY OF THE BIOLOGICAL EFFICIENCY OF MIXED FOOD CONTAINING PROTEIN CONCENTRATE FROM THE WINE YEAST SLUSH

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Addition of protein concentrate from the wine yeast slush to mixed food of broilers at 2.5 and 10% levels (calculated by weight), and to mixed food of younger poultry at 2.5; 5.0 and 7.5% levels (calculated by weight) substantially increases biological value of food and weight of the poultry.

Key words: *Broilers, Younger poultry, Protein concentrate, Wine yeast slush, Biological value of food.*

Methods for search of new sources and obtaining additional amounts of protein are actively studied in many countries of the world. According to the available data lack of protein in agriculture makes up about 20% of need [1]. Almost 50% of requirements for protein in stock-raising and poultry raising is covered by food grains.

In this regard it is very important to look for new sources of food protein. Most promising direction in this issue is obtaining of protein preparations from secondary resources, which make 20-23% of raw materials and contain substantial amount (8,3%) of slush with high content (38-40%) of protein [4].

By means of the method, elaborated in our laboratory, from the wine yeast slush can be obtained a concentrate with 80% content of protein. Carried out experiment proved that protein concentrate from the wine yeast slush is harmless and has high biological value, and it was decided to propose its use in agriculture as additional source of protein, particularly in poultry raising. Given work is devoted to the study of biological efficiency of mixed food containing protein concentrate from the wine yeast slush.

MATERIAL AND METHODS

We selected for the experiment 80 broilers 45 days of age at Gamarjveba poultry farm and put them into one section. Broilers were fed with control ration (mixed

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In the study participated Z. Mikautadze, 6. Grigorashvili, N. Maglaperidze.

food with 19.1% of protein) during 5 days, than we weighed them and began to give experimental ration containing the wine yeast slush protein.

During the experiments broilers were divided into 4 groups (20 in each group).

I group was given mixed food and the wine yeast slush protein concentrate in the ratio of 98:2 (according to weight), II group - 95:5, III group - 90:10, IV (control) group was given only mixed food. Duration of the experiment was 30 days.

Mixed food, used in the experiments, is commonly accepted in agriculture and contains (%): grain mix - 67.0; meal from fish bones-meat - 7.0; yeast hydrolysate - 5.0; table-salt - 2.9; grass meal - 3,0; cod-liver oil (vitaminized) - 2.0. Food was given in dry form. During last 3 days we carried out balance investigations, when the excrements were collected. Determination of total nitrogen in food and excrements was carried out by Keldal method [2]. As the cryteria for evaluation of biological effect of protein preparation served the data on food utilization and indices reflectung effect of protein addition on the poultry organism [3].

Biological efficiency of the wine yeast slush in mixed food was also studied while feeding younger poultry. Experiment was carried out at the scientific center of poultry farm. We selected so called 'Adatau' 1-day chicks, divided into 4 groups (50 in each group). During the experiment intensity of brooding, feeding, observation and other technological parameters were the same for all groups.

I group of poultry (control group) was given usual mixed food. Poultry of II-III and IV experimental groups were given mixed food and the wine yeast slush protein concentrate in ratio of 97.5:2.5; 95:5 and 92.5:75 (calculated by weight). Poultry of experimental groups received protein additive from the first day.

During the experiment we studied the following indices: survival, dynamics of live weight, total consumption of food and its compensation, development of internal organs.

RESULTS AND DISCUSSION

In the period of experiment we found the differences between the broilers of experimental and control groups in both the live weight and the daily gain in weight. We determined significant differences according to the levels of the wine yeast slush protein concentrate content in mixed food of broilers. Corresponding indices are shown in the Table 1.

Table 1

Indicators of broilers development

group	number of poultry	average live weight at the beginning of the experiment (gr)	average live weight at the end of the experiment (gr)	gain in during experiment	average daily gain in weight (gr)
I	20	897,0	1484,0	587,0	19,6
II	20	869,0	1510,0	641,0	21,5
III	20	847,0	1388,0	541,0	18,0
IV	20	879,0	1438,0	559,0	18,6

Poultry of control group were developing with less intensity then experimental ones. Weight of the latter was higher. Best indices were obtained in II group, where food contained 5% of protein concentrate. It is evident that given correlation of mixed food and protein the broilers better utilize concentrate. Despite the fact that poultry of III group received the mixture with higher (10%) content of protein concentrate, average gain in weight was lower then in II group. This could be explained by disorders of the ratio between some amino acids in case of high content of protein and discrepancy with their requirements.

With an aim to determine the biological efficiency of protein concentrate in experiment we specified indices of biological value of food: coefficient of food efficiency (FEC), protein efficiency coefficient (PEC) and consumption (Table 2).

Table 2

Indices of biological value of food

group	average daily gain weight (gr)	amount of consumed food gr/daily	food efficiency coefficient	number of consumed med protein gr	protein efficiency coefficient	exctreted nitrogen gr/daily	consumption%
I	50,0	66,0	0,76	13,1	3,8	1,3	37,0
II	55,0	66.6	0.84	13.9	4,0	1,2	40,0
III	44,0	66,0	0,61	15,2	2,7	1,7	33,0
IV	40,3	63,3	0,69	12,1	3,6	1,4	30,0

As shown in the Table , highest indices of biological value is fixed in poultry of II group, with 5% content of protein preparation in food. Besides, indices of biological value and consumption are higher then in control group.

Study of broilers showed that food, containing the wine yeast slush protein concentrate, has no negative effect on the organism of poultry (Table 3).

Table 3

Morphological and biochemical indices of broilers' blood during feeding them with food contaning the wine yeast slush protein concentrate

indicators	group			
	I	II	III	IV
total proteing	8,7±06	9,2±0,3	8,5±0,3	8,0±0,2
hemoglobin l0'2/l	13,7±0,1	13,2±0,6	13,5±0,6	13,4±02
erythrocytes l094	2,4±03	2,6±0,1	2,8±0,05	2,9±0,04
albumen,%	33,9±1,5	35,3±1,9	33,6±0,7	35,8±2,1
globulin,%	66,1±15	64,7±1,9	66,4±07	64,2±21
correlation	0,50 ± 0,04	0,55 ± 0,05	0,50 ± 0 02	0,56 ± 0,04

Study of morphological composition of broilers blood showed that reliable differences in amount of hemoglobin and erythrocytes between experimental groups in comparison with control group was not evident. Differences in total protein and protein fractions according to the groups were not determined.

Results of the experiment indicate on the perspectiveness of application of the wine yeast slush protein concentrate as additional source of protein for increasing broilers weight. Besides, as the study showed, adding 5% of protein concentrate to mixed food increases average live weight of broilers by 11.6%.

As it was mentioned above we studied the biological efficiency of the wine yeast slush protein concentrate in mixed food while feeding younger poultry.

Effect of the additive on the development of the poultry is shown in the Table 4.

Table 4

Dynamics of poultry live weight

As shown in the Table, feeding of poultry with mixed food containing the wine

group	in 4 weeks		in 7 weeks		in 13weeks		average daily gain in weight	
	Mfm	%	Mfm	%	Mfm	%	gr	%
I	142,0±2,95	100	303,0±12,2	100,0	716,0±22,1	100,0	7,5	100,0
II	140,0±2,9	98,6	332,0±7,9	109,6	793,0±21,3	110,8	8,3	110,7
III	148,0±3,9	104,2	376,0±9,6	124,1	905,0±26,0	126,4	9,6	128,0
IV	142,0±2,6	100,0	366,0±8,5	120,7	917,0±19,4	128,1	9,7	129,3

yeast slush protein concentrate during first 4 weeks did not produce statistically reliable difference in live weight. During the following period (7 and 13 weeks) poultry of experimental group were developing more intensively and their weight was by 9.6-28.1% higher then the weight of control group. It must be mentioned that the weight of cocks was increasing quicker then of the hens.

In experimental groups average daily gain in weight was higher then in control group. This index was the highest in IV group and exceeded control group by 2.2 g., or 29.3%.

Consumption of food by the poultry during the experiment is shown in the Table 5.

Table 5

Indices of food consumption by the poultry during the experiment

group	average consumption of food by one broiler		daily consumption by one broiler		consumption of food on 1kg gain in weight	
	g	%	g	%	Kg	%
I	3904,0	100,0	42,9	100,0	5,45	100,0
II	4177,0	107,0	45,9	107,0	5,26	96,5
III	4140,0	106,0	45,5	106,0	4,57	83,9
IV	3895,0	99,8	42,8	99,8	4,24	77,8

As shown in the Table, poultry of II and III experimental groups consumed more food in comparison with control group poultry, and in IV group this index equaled to the control group index.

Compensation of the food, i.e. its transition into meat, was more intensive in experimental groups then in control groups. On gaining 1 kg weight poultry of experimental group consumed 0.19; 0.88 and 1.21 kg less, i.e. 3.5; 16.1 and 22.2% less food then poultry of control group.

Observation of the poultry sustainability during the experiment showed that death-rate in both experimental and control groups did not differ statistically reliably.

At the end of the experiment, an analysis of development of poultry internal organs showed that the organs of vascular system, digestive, barrier-function and reproduction systems in experimental groups were not affected by the physiological load and their development did not differ from control group.

Thus, results of our experiments indicate the high biological qualities of non-traditional food additive - the wine yeast slush protein concentrate, which was reflected in increase of poultry sustainability, gain in weight and transition of food into meat.

The wine yeast slush protein concentrate can be efficiently used in food of younger poultry in amount of 5.0-7.5%.

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ღვინის ლექის ცილის კონცენტრატის შემცველი კომბინირებული საკვების ბიოლოგიური ეფექტურობის შესწავლა

ი.ფაღავა

საქართველოს ჯანმრთელობის დაცვის სამინისტროს გ.ნათაძის სახელობის სანიტარიისა და ჰიგიენის ინსტიტუტი, თბილისი

რ ე ზ ი უ მ ე

საბროილერე წიწილების კვებისას განსაზღვრულ იქნა ღვინის ლექის ცილის კონცენტრატის ბიოლოგიური მნიშვნელობა. გამოირკვა, რომ კომბინირებულ საკვებში ღვინის ლექის ცილის

კონცენტრატის შეტანა 2,5 და 10% რაოდენობით (წონით შეფარდებით) არსებითად ზრდის (13,1-37,7%-ით) კომბინირებული საკვების ბიოლოგიურ ღირებულებას. საკვების ბიოლოგიური ღირებულება მაქსიმალურად იზრდებოდა, როდესაც საკვებში 5% ცილა შეგვყავდა.

ბიოქიმიური და მორფოლოგიური მეთოდებით შესწავლილ იქნა ფრინველთა ორგანიზმის რეაქცია ჩვენს მიერ გამოყენებულ საკვებზე. გამოირკვა, რომ ცილის პრეპარატი ფრინველის ორგანიზმზე უარყოფითად არ მოქმედებს.

მოზარდი ფრინველის გამოკვებისას კომბინირებულ საკვებში ლექის ცილის კონცენტრატის შეტანა 2,5; 5,0 და 7.5% რაოდენობით (წონითი შეფარდებით) მნიშვნელოვნად ზრდის ფრინველის საშუალო სადღეღამისო წონამატს (10,7-29,3%-ით). ღვინის ლექის ცილის კონცენტრატი შეიძლება გამოვიყენოთ მეფრინველეობაში, დამატებით ცილოვან საკვებად, ფრინველთა წონამატის გაზრდის მიზნით.

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CYTOGENETIC DISORDERS, DUE TO DIFFERENT DOSES OF X-RAY IRRADIATION IN EXPERIMENTAL ANIMALS

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To study in bone marrow and peripheral blood cytogenetic disorders due to 3Gy; 4,5Gy; 6Gy and 8Gy X-ray irradiation, investigation, was carried out on 8 male dogs (totally 26 experiments). Investigations were conducted after 24, 72, 96 hours and on the 7, 12, 17th days from irradiation. Analyse of obtained data revealed a strong correlation of frequency of dicentrics (more specific chromosome aberration for irradiation) with exposed dose. Twice arm chromosomes were considered as dicentrics resulted from acrocentric chromosome fusion. Along with dicentrics, the second specific disorders for irradiation, were ring chromosomes, amount of which was significantly less, compared to dicentrics. Especial attention from chromatide aberration was paid on an appearance of tri- and quadriradials, which were not observed after 3Gy but are specific for 4,5Gy and high doses of X-ray irradiation. Chromatide aberrations, minutes, incresement of polyploid cell amount and sometimes multiple aberrations, were observed after all doses of irradiation. A correlation with morphological data was also revealed.

Key words: *Irradiation, Bone marrow, Lymphocytes, Chromosomal aberrations, Dicentrics, Dogs*

In recent years a special attention is paid on the investigation of changes in living organism due to the influence of ionizing irradiation. Cytogenetic data are frequently used as biological marker of absorbed dose, as for X-ray irradiation is responsible for the appearance of aberrant metaphases in bone marrow and peripheral blood lymphocyte culture. In aberrations are distinguished: unstable – dicentrics and rings and stable chromosome aberrations – consisted of reciprocal translocations, peri- and paracentric inversions, terminal and interstitial deletions, insertions, translocations of complex type, complex exchanges and complex non-identified rearrangements [12].

It must be mentioned, that unstable aberrations in irradiated organism are diminished gradually with time, compared with stable aberrations, which formed lately, but persisted longer in radiation-exposed individuals, because cells with monocentric (stable) aberrations have a higher probability of survival after cell division compared to multicentric or acentric aberrations (unstable) (5),

Furthermore, to determine the obtained irradiation dose by organism and to study biological effect of the influence on organism in earliest time after irradiation investigations of unstable chromosomal aberrations are generally accepted [9, 11].

According to a large body of data, in aberrations, due to X-ray irradiation, intrachromosomal exchanges are predominated compared with inter-chromosomal exchanges which in turn appears to be promoted by chromosomal organization in interphase nucleus, most probably due to close proximity of the two arms of a chromosome (10). Most chromosome aberrations produced by ionizing radiation, are thought to result from DNA double-strand breaks (4) and in particular, from misrepairing of them (3). Furthermore, in literature are available data, that within interchromosome aberrations dicentrics and translocations are produced in equal frequencies (6). Linear-quadratic dose-effect relationships for frequencies of dicentrics (2, 8, 11), rings (2, 7, 13) translocations (14) and multiple aberrations (pulverization and severe damage cells) was revealed [2].

The purpose of this study was to reveal the peculiarity of cytogenetic disorders, due to different doses of X-ray irradiation and to show the correlation with hematological changes. Investigations were carried on dogs. In the bone marrow and peripheral blood lymphocyte cultures of experimental animals cytogenetic data were studied.

It is known, that in addition to chromosomal apparatus, hemopoietic system is very sensitive system to the effect of ionizing irradiation. Consequently, morphological substratum was also investigated, detail results of which are given in our previous paper [1].

MATERIAL AND METHODS

The material for cytogenetic investigation are dividing cells of bone marrow and peripheral blood cultures of 8 male dogs. All experimental dogs were approximately the same size and their weight was varied in the range of 8-12 kg. Single irradiation with high doses of X-rays, in particular with 3Gy, 4,5Gy, 6Gy and 8Gy was performed by means of equipment "Run- 7" at room temperature during 20, 35, 60-60 minutes, accordingly. Voltage was 250 kv. Current power – 15 mA. Bone marrow taken from femur was investigated before experiment and after 24,72 hours from irradiation exposure. More lately from irradiation blood samples taken by venipuncture were also cytogeneticaly analysed. For bone marrow metaphase preparation was applied the "direct" method. In 7 ml of Eagle's medium with glutamine, were added 1,5 human AB+serum 1ml bone marrow and 0,3 mkg/ml 0,01% colchicine. Cells were cultured at 370 C during 2 hours, Hypotonic shock by 0,75 MKCL was carried on at 370 C for 20 minutes. After centrifugation the cells were fixed in threefold by addition of methanol-acetic acid (3/1 v/v). Suspension were spread on clean slides stained with 3% Giemsa and visualized by "Opton" microscope.

For peripheral blood metaphase preparation was applied culture method; 7ml of Eagle's medium with glutamine, 1,5 human AB+serum and 0,3ml of mitogenic agent Phytohemaglutinine [PHA] [Bulgaria] were added to 0,8ml whole blood. Cells were cultured at 370 C for 48 and 72 hours. After 48 hours on the medium very low output of mitosis was obtained therefore mitosis of 72 hours culture were also analysed. Colchicine 0,3mg, per ml was added into flask with culture, 2 hours before fixation. Hypotonization fixation and preparation of slides were similarly carried out as well as above mentioned for bone marrow cells.

Different types of aberrations such as chromatide breaks, intra-chromosome changes, fragments, rings and dicentrics were scored.

Furthermore, polyploidy, pulverization, association index and severe damage cells [cells with 10 or more aberrations of any type] were taken into account. Run parallel to this bone marrow and peripheral blood cells were analysed morphologically, according to methodology pointed in previous paper [1].

RESULTS AND DISCUSSION

Cytogenetic analyse was carried out on 8 male dogs. In each case was taken an attempt to analyse maximal amount of metaphases, as for on average 40-60 metaphases were investigated. Different types of aberrations were evaluated in percents. Control cytogenetic analyse conducted in all animals before experiment was shown that large amount of metaphases consisted of 78 chromosomes, and such chromosome aberrations as breaks, were single only in 2 dogs.

Two dogs each were irradiated with different [3; 4,5; 6; 8Gy] irradiated dosage. Cells of peripheral blood and bone marrow were investigated. It must be mentioned, that metaphase amount in bone marrow was always less, than in peripheral blood. In 3Gy irradiation case, 5 analyses were carried out, in particular, cells of bone marrow were investigated in threefold – after 24, 72 hours and on the 12th day after X-ray irradiation and peripheral blood cells – after 96 hours and on the 6th day from irradiation. After 24 and 72 hours changes in bone marrow were similar and by day 12, percent of aberrant cells was significantly decreased.

In peripheral blood where we managed to collect more metaphases for analyse, percent of aberrant cells was slightly low [Fig. 2], compared to the first day in bone marrow[Fig. 1]. Regardless, of this all types of aberrations – as chromosomal, as well as chromatide type were observed.

After 4,5Gy irradiation twofold investigation of bone marrow [after 24 and 72 hours] as well as peripheral blood [on the 7th and 17th day] was carried out.

In the case of 6Gy irradiation, bone marrow was studied twice and peripheral blood-only on the 7th day. Experimental animals obtained above mentioned dose

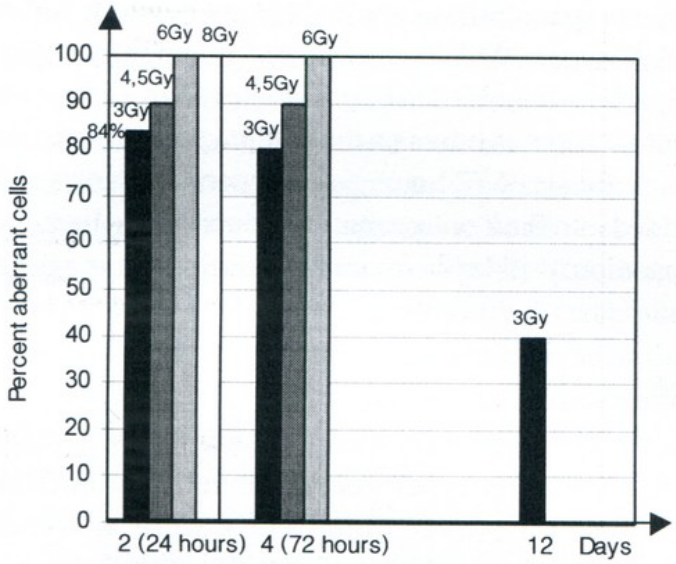


Fig.1. The dependence of percent of aberrant metaphase in bone marrow of experimental animals on time passed from different doses of X-ray irradion

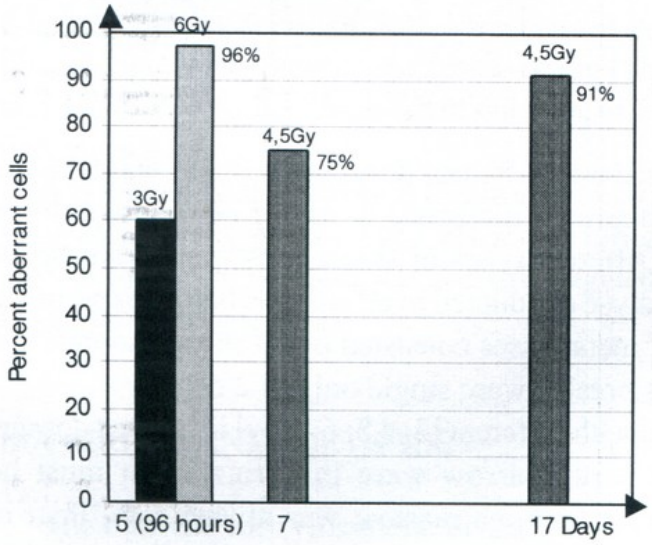


Fig.2. The dependence of percent of aberrant metaphase in peripheral blood of experimental animals on time passed from different doses of X-ray irradion

were died on the 10th and 11th day accordingly.

Compared to this, 8Gy irradiation dose was contributed to dog's early death in particular on the 3rd and 4th days. Bone marrow was investigated after 24 hours from irradiation. As for mitosis were in a small amount, metaphases were collected from great number of slides. None of normal metaphases were observed, because most metaphases were with multiple aberrations. In peripheral blood severe leukopenia was mentioned. Consequently, investigation of peripheral blood become impossible. Table 1 shows chromosome aberrations in all investigated cases. Analyse of obtained data manifested a strong correlation between frequency of the most specific for irradiation, chromosome disorders, in particular, dicentrics with exposed irradiation dose [Fig. 3, 4]. Twice arm chromosome in dogs were considered as dicentrics, resulted from centric fusion of acrocentric chromosomes [Photo 1]. Correlation of dicentric number with exposed irradiation dose is given on Fig 3, 4, but Table 1 shows, dicentric amount on

Table 1

Diapason of frequencies of more characteristic chromosome aberrations in bone marrow cells and peripheral blood lymphocytes of irradiated dogs

Dose of X-ray irradiation	Number of investigations	Diapason of analysed metaphase amount	Diapason of aberration frequency in percents					
			Aberrant metaphases	Dicentrics	Ring Chromosomes	Acentric fragmets	Atypical (marker) chromosomes	Chromatide aberrations
3Gy	10	30-100	40-84	15-66	0-3	1-50	0-8	0
4,5Gy	8	22-70	75-91	0-110	0-5	20-70	5-9	5-8
6Gy	6	30-60	69-100	30-140	0-5	40-200	0-16	8
8Gy	2	25 and 30	100	100-200	1-7	100-200	8-10	multiple

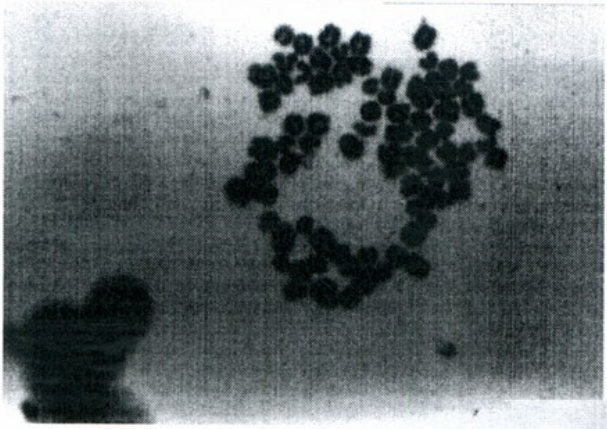
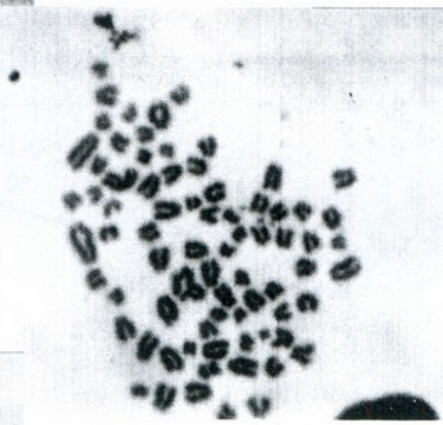


Photo 1

Photo 2



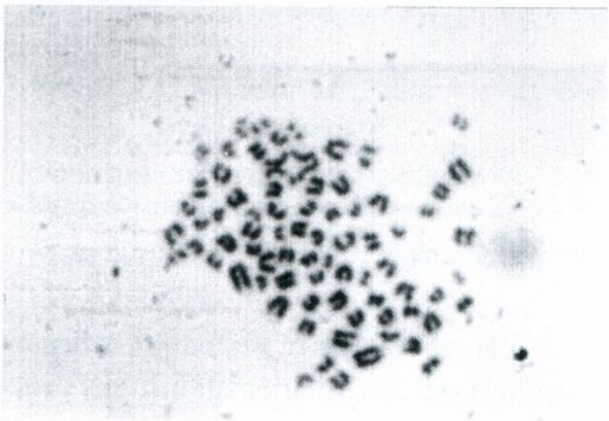


Photo 3

average in each irradiation dose. Along with dicentrics, rings are presented the second specific disorder for irradiation, but their number was significantly less, compared to dicentrics. Among chromatide aberrations a special attention must be paid on the appearance of tri- and quadriradials [Photo 2], which were not observed after 3Gy

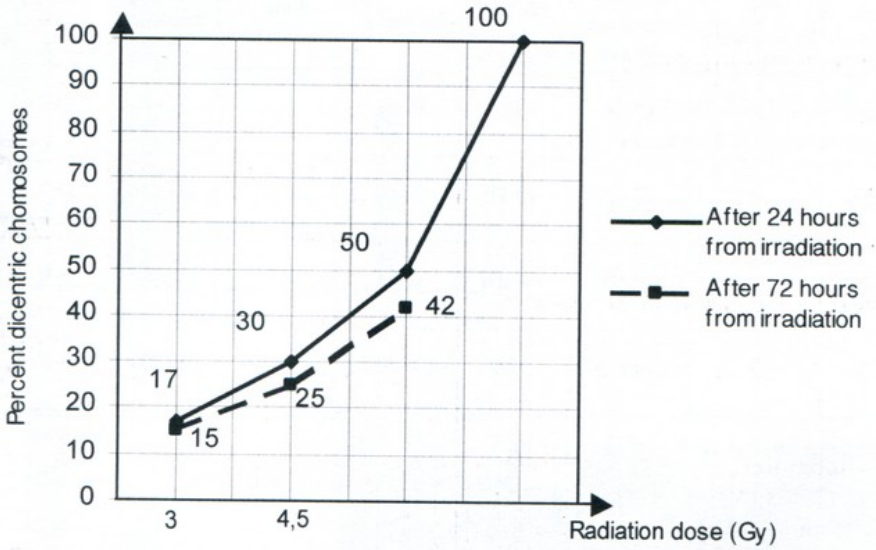


Fig.3. The influence of different doses of X-ray irradiation on dicentric number (in percents) in the bone marrow of experimental animals

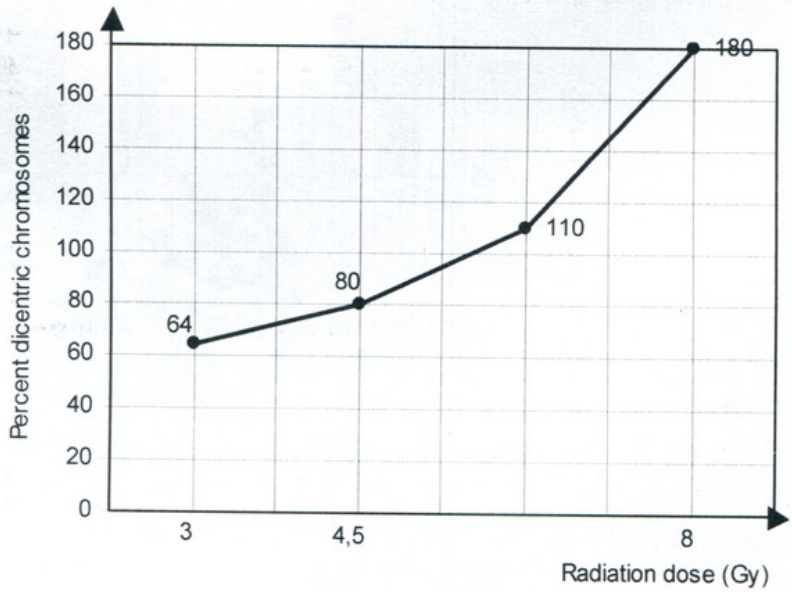


Fig.4. The influence of different doses of X-ray irradiation on dicentric number (in percents) in peripheral blood after 96 hours irradiation

irradiation, as for they raised after 4,5Gy and higher doses of irradiation exposure. Chromatide breaks and minutes were observed in all cases of irradiation in any period

of time. A special interest was paid on the appearance of polyploids more also indicated a normal mitosis disturbance.

According to data, available in literature, eccentric fragments and dicentrics are raised from similar kinetics [4].

Obtained cytogenetic data were indicated, a dose-effect relationship for an amount of aberrant metaphases, as well as for a total number of aberrations per cell, observed in experimental animals bone marrow after 24 hours from different doses of X-ray irradiation. Furthermore, a total amount of aberrations was decreased with time passed from irradiation [Fig. 1]. These data are in accordance with data available in literature [3, 9].

Morphological investigation of bone marrow and peripheral blood, which was run parallel with cytogenetic analyse, revealed that high doses of X-ray irradiation due to a deep myelokaryocytopenia, expressed already after 24 hours from irradiation and deepening with time. In the bone marrow of experimental animals were revealed availability of multiple destructed cells, blast cells, gigantic myelo- and metamyelocytes, pathological forms of mitosis, megaloblasts. It must be emphasized, that more lately, from irradiation in mononucleatic cells was observed an appearance of additional micronucleuses. These structures are presented lethal structures in their nature.

To conclude this paper, it must be mentioned, that different doses of X-ray irradiation are responsible for a deep morphological and cytogenetic alterations. Cytogenetic aberrations are characterised with a range of structural disorders, part of which are specific [dicentrics, rings] and in spite of their belonging to unstable chromosome aberrations, they persisted in peripheral blood for several times after irradiation.

Due to cytogenetic and morphological investigation of bone marrow and peripheral blood after high doses of X-ray irradiation, was revealed a correlation between degree of cytogenetic disorders with exposed dose as well as their simultaneousity with morphological alterations.

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სხვადასხვა დოზის X-სხივებით განპირობებული ციტოგენეტიკური დარღვევები ექსპერიმენტულ ცხოველებში

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რ ე ზ ი უ მ ე

ძვლის ტვინისა და პერიფერიულ სისხლში 3 Gy, 4,5 Gy, 6 Gy, 8 Gy-ით რენტგენის სხივების ზემოქმედებით განპირობებული ციტოგენეტიკური დარღვევების შესასწავლად გამოკვლევები ტარდებოდა ძაღლებზე დასხივებიდან 24, 72, 96 სთ-ის შემდეგ და მე-7, მე-12, მე-17 დღეს. გამოვლინდა რადიაციისთვის უფრო სპეციფიკური ქრომოსომული აბერაციების – დიცენტრული ქრომოსომების სიხშირის დამოკიდებულება დოზაზე. ორმხრიანი ქრომოსომები განიხილებოდა აკროცენტრული ქრომოსომების შერწყმის შედეგად წარმოქმნილ დიცენტრულ ქრომოსომებად. რადიაციისათვის

დამახასიათებელ მეორე სპეციფიკურ დარღვევას წარმოადგენდა რგოლური ქრომოსომები, რომელთა რაოდენობა მნიშვნელოვნად ნაკლები იყო დიცენტრულთან შედარებით. ქრომატიდული აბერაციებიდან განსაკუთრებულ ყურადღებას იპყრობს ტრი- და კვადრირადიალების წარმოქმნა 4,5 Gy და უფრო მაღალი დოზით რენტგენის სხივების ზემოქმედებისას. ნებისმიერი დოზით დასხივების ყველა შემთხვევაში აღინიშნებოდა ქრომატიდული აბერაციები, წერტილოვანი ფრაგმენტები, პოლიპლოიდური უჯრედების რაოდენობის გაზრდა და მრავლობითი აბერაციები. გამოკვლევებით დადგინდა ციტოგენეტიკური დარღვევების კორელაცია მორფოლოგიურ მონაცემთან.

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GENERAL AND SPECIAL MECHANISMS OF INFLUENCE OF RIDETHERAPY ON OSTEOCHONDROSE OF SPINE

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New method of conservative pathogenetic treatment of osteochondrose of spine – ridetherapy of therapeutic riding is presented in the work. General and special, or affecting motor segment of spine, mechanisms of its influence have been detailed and elaborated. High and stable therapeutic effect has been reached in patients with osteochondrose of spine.

Key words: *osteochondrose of spine, physical rehabilitation, conservative treatment, therapeutic riding, antigravitational system, muscles, muscular corset, intervertebral discus, motor segment of spine, dynamic stereotype, biomechanical mechanisms.*

The issue of prevention and treatment of osteochondrose of spine remains topical and pointed, because not decrease of morbidity, but its progress and rejuvenation are manifested. Long terms of treatment, its futility and frequent exacerbation makes necessary the search of new, efficient, pathogenetic methods. We [5,6,7] have elaborated completely new, pathogenetic method with high therapeutic effect – therapeutic riding of ridetherapy. It has been known since Esculap's age, but its revival in Europe has begun from 50th of our century and it had empirical character. It should be mentioned that almost simultaneously, irrespective of each other scientific research works have begun in both Europe and Georgia. In Georgia academician Dimitry Tsverava has led it. He investigated integrated biological system–"rider-horse". In 80th scientific and clinical researches carried out under his guidance took more differentiated character and developed new direction in ridetherapy. In particular, usage of therapeutic riding in psychosomatic diseases should be regarded as priority of academician Dimitry Tsverava and Georgia.

The purpose of the work is revelation and elaboration of general and special mechanisms of influence of ridetherapy on the osteochondrose of spine.

Two basic factors of influence are picked out in ridetherapy [6,8,9,10] – phsychogenic and biomechanical. The later has leading importance in treatment of osteochondrose of spine.

Biomechanical influence of ridetherapy is caused by following basic mechanisms: first – affection of vibrations formed from horse back both on the whole organism and directly on the motor segment of the spinal column; second – creation of new antigravitational system while riding, third – affection of different intensity and volume both on the whole muscular system as well as spinal muscles.

We'll pick out the factors of general influence and special or affecting motor segment of spine factors from mentioned mechanisms of influence.

Among general mechanisms we'll consider following:

1. Vibrations formed from the horse back and spread in three inter perpendicular planes differ by frequency and period according to pace of horse (walking, trot, gallop), but all the same they belong to vibrations of law frequency and amplitude (bumps). Their frequency is 0,5-1 hertz, and period – 1-1,6 seconds. Biological effect of vibrations is determined by their local influence, which causes direct alternative changes in tissues (contraction-stretching, budging, twisting, winding and etc) and transmitted changes, which are caused by activation of central and vegetative nervous and endocrine systems [1,2,3]. Mentioned mechanisms lay in the basis of vibrotherapy [11].

Vibrations formed from horse-back begin affection on the rider's organism from baroreceptors of parts of body that are in contiguity with saddle and horse, from these receptors excitation is transmitted by afferent ways to dorsal horns of spinal cord, and from there – to thalamus and afterwards brain hemispheres.

Simultaneously nodular cells of sympathic trunk perceive vibrations (bumps), by collaterals and sympathic nods of spinal cord, where from irritation is transmitted to appropriate metameres of body, which also affect inner organs. It's known that under influence of vibration blood circulation and metabolism in muscles are improved.

2. Important mechanism, which determines general influence of ridetherapy, is creation of new antigravitational system while riding, i.e. acquiring of balance or equilibrium on the horse.

For keeping balance on the horse by rider strain reflexes are activated. By means of them at first muscle-skeletal system creates steady support and then reflective contraction of antigravitaional muscular system begins. Both factors together determine holding of body weight and balance. Development and strengthening of balance while horse-riding needs certain time that is connected with creation and consolidation of new reflexes. While sitting on the horse rider's center of gravity coincides with center of gravity of horse. In this posture postural, or maintaining posture reflexes and their realization is determined by reflective contractions of antigravitational, postural muscles. Every deviation of the rider from vertical position causes movement of his center of gravity, then next group of postural reflexes begins acting and causes development of defensive movements and maintain balance while affection of rider by different forces. Thus, almost all muscles participate in keeping of balance. Certain load and training receive also optic and vestibular systems.

3. Riding does not belong to habitual sphere of movement for men. Movement coordinating system undergoes big loading; Formation of new conditioned and unconditioned reflexes takes place. Gradually is developed new motor stereotype, which has therapeutic effect both in the process of development and afterwards.

4. New field of proprioceptive excitation, which is transmitted from rider's muscles, tendons, ligaments, joint surfaces, joint capsules and fasciae to spinal cord and then hierarchically to cerebral cortex should be recognized as one of general mechanisms of ridetherapy. Proprioceptive irritation forms new refectory ways, which improve conditional function of rider's nervous system; forms new positive zones of excitation in cerebral cortex and suppresses pathologic nidi by means of ability of induction.

As we have already reported in the system of physical rehabilitation of osteochondrose of spine ridetherapy is prescribed when the disease is in subacute phase or remission. That is why, those mechanisms of disease, which act in acute phase and restrict usage of remedial exercises, and the more ridetherapy, do not represent any more the factors hampering the beginning of ridetherapy in subacute phase or remission. Despite the fact, that positive dynamics of disease can be achieved by drug therapy carried out in acute phase, or activation of mechanisms of sanogenesis, pathologic substratum and pathogenetic mechanisms still remain.

Special mechanisms of ridetherapy affect both different elements of motor segment of spinal column and also remote regions.

1. Vibrations formed from horseback spread in the three inter perpendicular directions. Among them vibrations of two types in horizontal plane – front-back Z direction and right-left X direction, and third – in vertical plane up-down Y direction (Fig. 1 a).

While riding center of gravity of rider-patient goes through spinal column. That is why vibration affects directly the place of pathologic process – intervertebral discus.

Vibrations acting in horizontal plane in X direction affect pulpy nucleus moving from center to right or left and cause its movement towards center. (Fig. 1 b,c). Vibrations acting in horizontal plane in Z direction affect pulpy nucleus moving from center forward and backward and cause its certification (Fig. 2 a, b, c).

Thus, under influence if vibration acting in horizontal plane attempt of restoration of anatomic structure of intervertebral discus takes place.

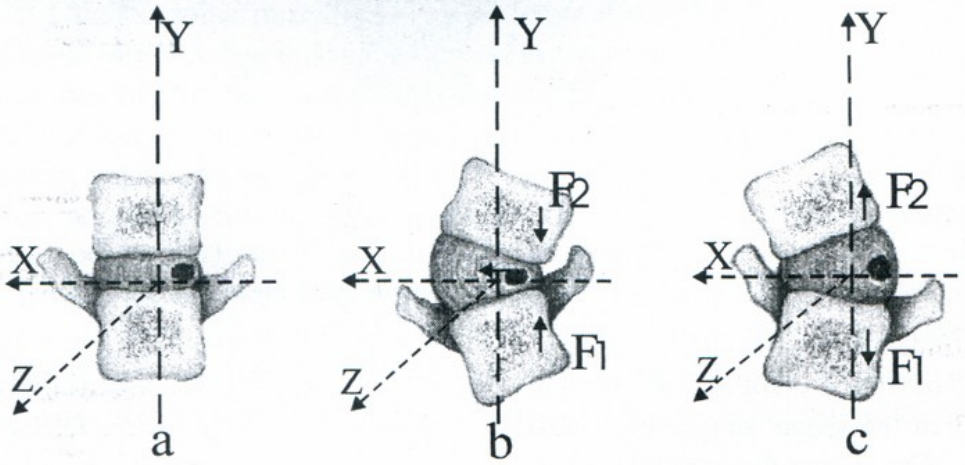

Fig. 1 Influence of vibrations formed from horse back in horizontal plane in X direction.

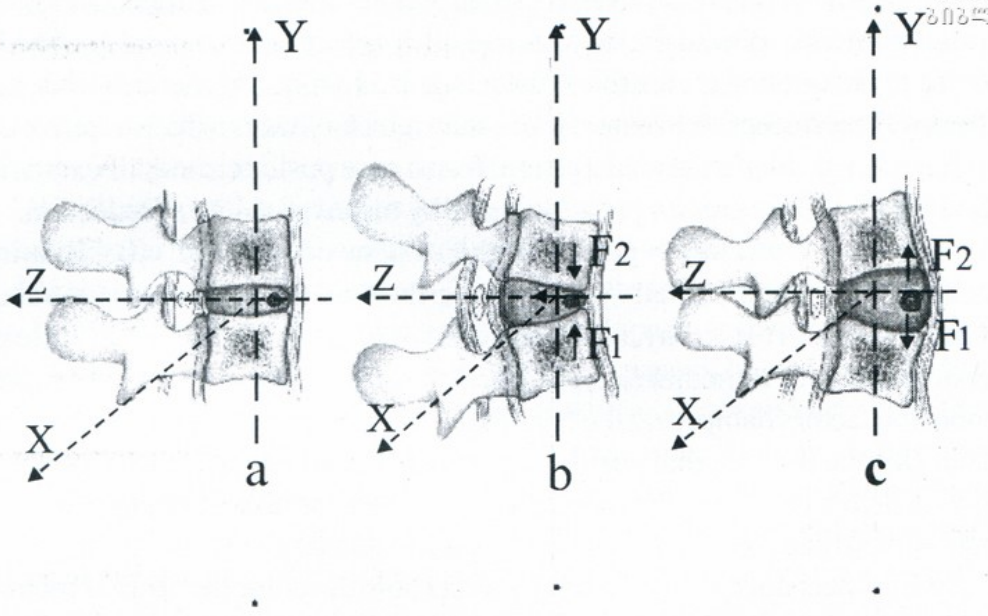

Fig. 2 Influence of vibrations formed from horse back in horizontal plane in Z direction.

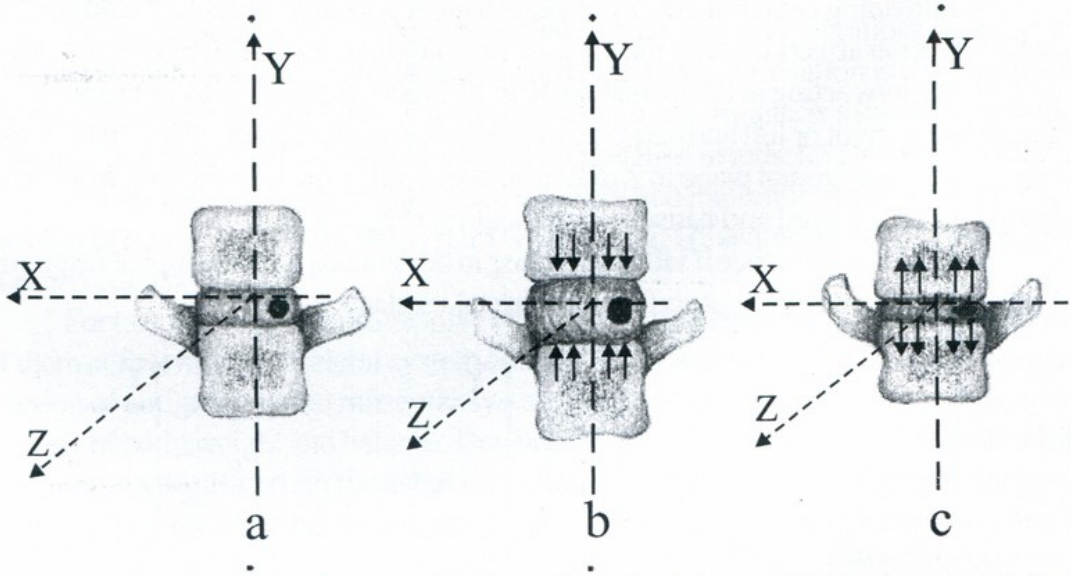

Fig. 3 Chart of strengthening of vertical forces (vibrations in Y direction) on intervertebral discus and metabolism in it.

In vertical plane act vibrations in up-down or Y direction. While affection occurs a kind of "massage" of intervertebral discus, all its elements (pulpy nucleus, hyaline plates, fibrous angulus) and fixative tissues (anterior and posterior descendent, yellow and other tendons, also joints). Vertically acting forces cause active stretching of intervertebral foramen and intervertebral discus. This process is important for anatomic structure of intervertebral foramen, because under influence of these forces occurs decompression of nerve root and blood vessels, elimination of reactive inflammation

around the root, stretching of connective tissue adhesions and others. Influence of these vibrations undoubtedly causes diffuse improvement of nutrition, because at each bump a kind of "vacuum" is formed, during which nutritional substances are attracted; and during contraction the tension growth, and it should be assumed that penetration of nutritional substances to cells is reinforced. Taking out of remainders is quickened under affection of the same forces and mechanisms (Fig.3 a, b, c,). Metabolism is improved in motor segment of injured spinal column and joints (mainly hip) that impedes degenerative-dystrophic processes going in them.

2. Vibrations formed from horseback and creation of new antigravitational system have significant influence on the whole muscular system, and especially muscles of spinal column and lower limbs of the patient (rider). New proprioceptive field, which is formed while riding improves metabolism first of all in analyzer motor neurons by intensification of their vascularization, and afterwards has trophic influence on muscles of trunk and limbs, in particular – muscles of spinal column.

While sitting on the horse muscles undergo both dynamic (isotonic) and static (isometric) strain. Each vibration (bump) of horse is followed by training of muscles of all parts of spinal column (cervical, thoracic, lumbar, and saccro-coccygeal) and groups (large, middle, small, superficial and deep), abdominal press and lower limbs, especially adductors. Consequently develops needed for balance "muscular memory" and new dynamic stereotype.

Affection on muscles changes according to pace: muscular strain is much lower at a walk, then at a trot. Above mentioned muscles contract and relax 60-80 times at a trot. Alternation of isotonic and isometric physical strains is especially significant at a light trot. Every rise from saddle and squatting causes isometric strain of muscles with subsequent relaxation, that intensively develops muscular mass and force, mobilizes motoneurone apparatus and promotes for quick restoration of impaired function.

While ridetherapy affection on the whole muscle-skeletal system is symmetrical. Osteochondrose of spine is characterized by asymmetric strain of muscles. That's why during ridetherapy spinal muscles undergo different affection: in particular, in stretched (lengthened) muscles their tone increases and in contracted (strained) muscles process of fatigue begins. Afterwards muscles relax and in the end its tone becomes normal. Influence of forces symmetrically acting on the horse causes development of strong muscular corset around the spinal column. Creation of muscular corset and re-distribution of supporting function is the main tusk for pathogenetic treatment of patients with osteochondrose of spine.

3. Mechanism of influence of ridetherapy on joints should be considered separately. Joints system, especially hip and knee joints receive significant load at different horse paces. Consequently, metabolism in mentioned joints increases that hampers development of dystrophic process, which very often accompanies osteochondrose of spine.

On the basis of general and special mechanisms of ridetherapy, which occur while riding we elaborated appropriate methodology [4,9] and treated 232 patients of both sexes in age from 19 to 52 years old. The patients had diagnosis of osteochondrose of neck, thorax and waist in subacute phase or remission. In I – basic group complex treatment was carried out using remedial exercises, massage and ridetherapy, in II –

control group - remedial exercises and massage only. All patients had general clinical, X-ray and functional investigations, data of which were studied in dynamics.

In order to determine efficiency and stability of therapy we also studied the degree of clinical picture, frequency of exacerbation and their duration, character of remission in three months of treatment and after a year from treatment.

Analysis of results shows that therapeutic effect after treatment is quite high in both groups –in I - 96,67%, and in II – 88,89% of cases, but is higher in basic group ($p<0,05$) and is maintained in data obtained in a year after treatment ($p<0,005$); in the same way frequency of exacerbation ($p<0,001$) decreased and degree of clinical picture improved ($p<0,001$) more n the I group. Reliable difference was not manifested between the groups in duration of exacerbation.

The character of remission differed significantly in the basic group both by the end of treatment ($p<0,001$) as well as in a year after therapy ($p<0,05$) – complete and "a" type remission was achieved in 87,08% of cases in the basic group, and in 60,97% - in control group. Analogous results were manifested in data obtained in a year after treatment.

Thus, we can make following conclusions:

1. Following general mechanisms act during ridetherapy - vibrations formed from the horse back and spread on the patient cause local and general changes in him (her). Frequency and amplitude of vibrations positively affects nervous and endocrine systems of the patient and metabolic processes in tissues.

During ridetherapy a patient develops new motor stereotype and new, extensive proprioceptive field is created, which condition significant therapeutic effect.

2.Following special mechanisms are revealed in ridetherapy: - under affection of vibrations created from horse back on motor segment of spine occurs – centering of decentred nuclei, improvement of metabolism in intervertebral discus and tissues around, decompression of nerve trunk and blood vessels.

- Development of significant muscular corset in isotonic and isometric regimen at different horse paces.

- Antidystrophic, antiarthrose affection caused by intensification of metabolism in joints during riding.

3. Due to methodology of ridetherapy proposed by us high and stable therapeutic effect was achieved in-patients with osteochondrose of spine.

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რაიტთერაპიის ზოგადი და სპეციალური ზემოქმედების მექანიზმები ხერხემლის ოსტეოქონდროზის დროს

მ.რუხაძე

თბილისის ექიმთა დიპლომის შემდგომი განათლების სახელმწიფო სამედიცინო აკადემია

ნაშრომში წარმოდგენილია ხერხემლის ოსტეოქონდროზის კონსერვატიული მკურნალობის, პათოგენეზური ხასიათის ახალი მეთოდი სამკურნალო ცხენოსნობა ანუ რაიტთერაპია. სამკურნალო ცხენოსნობა სომატიური დაავადებების სამკურნალოდ, სრულიად ახალი მიმართულებაა რაიტთერაპიაში, რომლის სამეცნიერო-პრაქტიკული შესწავლა მიმდინარეობს საქართველოში აკადემიკოსს დიმიტრი წვერავას ხელმძღვანელობით.

რაიტთერაპიის ბიომექანიკური ზემოქმედება განპირობებულია შემდეგი ძირითადი მექანიზმებით: პირველი - ცხენის ზურგიდან წარმოქმნილი რხევების ზემოქმედება როგორც მთელ ორგანიზმზე, ასევე მიმართულად ხერხემლის სვეტზე; მეორე - ცხენზე ჯდომისას (ამხედრებისას) ახალი ანტიგრავიტაციული სისტემის შექმნა და მესამე - ორგანიზმის მთელ და მიმართულად ხერხემლის კუთნებზე სხვადასხვა ინტენსივობისა და მოცულობის ზემოქმედება.

აღნიშნული მექანიზმებიდან ჩვენ გამოვყავით ზოგადი და სპეციალური ანუ ხერხემლის მამოძრავებელ სეგმენტზე, მოქმედი ფაქტორები.

რაიტთერაპიის დროს მოქმედებენ შემდეგი *ზოგადი* მექანიზმები:

1. ცხენის ზურგიდან წარმოქმნილი რხევები, რომელიც ავადმყოფზე ვრცელდებიან, იწვევენ მასში ლოკალურ და ზოგად ცვლილებებს. რხევების სიხშირე და ამპლიტუდა დადებითად მოქმედებს ავადმყოფის ნერვულ, ენდოკრინულ სისტემებზე, ხოლო ქსოვილებში - მეტაბოლურ პროცესებზე.
2. ცხენზე ჯდომისას ახალი ანტიგრავიტაციული სისტემის შექმნა იწვევს ახალი დაცვითი და პოსტურალური რეფლექსების ამოქმედებას, რაც ავითარებს პოსტურალურ კუნთებს, მხედველობით და ვესტიბულარულ სისტემებს.
3. რაიტთერაპიისას ავადმყოფს უყალიბდება ახალი მოძრაობითი სტერეოტიპი და წარმოიშვება ახალი, ვრცელი პროპრიოცეპციული ველი, რომლებიც მნიშვნელოვან სამკურნალო ეფექტს განაპირობებენ.

რაიტთერაპიისას გამოვლენილია შემდეგი *სპეციალური* ზემოქმედების მექანიზმები:

1. ცხენის ზურგიდან წარმოქმნილი რხევების ზემოქმედებით ხერხემლის მამოძრავებელ სეგმენტებში ხორციელდება - დეცენტრირებული ბირთვების ცენტრირება, მალთაშუა დისკისა და მის ირგვლივ მდებარე ქსოვილებში ნივთიერებათა ცვლის გაუმჯობესება, ნერვული ღეროსა და სისხლძარღვების დეკომპრესია.
2. ცხენის სხვადასხვა ალურისას კუნთების იზოტონური და იზომეტრული რეჟიმით ხერხემლის ირგვლივ მნიშვნელოვანი კუნთური კორსეტის განვითარდება.
3. ცხენზე ჯდომისას სახსრებში ნივთიერებათა ცვლის გაძლიერებით გამოწვეული ანტიდისტროფიული ზემოქმედება.

ჩვენს მიერ მოწოდებული რაიტთერაპიის მეთოდიკის გამოყენებით მიღებულია მაღალი და სტაბილური სამკურნალო ეფექტი ხერხემლის ოსტეოქონდროზით დაავადებულ ავადმყოფებში.

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THE ROLE OF PERIPHERAL ADRENERGIC STRUCTURES IN INTERRELATION BETWEEB SECRETORY AND EXCRETORY FUNCTIONS OF THE STOMACH

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According to the data obtained in experiments carried out in the dogs with Pavlovian stomach pouch, it could be suggested that pharmacological and surgical desynpathization induce alterations of secretotory and excretory processes, which could be mainly attributed to the vascular mechanisms, with decrease of the mucous blood supply. Accordingly, it could be concluded that intensity of gastric excretory function is dependent on the concentration in the blood of a substance to be excreted. Moderate excretion is stimulated during feeding. Depression of the central adrenergic structures elicits attenuation of gastric excretory ability, while the changes in the functional state of the peripheral adrenoreactive structures induces disorders in the correlational course of gastric secretory and excretory functions.

Key words: *Stomach, Secretion, Excretion, Adrenergic structures, Dogs*

Notwithstanding significant breakthroughs in the gastroenterology, treatment of the gastric ulcer and duodenal ulcers still pose a major problem. The most authors consider that the main factor of ulcerogenesis is a disbalance between the protective and aggressive factors affecting the gastric mucous, which, at a short run, is reduced to the mechanisms of acide-pepsinic or bacterial influences (for references see [1]), while the literature on the putative role of gastric excretory function in the ulcer pathogenesis is scarce [2, 3, 4, 5, 6, 7]. Hence, the role of gastric secretory and excretory functions and their regulatory mechanisms in development of the disease remains vague. Therefore, investogation of the poorly studie problem of interrelation between these two gastric functions seems to be expedient. It is important as well to establish the role of peripheral adrenergic controlling structures in the above interrelation.

With an aim to reach above goal the following successive tasks have been drawn: to study a dependence of the gastric excretory function on intensity of secretion, and to reveal a role of the functional state of adrenergic structures in the processes of interrelation between gastric secretory and excretory fuinctions.

MATERIAL AND METHODS

Two male dogs, weighing 18-22 kg, with a small stomach pouch according to the Pavlov method, were used in the experiments. With an aim to evaluate the stomach secretory function, on the bachground of various stimuli (meat, bread, milk), the secretion latency, and the volume of gastric juice (for 4 hour period) were investigated; in each hourly portion of the juice the free hydrochloric acid concentration and general acidity in a titering units were measured; a digestive power, according to the Metti method, was evaluated.

For the study of stomach excretory function the radionuclide (NaI^{131}) method was emplyed. The dogs were intravenously injected with 0.5, 2.5, and 5.0 mega-Becquerel (MBc) of sodium iodide. The stomach pouch was washed out with 50 ml of physiological saline. In a 4-hour intervals, every 30 min, the wash-down specimens were collected. Evaluation of the radioactivity was made by the automated spectrometer (GAMMA NK 350, Hungary), in each 50 ml specimen of the mixed solution of gastric juice.

With an aim to alter functional state of the brainstem reticular formation and central adrenergic structures chlorpromazine administration was used in the dose of 0.1 and 1.0 mg/kg; peripheral adrenergic structures were manipulated by adrenaline (0.4 mg/kg), ergotamine (1.0 mg/kg), and surgery - unilateral retroperitoneal splanchnotomy.

The digital data obtained in the experiments were processesd by the variation statistics - Student's *t*-criterion.

RESULTS AND DISCUSSION

Following intravenous administration of 0.5 MBc NaI^{131} maximal intensity of its excretion was was observed at 6th and 90th minutes; then the process decreased stepwise. After the four hours the gastric mucous excreted 0.69% of initial NaI^{131} dose. Intravenous injection of 2.5 and 5.0 MBc NaI^{131} resulted in almost equally high excretion indices per specimen, along the whole period of observation. Four hours after the administration 0.87% of initial amount was excreted. Therefore, in the further experiments the animals were given 0.5 and 2.5 MBc of NaI^{131} only (Tables 1, 2, 3).

Investigation of NaI^{131} excretion on the background of the gastric secretion stimulation with various foods has shown: Intravenous administration of 0.5 MBc NaI^{131}, especially after the meat intake, resulted in the statistically reliable increase of excretion. During administration of 2.5 MBc, the gastric secretion stimulation with any kind of food, did not elicit statistically reliable alteration of excretion (Tables 2, 3). Thus, in a case of high concentration of the substance to be excreted, stimulation of the secretion is not an esential factor in the excretion modulation. Investigation of various doses of chlorpromazine in the secretotory and excretory

Table 1

Percentage of NaI^{131} total excretion indices, followinf administration of the substance in various doses

Quantity of NaI^{131}, MBc	Time elapsed after the substance administration, min.							
	30	60	90	120	150	180	210	240
0.5	10	24	38	49	59	63	66	69
2.5	15	31	47	65	83	99	115	129
5.0	20	38	45	49	65	73	77	87

Table 2

Percentage of 0.5 MBc of NaI^{131} total excretion, on the background of various food stimuli

Food stimuli	Time elapsed after the radioactive substance administration, min.							
	30	60	90	120	150	180	210	240
No food	10	24	38	49	59	63	66	69
200 g of meat	15	33	51	66	79	91	104	113
200 g of bread	10	26	41	57	64	73	79	84
500 ml of milk	15	30	43	59	71	80	89	95

Table 3

Percentage of 2.5 MBc of NaI^{131} total excretion, on the background of various food stimuli

Food stimuli	Time elapsed after the radioactive substance administration, min.							
	30	60	90	120	150	180	210	240
No food	15	31	47	65	83	99	115	129
200 g of meat	18	35	50	71	91	111	129	144
200 g of bread	16	34	52	69	86	104	123	139
500 ml of milk	16	34	55	74	95	112	130	144

functions has show that intramuscular injection of 0.1 mg/kg of the drug, on the background of any kind of food intake, resulted in statistically reliable elevation od all the indices of the gastric secretory function and activation of the excretory function, during the first two hours of observation. Intramuscular injection of 1.0 mg/kg chlorpromazine, on the background of all kinds of the food stimuli, elicited suppression of both secretory and excretory functions. It should be noted that

chlorpromazine injection in a dose of 0.1 mg/kg, in absence of the food stimuli, practically did not alter excretion indices, while the dose of 1.0 mg/kg, in the first hour of the observation, statistically reliably decreased excretion indices. Thus, following the chlorpromazine action the unidirectional changes in the secretory and excretory functions are revealed (Tables 4, 5).

Table 4

Indices of the NaI^{131} 4-hour excretion in the stomach, on the background of various influences

Food stimuli	Statistical parameters	Norm	Chlorpro-mazine, 0.1 mg/kg	Chlorpro-mazine, 1.0 mg/kg	Adrenaline 0.04 mg/kg	Ergotamine 1.0 mg/kg	Splanch-notomy
Meat, 200 g	M+m	113+1.83	149+2.16	78+1.41	119+1.58	130+1.91	151+1.39
	D		+36	-35	+6	+17	+38
	t		12.57	15.21	2.47	6.49	16.52
	P		<0.001	<0.001	<0.05	<0.001	<0.001
Bread, 200 g	M+m	84+1.66	120+1.38	58+1.06	133+1.13	120+1.29	153+1.42
	D		+36	-26	+49	+36	+69
	t		18.84	13.19	24.50	16.07	31.50
	P		<0.001	<0.001	<0.001	<0.001	<0.001
Milk, 500 ml	M+m	95+0.93	133+1.82	56+0.92	127+1.13	116+1.42	139+1.98
	D		+38	-20	+32	+21	+44
	t		18.62	22.30	21.91	12.42	20.09
	P		<0.001	<0.001	<0.001	<0.001	<0.001

Intramuscular injection of adrenaline (0.04 mg/kg) induced suppression of gastric secretory function, regardless the kind of food given. The similar injection induced a significant activation of gastric excretory function. At the first 90th-120th minutes the marked synpathico-activating influence of adrenaline was found on the gastric secretion, while parasympathico-activating - on the excretion process. Subcutaneous administration of ergotamine in a dose of 1 mg/kg, on the background of any kind of food stimuli, statistically reliably activated gastric secretory function (except for the secretion latency). The gastric excretory function was activated as well: in a case of feeding with meat and bread - during the first 2.5-3 hours, and in a case of feeding with milk - during 1.5 hours (Table 4, 5).

Bilateral splanchnotomy, on the background of any kind of food stimuli, elicited statistically reliable and significant activation of gastric secretory and excretory functions (Table 4, 5).

According to the foregoing data it could be suggested that pharmacological and surgical desynpathization induce alterations of secretotory and excretory processes, which could be mainly attributed to the vascular mechanisms, with decrease of the mucous blood supply. Accordingly, it could be concluded that intensity of gastric excretory function is dependent on the concentration in the blood of a substance to be excreted. Moderate excretion is stimulated during feeding. Depression of the central adrenergic structures elicits attenuation of gastric excretory ability, while the changes in the functional state of the peripheral adrenoreactive structures induces disorders in the correlational course of gastric secretory and excretory functions.

Table 5

Secretory function of the stomach on the background of various food stimuli: following chlorpromazine (CPZ), adrenaline (AE), and ergotamine (ET), and after the splanchnotomy (ST)

Food stimuli	Drug dose, mg/kg	Statistical parameters	Secretion latency	Volume of excreted gastric juice, ml				Volume of juice during 4 hours	Titration units of the free hydrochloric acid in the hourly specimens				Titration units of the general acidity in the hourly specimens				Digestive power of gastric juice during 4 hours
				1 hr	2 hr	3 hr	4hr		1 hr	2 hr	3 hr	4hr	1 hr	2 hr	3 hr	4hr	
Meat, 200 g	CPZ 0.1	D	-1	+6.9	+3.8	+2.3	+1.0	+12.5	+11	+9	+7	+9	+16	+15	+9	+11	+2.6
		P	<0.05	<0.10	<0.05	>0.05	>0.05	<0.01	<0.01	<0.01	<0.05	<0.05	<0.01	<0.01	<0.01	<0.01-15	<0.01
	CPZ 1.0	D	+6	-2.4	-2.4	-1.6	-1.0	-8.4	-19	-23	-26	-20	-18	-20	-24	<0.01	-2.4
		P	<0.01	<0.01	<0.01	<0.05	<0.05	<0.01	<0.01	<0.01	<0.01	<0.05	<0.01	<0.01	<0.01	-42	<0.01
	AE 0.04	D	+12	-3.4	-3.2	-1.8	-0.8	-9.2	-38	-59	-52	-42	-36	-48	-40	<0.01	-3.9
		P	<0.01	<0.01	<0.01	<0.05	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	+3	<0.01
	ET 1.0	D	-1	+2.1	+0.6	+0.2	+0.4	+3.0	+6	+7	+8	+6	+9	+7	+5	<0.05	+2.0
		P	>0.05	<0.05	>0.05	>0.05	>0.05	>0.05	>0.05	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	+53	<0.01
	ST	D	-4	+10.5	+8.7	+6.5	+4.5	+29.3	+48	+39	+35	+25	+73	+70	+68	<0.01	+4.3
		P	<0.05	<0.05	<0.01	<0.01	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		<0.01
Bread, 200 g	CPZ 0.1	D	-2	+2.7	+2.4	+0.8	+1.1	+6.0	+22	+16	+16	+14	+23	+16	+29	+14	+2.0
		P	>0.05	<0.05	<0.05	>0.05	>0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	CPZ 1.0	D	+6	-2.5	-0.7	-0.7	-0.8	-4.6	-26	-27	-28	-17	-41	-43	-21	-18	+4.3
		P	<0.01	<0.05	>0.05	>0.05	>0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	AE 0.04	D	+13	-4.3	-1.8	-1.8	-1.1	-11.2	-40	-36	-40	-22	-38	-36	-40	-30	+2.0
		P	<0.01	<0.01	<0.05	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05
	ET 1.0	D	-2	+2.0	+0.8	+0.6	+0.4	+4.0	+5	+6	+9	+3	+6	+6	+4	+2	-1.7
		P	>0.05	>0.05	<0.01	<0.05	>0.05	>0.05	<0.05	<0.05	<0.01	<0.05	<0.05	<0.01	>0.05	>0.05	<0.05
	ST	D	-4	+8.5	+6.6	+5.0	+4.1	+15.1	+52	+52	+49	+51	+56	+49	+64	+58	+4.9
		P	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Milk, 500 ml	CPZ 0.1	D	-3	+0.9	+1.1	+1.3	+0.7	+3.9	+16	+16	+15	+12	+18	+14	+18	+9	+1.6
		P	<0.05	>0.05	>0.05	>0.05	>0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05
	CPZ 1.0	D	+9	-4.0	-2.3	-0.7	-5.2	-7.2	-24	-19	-22	-24	-25	-25	-31	-30	-1.8
		P	<0.01	<0.05	>0.05	>0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	AE 0.04	D	+17	-3.6	-3.0	-1.2	-0.6	-9.4	-36	-30	-28	-26	-38	-42	-36	-30	-2.8
		P	<0.01	<0.01	<0.01	<0.01	>0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	>0.01	<0.01
	ET 1.0	D	-1	+1.8	+0.6	+0.4	+0.3	+3.0	+4	+6	+5	+3	+5	+4	+3	+3	+1.6
		P	>0.01	<0.01	>0.01	>0.01	>0.01	>0.01	<0.05	<0.05	<0.05	<0.05	>0.05	>0.05	>0.05	>0.05	>0.05
	ST	D	-3	+6.8	+6.3	+4.2	+3.0	+20.3	+45	+39	+39	+34	+59	+50	+39	+25	+3.1
		P	>0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

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პერიფერიული ადრენერგული სტრუქტურების როლი კუჭის სეკრეტორული და ექსკრეტორული ფუნქციების ურთიერთობაში

გ.სარიშვილი, ნ.კოჭლავაშვილი

თბილისის ექიმთა დიპლომისშემდგომი განათლების სახელმწიფო აკადემია

რ ე ზ ი უ მ ე

ორ ზრდასრულ ძაღლზე, პავლოვის მცირე კუჭის მოდელზე, შესწავლილია პერიფერიული ადრენერგული სტრუქტურების აქტივაციის და ინაქტივაციის გავლენა კუჭის სეკრეციული და ექსკრეციული ფუნქციების ურთიერთობაზე. სხვადასხვა სახის საკვების (ხორცი, პური, რძე) მიწოდების ფონზე, გამოყენებულია ადრენალინის, ამინაზინის და ერგოტამინის შეყვანა, აგრეთვე რეტროპერიტონეალური სპლანქნოტომია. კუჭის ექსკრეტორული ფუნქციის გამოსაკვლევად გამოყენებულია რადიოაქტიური ნატრიუმის იოდიდი.

დადგინდა, რომ ფარმაკოლოგიური და ქირურგიული დესიმპათიზაცია ცვლის კუჭის სეკრეტორულ და ექსკრეტორულ ფუნქციებს, რაც დაკავშირებული უნდა იყოს ლორწოვანი გარსის სისხლით მომარაგების შემცირებასთან. შესაბამისად, შეიძლება დავასკვნათ, რომ კუჭის ექსკრეტორული ფუნქციის ინტენსიურობა დამოკიდებულია სისხლში საექსკრეციო ნივთიერების კონცენტრაციაზე.

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Changes in arterial blood pH and gas composition at acute CO - intoxication and subsequent normo- and hyperbaric oxygenation of white rats

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High effectiveness of hyperbaric oxygenation in case of CO-intoxication was revealed in experiments on rats. The maximal period of time after CO-intoxication, when application of hyperbaric oxygenation will be still effective and whether it can prevent later manifestation of a neurological deficit has to be investigated additionally.

Key Words: *CO-intoxication, Rats, Hyperbaric Oxygenation.*

One of the most prevalent reasons, of both accidental and deliberate poisoning, is the intoxication by Carbon Monoxide (CO). Revelation of CO-intoxication is rather simple process (by estimation of Carboxyhemoglobin level), but the estimation of intoxication depth is interlinked to major difficulties and inconsistencies [3, 6].

It is accepted, that in case of CO - intoxication the treatment by respiration of 100 % Oxygen is indicated [6]. The physiological effect of Oxygen (especially during hypoxia) is multilateral. In patients with respiratory insufficiency Oxygen breathing can increase O_2 tension in both alveolar air and blood plasma [5, 7]. At the same time the concentration of an oxyhemoglobin in arterial blood increases, the metabolic acidosis is reduced, the activity of chemo receptors changes, catecholaminemia decreases, the heart rate is normalized, reparative and trophic processes in tissues are improved [1, 2].

In the present study attempt is made to reveal changes in acid-base balance and gas composition of arterial blood if white rats during acute CO-intoxication and subsequent normo- and hyperbaric oxygenation.

MATERIAL AND METHODS

32 Wistar-line white rats, weighing 250-270g at the beginning of CO-intoxication, were used in this investigation. The animals had a free access to water and gained a standard dose of dry granulose nutrition.

As an experimental model of acute carbon monoxide intoxication we have used a model offered by Jiang and Tyssebotn [4]. Under a mild chloralhydrate narcosis

2-3 days prior to intoxication the unilateral occlusion of a left common carotid artery and catheterization of a femoral artery were done. After wound healing and adaptation to the experimental conditions (lasting 2-3 days) animals one hour were exposed to mixture of air and 0.27%CO. The results obtained by mentioned authors show that such experimental model is quite adequate to study the development of pathological processes taking place during CO-intoxication, and testing of this or other methods for their prevention and treatment [4]. The usage of given experimental model have shown that approximately in 84% of cases, the cerebral edema develops (ipsilateral to the side of common carotid artery occlusion), 76% of animals perish in 8,7±1,7 hour after an intoxication [4].

Procedure of experiment: In two days after the surgical operation the rats were put in the chamber for movement restriction (in which beforehand were within 18 days) and the chamber were placed in 4.5 liter volume flow-through box.

After completion of background measurements the mixture of 0.27% CO and air within 60 minutes were given to animals through the gas mixer in the flow-through box. 18 animals of 24 (survived after CO-intoxication) were divided into 3 groups (6 animals in each): I group was for a control, II group - intended for normobaric, and III - for a hyperbaric oxygenation.

On completion of CO breathing, flow-through box on a short time (some minutes) were vented (for complete elimination of residual CO) by air for I and III groups and 100 % Oxygen - for II group.

Animals of III group were placed into the hyperbaric chamber (3.5 liter in volume special steel chamber, having possibility to control gas pressure. The pressure in hyperbaric chamber was increased by addition of pure Oxygen with the rate of 50 êÐà/min. In 35 minutes (with allowance of ventilation) the balance pressure in 300 kPa was reached in the chamber, which was maintained within 60 minutes. Before decompression the air (10kPa/min) was feed to the chamber. After that animals of III group were placed into the flow-through box for breathing by normal air.

Finally the animals of all groups were reverted in the normal cages. After 24 and 48 hours animals once again for a short time were placed into the "movement restriction" chamber for blood sampling, and then were killed by injection of a lethal dose of Nembutal.

The measurements of ðÍ and gas composition of an arterial blood (ÐàÎ and ÐàÑÎ) were conducted by means of pH/Blood gas Analyzer OP-215 (Radelkis, Hungary). After each sampling the catheter washed out by a normal saline solution.

The general state of the animals, besides the above-mentioned parameters was estimated by locomotor activity.

THE OBTAINED RESULTS

The control levels of studied parameters in all groups of rats were comparable among themselves (see the Table).

During intoxication, after 15-20 minutes of respiration by mixture of air and

carbon monoxide, animals almost have appeared in a state of a semi-coma. Arterial tension of a Carbonic acid (ÐàÑÎ) and arterial ðÍ were progressively decreased without any noticeable shifts in a level of arterial oxygen tension (ÐàÎ). Upon termination of respiration all survived animals, to all appearances were in identical condition of intoxication.

After CO - intoxication ÐàÑÎ has reached a control level less, than for 1 hour in II and III groups and after 4 hour in I group. Within 1-4 hours of the period of post-intoxication the level of a blood ðÍ in II group was much lower than in all other groups of animal. In one hour after CO - intoxication ÐàÎ in normoxic group considerably exceeded control levels. The dynamic of arterial blood ðÍ after CO - intoxication was in inverse correlation with changes of ÐàÎ in I group ($r = -0.9$, $P < 0.001$) and II group ($r = -0.8$, $P < 0.001$). During normobaric oxygenation ÐàÎ increased up to 500 mm Hg and up to 1000 mm Hg in case of hyperbaric oxygenation. At least within 2 hours after a session of a hyperbaric oxygenation in III group (in comparison with group 1) the level of ÐàÎ was higher ($P < 0.05$).

The survival rate in III group (80%) was much higher in comparison with I (22%, $P < 0.001$), and II (44 %, $P < 0.05$) groups.

30 minutes after termination of CO-intoxication animals of first group have restored normal behavior. As against them the animals of second group have revealed progressive neurological disorders characterized by a hemiplegia, lethargic states, spontaneous rotation and have perished on the average in 2.5 ± 0.15 hours. Normobaric oxygenation has prolonged time of a survival on the average up to 3.5 ± 0.2 hours. The hyperbaric oxygenation has prevented development of the indicated disorders in III group, in which spontaneous rotations was not observed. All animals of this group were alive over 6 hours, though the hemiparesis and drowsiness was observed in some of them.

The inverse correlation obtained between arterial ðÍ and Oxygen tension directs on thought, that after termination of CO - intoxication low ðÍ can boost respiratory ventilation and by means of arterial carbonic acid elimination can normalize arterial pH. At the same time, the action of low-level ðÍ can be changed by the increased arterial Oxygen tension, especially at 100 % Î breathing at once after completion of a CO - intoxication. Judging by obtained data it is difficult differentiate whether low arterial ðÍ or normobaric oxygenation, have an effect on the initial stage of recovery period.

Normobaric oxygenation also has reduced period of a semi-comatose state and has prevented animal's death during this process of oxygenation. However, there were cases, when animals suddenly perished after completion of normobaric session, though any tags hinting at such outcome in the process of oxygenation was not observed.

The mortality of animal and neurological disorders did not vary within four hours at normobaric oxygenation, but were considerably reduced within the first hour of a hyperbaric oxygenation started in 35 minutes later after completion of CO - intoxication. In the conclusion of this discussion it is possible to tell, that the data, obtained by us, confirm high effectiveness of a hyperbaric oxygenation, but rise a problem on clarification of maximal period of time after CO - intoxication, when the application of a

hyperbaric oxygenation will be still effective and whether it can prevent, later manifestation of a neurological deficit.

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არტერიული სისხლის **pH**-ის და აირთა შემცველობის ცვლილებები თეთრი ვირთაგვების მწვავე **CO**-ინტოქსიკაციისა და მომდევნო ნორმო- და ჰიპერბარული ოქსიგენაციის პირობებში

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ექიმთა დიპლომისშემდგომი განათლების სახელმწიფო სამედიცინო აკადემია

რეზიუმე

თეთრ ვირთაგვებზე ექსპერიმენტებში ნაჩვენებია ჰიპერბარული ოქსიგენაციის მაღალი ეფექტურობა CO-ინტოქსიკაციის დროს. საჭიროებს დამატებით კვლევას დროის იმ მაქსიმალური პერიოდის დადგენა, როდესაც ასეთი სახის მკურნალობა არ კარგავს ეფექტურობას და შეუძლია მოგვიანო ნევროლოგიური დეფიციტის პრევენცია.

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CARBON MONOXIDE POISONING AND HYPERBARIC OXYGEN TREATMENT: THE MAJOR RESULTS OF LATEST EXPERIMENTAL STUDIES

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The study on white, conscious rats with occluded left carotid artery analyses the correlation between cerebral edema developed after carbon monoxide intoxication and intracranial pressure. Comparative therapeutic effectiveness of normobaric and hyperbaric oxygenation is evaluated. The results demonstrated that the hyperbaric oxygenation can prevent increase in cerebrospinal fluid pressure and all negative consequences of this increase.

Key words: *Carbon monoxide poisoning, Cerebrospinal fluid pressure, Hyperbaric oxygenation, Cerebral edema, Rats.*

Carbon monoxide poisoning is the leading cause of poisoning death in many developed and developing countries. Common morbidity involves myocardial and/or neurologic injury including delayed neurologic sequelae [4, 7]. The pathophysiology of this entity is complex, involving hypoxic stress on the basis of interference with oxygen transport to the cells and possibly impairing electron transport [1, 10]. Carbon monoxide can also affect leukocytes, platelets and the endothelium, inducing a cascade of effects resulting in oxidative injury [5].

Oxygen therapy is accepted as a key treatment of carbon monoxide intoxication [6, 8, 9] and hyperbaric oxygen has been shown to interdict and improve clinical outcome in patients and that stimulates worldwide intensification of experimental study of behavioral, cardiovascular, neurologic, biochemical changes cause by carbon monoxide intoxication and following hyperbaric oxygen treatment.

In investigations of Jiang and Tyssebotn [2] experiments were designed to establish an animal model of acute carbon monoxide (CO) poisoning in awake habituated rats. On the day before CO-exposure, under a brief anesthesia, a Levine preparation (unilateral common carotid artery occlusion) was performed on group 1 and 2 but not on group 3 rats. Group 1 rats were exposed to air as control. Groups 2 and 3 rats were exposed to 0.27% CO in air for 60 min followed by a 3-day recovery in air. The

Levine preparation per se did not induce any detectable physiologic effects on group 1 rats. Identical cardiovascular and metabolic responses to CO occurred in groups 2 and 3. After the CO exposure, all group 3 rats lived for 2 days with normal neurologic index. In group 2 84% of the rats showed increase of neurologic index and edema of the ipsilateral cerebral hemisphere, and 76% of the rats died in about 8 hours after the CO exposure. Neurologic index correlated with the brain edema and inversely correlated with the survival tome after the CO exposure. The authors therefore concluded that exposure of the Levine-prepared rats to 0.27% CO in air for 60 min will provide a valuable model for testing of different treatments for CO poisoning.

Based on a described model of acute carbon monoxide poisoning in rats with an occluded left carotid artery, we have evaluated the effects of normobaric oxygen and hyperbaric oxygen on mortality and morbidity. The results our investigation practically have confirmed data received in similar study by Jiang and Tyssebotn [3]. After exposure to 0.27% of carbon monoxide in air for 60 minutes, the rats were grouped and treated with air (control group) and 100-kPa oxygen for 3. hours (I experimental group), 300 kPa normoxia (II experimental group) and 300 kPa oxygen for 1 hour (III experimental group). The air breathing were started immediately after CO exposure, whereas hyperbaric oxygen breathing began 30 minutes after the end of carbon monoxide exposure. In accordance with data concerning the hypothermia, hypocapnia, drop in mean systemic arterial pressure and acidosis the all group of animals suffered identical level of poisoning. About 40-50 hours after the end of the carbon monoxide exposure, mortalities were 75, 60, 75 and 15% in control and experimental groups respectively. The neurologic morbidities, indicated by abnormal motor behaviors and edema in the left cerebral hemisphere, were 85% in control group, and 65, 81 and 40% in I, II and III experimental groups of animals respectively. Compared to the normoxic treatments, the hyperbaric, but not normobaric one, significantly reduced the mortality and neurologic morbidity. Hyperbaric oxygenation was also markedly better than normobaric in increasing surviving time and survival rate. These results support the value of hyperbaric oxygenation in improving short-term outcome of acute carbon monoxide poisoning in this rat model.

In other series of experiments using the same model of carbon monoxide poisoning the influence of cerebral edema on cerebrospinal fluid pressure and evaluation of the therapeutic effectiveness of normobaric and hyperbaric oxygen were studied.

The cerebrospinal fluid (CSF) pressure was continuously measured via cannula inserted in the left cerebral ventricle before, during and for up 5 hours after exposure 0.27% CO for 60 minutes. A non-sustained small increase in the CSF pressure identical degrees of hypoxemia, hypocapnia, arterial hypotension and acidosis were found during exposure in all rats. After the CO exposure, all non-edema control rats without carotid artery ligation recovered completely with normal CSF pressure, behavior and brain water content. All untreated and normobaric oxygen treated rats developed a severely increased CSF pressure (over 50-100 mm HG) with neurologic motor dysfunction, and died with considerable cerebral herniation. All hyperbaric oxygen treated rats (300 kPa oxygen for 1 hour, beginning at 30 minutes after CO exposure) survived with significantly less neurologic motor dysfunction and less left hemispheric edema than those in untreated and normobaric oxygen treated rats. These results clearly demonstrate that the increase in the cerebrospinal fluid pressure was related to the left hemispheric edema, and that the cerebral herniation is the predominant cause of death after carbon monoxide intoxication.

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ნახშირჟანგით მოწამვლა და მისი მკურნალობა ჰიპერბარული ოქსიგენაციით: უკანასკნელი ექსპერიმენტული კვლევის ძირითადი შედეგები

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ექიმთა დიპლომისშემდგომი განათლების სახელმწიფო სამედიცინო აკადემია

რეზიუმე

მარცხენა საერთო საძილე არტერია ოკლუზირებულ თეთრ ვირთაგვებზე გამოკვლეულია კორელაცია ნახშირჟანგით ინტოქსიკაციის შედეგად განვითარებულ თავის ტვინის შეშუპებასა და ქალასშიდა წნევას შორის. შედარებულია ნორმო- და ჰიპერბარული ოქსიგენაციის თერაპიული ეფექტი. ნაჩვენებია, რომ ჰიპერბარული ოქსიგენაცია აფერხებს თავის ქლასშიდა წნევის ზრდას და მის შედეგად გამოწვეული ყველა უარყოფითი მოვლენის განვითარებას.

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ON SIGNIFICANCE OF GENDER-DEPENDENT RISK-FACTOR IN CHILDREN'S ACUTE HEMOBLASTOSIS

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Number of the boys was shown to exceed that of the girls in the groups ef high risk patients with acute leukemia. The data obtained confirm the suggestion on significance of sex-dependent risk-factor in children's acute hemoblastosis.

Key words: *Acute leukemia, Children, Sex, Risk-factor.*

The term "Hemoblastosis" implies a tumor formation, developed from the hemopoietic cells. Its primary center may be localized in the bone marrow (in a case of leukemia), or outside the latter (in a case of lymphomas). Development of the tumor center and violation of the hemopoiesis indicate existence of the primary indices of hemoblastosis. Infectious and necrotic processes, alterations in hemodynamics, and hemorrhages serve as the secondary indices (2,7, 8}. In the acute hemoblastosis infiltrations into skin and testicles [3,4, 5], central nervous system [5, 6], and lymphatic nodes [5,6, 7, 8] do occur, which promotes development of the heavy relapses. Among the concomitant with hemoblastosis diseases the following are considered - sepsis [4], and gastro-intestinal- and circulation systems' lesions [7, 8]. Although the modem means of acute hemoblastosis treatment are highly efficient in rapid remission attainment, they, however, may induce lesions in the internal organs and central nervous system of a patient [2.3,5,6].

All the aforementioned undoubtedly points at an importance of urgent and detailed investigation of the acute hemoblastoses, considering, especially, that this form of pathology is characteristic of the juvenile age and its incidence is very high in the Georgian population. Particularly, the acute leucosis is found in one child per two thousand, and the disease outcome, regardless the efficiency of the modem therapeutic methods, in a number of cases, remains lethal {4, 5].

A disease prognostics and choice of therapeutic intervention strategy represent a major link in treatment of the acute leucosis [1-3, 5, 7-9}. This, in its turn, is directly concerned with determining of the risk-group of a patient. There are determined

standard-, medium-, and high risk-groups of patients, according to the phenotype of the blast cells, number of leucocytes. presence of spleen: and liver cells' hyperplasia, remission status, chromosomal alterations, response to prednisolone, and to the primary lesion of central nervous system.

In 1998 Georgian hematologists published the data îÿ importance of the patient's sex for determining his/her risk-group [5]. The present work provides the new evidences for importance of a patient's sex as of the risk-factor in the juvenile acute leucosis.

MATERIAL AND METHODS

Total of 138 children with acute leucosis (average age of the patients- 6.3 years) have been investigated. Out of these patients the 103 individuals have been completely recovered as a result of treatment, while the rest were returned to the clinic (2-2.5 years following the end of treatment course), because of the disease relapse (Group A and Group B, respectively). Determination of the risk-group of the patient was made at the moment of admission into clinic, according to the common hematological criteria for the acute leucosis as indicated by the Program ALL-BFM-90 [5,8]. The percent ratio of boys and girls was calculated per each risk-group (Standard, Medium, High) of the A and Â patients, separately.

RESULTS AND DISCUSSION

The Figure 1 shows that in, the Standard risk Group A number of giri & exceeds the number of boys by 10%, while in the Medium risk group the sex ratio is practically equal, and in the High risk group number of the male patients is prevailing. Thus, in the patients, completely recovered after the Program ALL-BFM-90 treatment of the acute leucosis, the least favorable prognosis for the disease course, according to attributing the patients to the High risk group, could be made in boys rather than in girls.

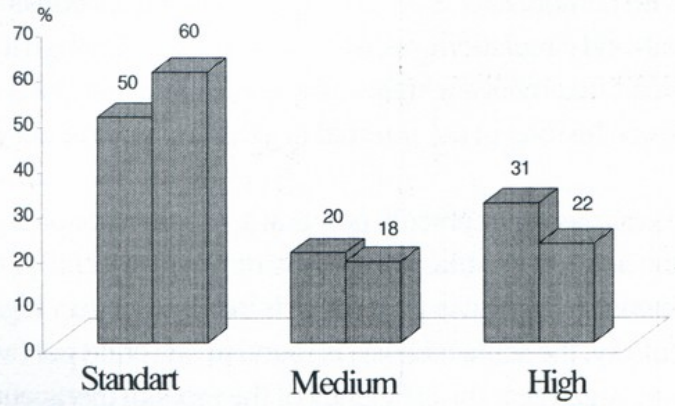

Fig.1. Percent ratio of boys and girls in the acute leucosis patients of the Croup A.Closed bars- boys, open bars-girls.

In the Group B (Fig. 2) number of girls in the Standard risk group is higher than that of boys, while in the Medium- arid High risk groups boys are prevailing. Thus, in a case of leucosis relapse, an unfavorable prognosis, according to attributing the patients to the High risk group, should be made more frequently in boys rather than in girls.

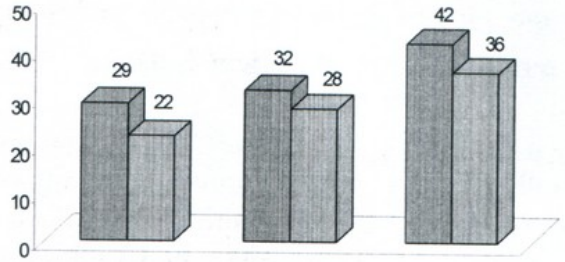

Fig.2. Percent ratio of sexes in the acute leucosis patients of the Group B. All notation as in Fig.1.

In a whole, the results obtained by us support the notion [5] that the sex should be viewed as a risk-factor in the children's acute leucosis. Apparently, at the stage of the disease manifestation, the boys are more vulnerable than the girls. Respectively, prognosis in the boys is less favorable than in the girls and hence the former require more radical therapeutic intervention.

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სქესზე დამოკიდებული რისკ-ფაქტორების მნიშვნელობა ბავშთა მწვავე ჰამობლასტოზებში

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საქართველოს ჯანმრთელობის დაცვის სამინისტროს ჰამატოლოგიისა და ტრანსფუზიოლოგიის ინსტიტუტი, თბილისი

რ ე ზ ი უ მ ე

ნაჩვენების, რომ მწვავე ლეიკემიით დაავადებულთა მაღალი რისკის ჯგუფში ბიჭების რაოდენობა ჭაებობს გოგონებისას. მიღებული მონაცემები განამტკიცებს მოსაზრებას, რომ ბავშთა მწვავე ჰემობლასტოზებში სქესი მნიშვნელოვანი რისკ-ფაქტორის როლს თამაშობს.

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ON THE TREATMENT STRATEGY IN NON-HODGKIN'S LYMPHOMA, ACCOSSING TO THE RISK-GROUP OF THE PATIENT

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Long-lasting investigations have shown that daring treatment of the non-Hodgkin's lymphomas in children, it is possible to exclude irradiation of the brain, if the patient belongs in the Standard risk-group. Exclusion of radiation therapy may significantly attenuate possible complications elicited by irradiation.

Key words: *Now-Hodgkin 's lymphomas. Radiation therapy. Brain, Risk-groups*

The non-Hodgkin's lymphomas ate frequently encountered in the children's hemoblastoses [1, 4]. In treatment of this disease a priority is often given to the polychemiotherapy, and the treatment tactics is executed according to the principles implied in the treatment method» for the acute leucosis m children. Regardless the primary localization of the process, treatment of the children's lymphosarcomas is initiated with the means for remission induction, utilizing various schemes of the polychemiothetapy (except for the patients with abdominal lesions, who are frequently subjected to the urgent surgery). In the period of remission, induction the measures, against infiltration of the tumor cells into the central nervous system, should be taken [f,4]. With this aim the intrathecal administration of Metathrexate and irradiation of the brain are carried out [1, 4].

Unfortunately, both the polychemiotherapy and the brain irradiation result in a number of the side-effects. Application of the chemical substances induces lesions in the internal organs [1, 4], which significantly, complicate the patient's state. Georgian clinicians have revealed the cases of the central nervous system's damage as a result of irradiation of the brain [2, 3], which induced consecutive retardation of intellectual development in the patients [2].

Therefore, a serious problem does arise. On the one hand, the hemoblastoses treatment is impossible without chemiotherapy, and, in some cases, without irradiation of the central nervous system as well. On the other hand, the above-mentioned therapeutic means may induce lesions in a number of the patient's organ systems. The Georgian hematologists [2] suggest that in the patients, who have been diagnosed as belonging to the standard risk-group, physician may refrain from the brain irradiation. Thus, determination of the risk-group has a high importance not in the choice of a treatment strategy only, hut in precaution means against the side-effects of chemical-

and radiation therapies as well, which may induce the complications in the patient.

Hence, according to the aforementioned, we consider it highly important to possess an information on frequency of attributing of the non-Hodgkin's lymphoma patients to one or another risk-group (Standard, Medium, and High, resp,). The present investigation was carried out with an aim to collect the data on this very subject.

MATERIAL AND METHODS

Total of 231 children (aged 6.1 years, at an average), who have been treated in the period of 1992-1995, with diagnosis of the non-Hodgkin's lymphoma, served as the subjects of investigation. Due to the wide-adopted criteria, the patients, treated according to the Program BFM-90 for the acute lymphoblastoses, were re-distributed into the three risk-groups - Standard, Medium, and High.

RESULTS AND DISCUSSION

The percent values of the ill children, attributed to the different risk-groups, at the moment of diagnosing of the non-Hodgkin's lymphoma, are presented at the Fig. 1. Total number of the patients (231 individuals) are considered as 100%. According to the data obtained, the standard risk-group patients are prevailing in the total of ill children. Bearing in mind the above-mentioned recommendation [2] on the strategy for treatment of such patients, our results could be considered as promising in a sense that during last three years (1992-1995) about half of the children with the non-Hodgkin's lymphoma, under our surveillance, comprised the group in which irradiation of the brain could be omitted from the treatment scheme.

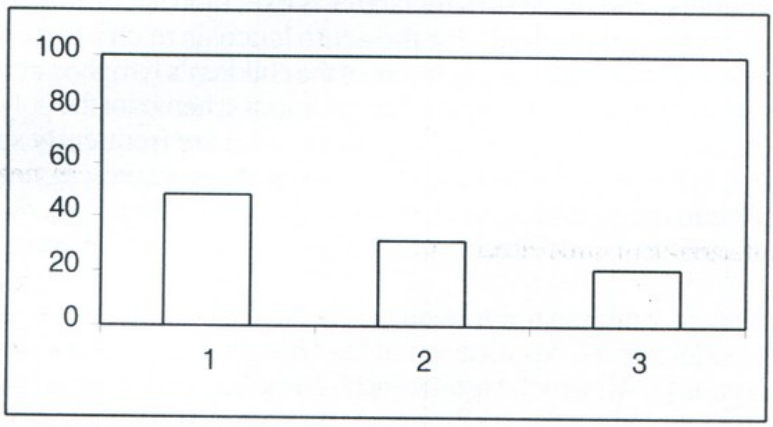

Fig. 1. Number of patients in the groups of Standard (1), Medium (2), and High (3) risk.

If further investigations will support a possibility of avoiding-the irradiation therapy in the Standard risk-group of the patients, and infiltration into the central nervous system could be avoided, treatment methods of the hematological patients (at least the part of them) may be more sparing.

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არა-ჰოჯკინის ლიმფომის მკურნალობის სტრატეგიის შერჩევა ავადმყოფთა რისკ-ჯგუფის მოხედვით

ზ.შაქარაშვილი, ა.შენგელაია

საქართველოს ჯანმრთელობის დაცვის სამინისტროს ჰემატოლოგიისა და ტრანსფუზიოლოგიის ინსტიტუტი, თბილისი

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ხანგრძლივი გამოკვლევებით დადგინდა, რომ არა-ჰოჯკინის ლიმფომით დაავადებულ ბავშთა მკურნალობისას შესაძლებელია თავი ავარიდოთ თავის ტვინს რადიაციულ დასხივებას, თუ ავადმყოფი სტანდარტული რისკის ჯგუფს მიეკუთვნება, და ამით შევამციროთ რადიოთერაპიით გამოწვეული გართულებების შესაძლებლობა.

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PSYCHOPHYSIOLOGICAL APPROACH IN THE EARLY DIAGNOSIS OF GLAUCOMA

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The eyes with preclinical stage of glaucoma and with primary open angle glaucoma (POAG) have been investigated with an aim to reveal characteristics of early disorders of the visual function in initial glaucoma; The topographical study of contrast sensitivity in the On-Off channels of the retinal cone system was used as a method. A new specific psychophysical symptom of initial POAG - predominant decrease of sensitivity in the Off-channels (to the opponent colors), exhibited in deceleration of the sensorymotor reactions (SMR), and preservation or insignificant decrease of sensitivity in the On-channels - has been revealed.

Key words: *Glaucoma, Chromatopsia, On-Off channels, Human*

In the meantime there exists a notion concerning the glaucoma, as of disease characterized with increased intraocular pressure over the tolerable level for the particular eye, development of specific atrophy with excavation of the optic nerve disc, and deterioration of the visual function [6, 7, 8].

The problem of early diagnosis of glaucoma remains complicated. In the last decade a number of new psychophysical and electrophysiological methods, for revealing the early alterations in the visual function in glaucoma, were proposed. However, character, topography, and specificity of disorders revealed are studied insufficiently, which necessitates search for new, more informative methods of the visual functions' study, providing for determination of initial glaucoma symptoms at the preclinical stage [8, 10, 11]. Therefore, in the present study it was endeavored to detect early psychophysical symptoms of this disease.

MATERIAL AND METHODS

Total of 125 human subjects aged 43-70, with visual acuity of 0.8-1.0, were divided into the following three groups: Group I - 30 healthy control individuals (60 eyes); Group II - 60 eyes with POAG; Group III - 35 eyes with no clinical signs of glaucoma, although suspected.

Presence of morphologically and functionally heterogeneous On-Off channels in the retinal cone system, serving recognition of the opponent colors (black-white, red-green, yellow-blue), provides for suggestion on their different reactions when the pathological processes are localized in different structures of the visual analyzer [1, 2, 3, 4, 5, 12, 15].

Investigations were carried out according to the method by Shamshinova *et al.*[9, 11, 13, 14, 15] designed for the studies of activity in On-Off channels of the retinal cone system. In mesopic conditions, achromatic and chromatic stimuli, on the respective opponent backgrounds, are displayed on the computer monitor screen. The stimuli are presented in a random order, in each predetermined by schedule point, in a zone of 2, 5, and 12.5 degrees. The stimulus luminosity increases stepwise from the darker than background, to the brighter than background one, with the step value of 0.5 cd/m^2. Luminosity difference between stimulus and opponent background (the luminosity contrast) is assessed according to the SMR time value. Results are presented as a histogram of dependence of the SMR time on the stimulus luminosity. Testing was carried out monocularly, distance between the eye and the screen was 33 cm. All the numerical data obtained were processes statistically.

RESULTS AND DISCUSSION

During investigation of the contrast sensitivity topography in the central zone of visual field (the On-Off channels' activity) in normal (Group I) subjects, it was found that in a case of presentation of both achromatic and chromatic stimuli on the opponent background, if the stimuli were closely illuminant as compared to the background, the zone was evident, where the SMR time was significantly higher than in a case, when the stimulus and the background difference was visible. Along with increasing difference between the stimulus and the background, towards either the darker than background, or the lighter than background stimuli, the SMR time symmetrically and evenly decreased and, since the reaching the certain value, did not change. Increasing of receptive fields from the center to the periphery was considered during choice of the optimal dimensions of stimulus aimed to obtain the maximal response in the norm. During presentation of 2 mm stimulus into the zones of 2 and 5 degrees, and of 3 mm stimulus into the zone of 12.5 degrees, the averaged SMR-time/stimulus-luminosity curves coincided between each other and were symmetrical against the conditional axis.

In the patients with initial POAG (Group II), in 100% of the cases, increase of the SMR time to the stimuli darker and lighter against the opponent background was observed in the zones of 2, 5, and 12.5 degrees. The SMR-time to the stimuli (chromatic and achromatic) darker than the background of high contrast, increased 2.2$\pm$0.2 times; in a case of medium contrast - 3.5$\pm$0.3 times; in a case of low contrast - 2.6$\pm$0.1 times;

while in a case when the stimuli were brighter than the opponent background, the SMR-time, at high contrast, increased 1.5±0.15 times; at medium contrast - 1.7±0.2 times; at low contrast - 1.5±0.1 times.

In 75% of the patients, which at the time of examination had no clinical signs of glaucoma (Group III), the same regularity was observed, which allows to speak on existence of psychophysical symptoms preceding the clinical manifestations of the disease.

Thus, a new specific psychophysical symptom of the initial POAG has been determined - predominant decrease of sensitivity in the Off-channels of the retinal cone system, which is displayed in deceleration of the SMR-time, along with unaltered or insignificantly decreased sensitivity in the On-channels.

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ფსიქოფიზიოლოგიური მიდგომა გლაუკომის ადრეულ დიაგნოსტიკაში

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გლაუკომის კლინიკამდელ სტადიასა და პირველადი ღიაკუთხოვანი გლაუკომის (პღკგ) შემხვევებში ჩატარებულია თვალების გამოკვლევა, რათა გამოგვევლინა მხედველობის ფუნქციის საწყისი დარღვევების მახასიათებლები ადრეული გლაუკომის დროს. ამ მიზნით გამოყენებულია On-Off-არხების კონტრასტული მგრძნობელობის ტოპოგრაფია ბადურის კოლბების სისტემაში. დადგენილ იქნა ახალი, სპეციფიკური პღკგ-ის ფსიქოფიზიკური სიმპტომი - Off-არხების (ოპონენტურ ფერებზე) მოხედვით მგრძნობელობის უპირატესი დაქვეითემა, რაც გამოიხატებოდა სენსომოტორული რეაქციის დროის შენელებაში მაშინ, როდესაც On-არხების მიხედვით მგრძნობელობა უცვლელი რჩებოდა, ან მცორდებოდა უმნიშვნელოდ.