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Laser Correlation Spectroscopy: Nutritional, Ecological and Toxic Aspects

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1. Introduction

Changes in physiological status of the organism under the effect of environmental factors modulate metabolic processes, which in turn, affects the composition of biological fluids and possibly of bioelements – substances, important for building and maintenance of the living matter. Bioelements are the elemental functioning units of living matter, which are biologically active complexes of chemical elements as atoms, ions and nanoparticles with organic compounds of exogenous (primary) or biogenous (secondary) origin (Skalny, 2009, 2011). Evaluation of subfraction composition of samples provides information on percentage of biosubstrate constituents and yields integral characteristics reflecting the function of systems and their readiness to adequate protection of the body. This initially biophysical method, named laser correlation spectroscopy (LCS) is used for screening of large groups of conventionally healthy individuals for diagnosis of body functional strain and detection of risk groups (Karganov et al., 2009). Optimized software takes into account individual peculiarities in internal sanogenetic systems of the organism when determining consequences for health in persons exposed to low doses of potentially harmful factors (Kryzhanovsky, 2004). LCS allows measuring particle size in native biological fluids. It is successfully used in clinical practice for evaluation of the disease severity and treatment efficiency in patients with asthma, diabetes mellitus, myasthenia, some haematological diseases, and other pathologies (Kovalev et al., 2002; Piruzyan et al., 2004; Karganov et al., 2006).

2. Principles of laser correlation spectroscopy

Laser correlation spectroscopy (LCS) (analogs: spectroscopy of quasi-elastic light scatter, optical mixing spectroscopy, photon-correlation spectroscopy) consists in measurement of spectral characteristics of light scattered in quasi-elastic mode from the spectrum of intensity fluctuations of the recorded light (Gulari et al., 1979; Hautz et al., 1981; Horn, Dalgleish, 1985; Hwand, Cummins, 1982; Chu, 1974). Two schemes of measurements can be used: homodyne (selective recording of the light scattered by the system) and heterodyne

(recording of beats between the scattered light and reference fixed high-intensity light). The spectrum of light intensity fluctuations is a Fourier-transform of a correlation function of intensity fluctuations of the recorded field. In the device used by us, a heterodyne scheme (Fig. 1) is applied (Lebedev et al., 1997). Helium-neon laser serves as a source of light (2).
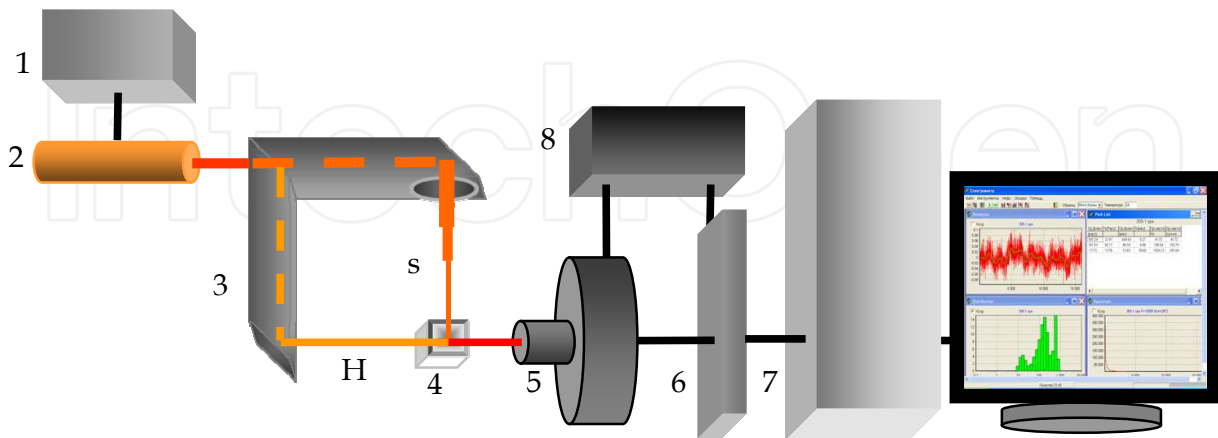

Fig. 1. Scheme of laser correlation spectroscope.

Laser beam is divided by a plate (3) and about 0.1% light beam (S) is separated from the main beam, is transmitted to a photorecorder (5), and is mixed with the scattered portion. Not the spectrum of light scattered by the studied system, but the spectrum of photoelectric fluctuations at the photorecorder (5) output is directly recorded in LCS. This spectrum represents a result of mutual beats of electromagnetic filed harmonics and is located in a low-frequency band.

The fluctuation spectrum of photoinduced current $I(\omega)$ at uniform size of scattering particles is described by Lorenz curve:

$$I(\omega) = A \; \frac{\Gamma}{\Gamma^2 + \omega} \; , \tag{1}$$

where $\Gamma = D_T q^2$ is width at half peak of the spectrum and q is a transmitted wave vector of the light scattered by the sample,

$$q = \frac{4\pi n}{\lambda} \sin\frac{\theta}{2} \; ,$$

where n is medium refraction coefficient,
λ is wavelength,
θ is scattering angle,
and D_T is a coefficient of translation diffusion of scattering particles.

The size of scattering particles can be determined from D_T. The relationship between diffusion coefficient D_T and hydrodynamic radius of particles R_h is described by Einstein-Stokes formula:

$$R_h = \frac{kT}{6\pi\eta D_T} \; ,$$

where T is absolute temperature,
k is Boltzmann constant,
and η is medium viscosity.

Correlation spectroscopy analyzes not the determinate, but the stochastic signal. Therefore, the measured parameter of the spectrum has a statistical error related to stochastic nature of light scattering. For improving the accuracy, measurements should be performed in many independent realizations and the results should be averaged.

The size of particles can be evaluated by measuring the peak width in current fluctuation spectrum. However, biological fluids are as a rule not monodisperse and contain particles of different sizes. The spectrum of light scattered by monodisperse particles is approximated by a Lorentz curve (1), whereas for polydisperse system it is a sum, or, for continuous distributions, is an integral of Lorentzians with different widths.

In this case, the spectrum I (ω) is described by the formula:

$$I(\omega)=A \int \frac{A(\Gamma)\Gamma}{\Gamma^2 + \omega}d\Gamma \; , \tag{2}$$

where $A(\Gamma)$ is a function describing the distribution of particles by diffusion coefficient ($\Gamma = D_T \, q^2$, see above), and, hence, by their size. Assessment of particle size distribution consists in solution of the integral equation (2) with Lorentz kernel. This type of tasks is characterized by significant instability of the solution relative to variation of the experimental data. Generally, this is solution of Fredholm integral equation of the first kind. Using regularization method we can find approximate solutions for incorrectly posed inverse problems. This substantially extends the possibilities of experimental data interpretation.

Model experiments showed that for obtaining precise distribution characteristics the noise level σ of the experimental spectrum should not exceed ~0.1-0.3%. At the same time, actual spectra at 10 min accumulation time have noise level of 1% (2000 Hz band) or even 3% (200 Hz). Preprocessing of the experimental data improves analysis of these spectra due to intensive smoothening without loosing information on the true distribution. The smoothening procedure can be repeated, this will reduce the scatter of experimental points.

Parameters of the reconstructed distribution appreciably approximate to actual values. The required information on the distribution shape can be obtained from experimental curves with noise level of 0.5-1%.

In the given algorithm, the regularization parameter is determined on the basis of known error of experimental data. For the analysis of recorded spectra, the noise level of spectral curve should be determined with an accuracy of 15-20%. Overestimated error increases the bias caused by regularization procedure, while underestimated errors can lead to the appearance of additional false components (Braginskaya et al., 1983). Thus, the developed protocol of mathematical processing (regularization procedure) of spectrum $I(\omega)$ yields function $A(\Gamma)$ describing particle distribution by scattering characteristics. Setting a certain model of scattering particles allows evaluation of the weight distribution of these particles by their size.

It is known that the major contribution into light scattering is made by proteins and their complexes that have spherical shape. The hydrodynamic radius of a spherical scattering particle somewhat surpasses the geometrical radius of a dry particle due to the formation of a shell consisting of solvent molecules. For globular proteins, the hydrodynamic size is close to the diameter of the macromolecule. For non-spherical particles, the contribution of orientation dynamics (and conformation dynamics for deformable particles) is significant. Therefore, the hydrodynamic size of non-spherical protein molecules is a rather rough parameter. At the same time, the coefficient of translation diffusion is an objective physical characteristic of the protein particle reflecting structural changes in macromolecules: aggregation, conformational transitions, enzyme-substrate interactions *etc*.

It should be noted that light scattering capacity of high-molecular-weight particles is higher than that of low-molecular-weight particles. Therefore, the method is more sensitive to large objects. In case of relatively high concentration, they can shield the low-molecular-weight fractions in LC spectrum.

A histogram of typical size distribution is presented in Fig.2. The size scale is discrete and consists of 32 points.
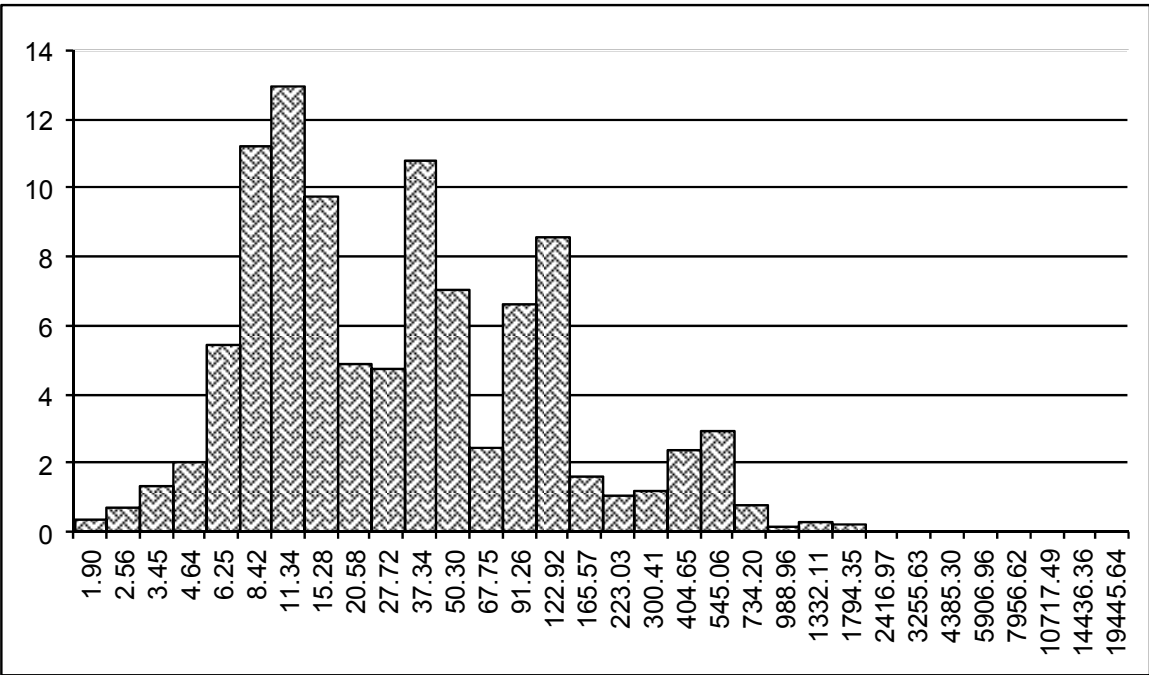

Fig. 2. Histogram of size distribution of particles in blood serum. Ordinate – contribution of particles of the corresponding zone into light scattering (%). Abscissa – particle size (nm).

The distribution histograms in the serum provide qualitative information on the mean particle sizes and their relative content. Strict correspondence of certain fragments of the spectrum to biological nature of serum components can be determined after additional studies.

Visual analysis of histograms is low-effective for screening studies; special classification programs are required to enable analysis of data bulk over a short time. The algorithm of classification analysis is based on methods of the theory of groups. For each serum n from group v the histogram is described by a vector $A_i n^{(v)}$ and represent a point in a 32-space (i =

1,…..32; n = 1,…..N, where N is the number of patients in the group). For a group of patients with certain pathology, the points will be located in certain region of the space. After examination of a representative group of patients, a region corresponding to $A_i^{n(v)}$ values can be determined, which will allow qualitative attribution of a particular case to this group. The regions corresponding to normal health or different diseases can overlap, which means that the diagnosis cannot be made from LCS data only. Examination of a group of patients allows outlining a specific LCS data region in the 32-space. However, this comprehensive information requires a huge sample (if N cases is sufficient for one parameter, 32 parameters require N^{32} cases). It should be noted that A_i are obviously not independent values: mathematical analysis shows that the histogram contains information on 4-5 independent parameters. Moreover, not all parameters are of diagnostic value. The next approximation implied consideration of mean particle size in fractions 0-20 nm, 20-120 nm, and >120 nm and the relative number of particles in these fractions. Preliminary analysis showed that these parameters most adequately reflect relevant (from medical viewpoint) information contained in $\{A_i\}$ set. From mathematical point of view, this corresponds to projection of the 32-space to a 5-space. This reduction means certain information loss. On the other hand, this modification makes the entire procedure more resistant to stochastic measurement errors and reduces the necessary size of referent groups to acceptable values.

3. Basis for semiotic classifier

In case of blood plasma or serum, the total spectrum range is divided into 5 discrete intervals (by the size of scattering particles): I - 0-10 nm; II - 11-30 nm; III - 31-70 nm; IV - 71-150 nm; V - >150 nm. The first interval primarily includes low-molecular-weight monomer albumins and free glycolipid complexes; the second interval comprises globular proteins and low-molecular-weight lipoprotein complexes; the third interval contains larger lipoprotein complexes, RNP and DNP particles, and immune complexes with the lowest molecular weight; the fourth interval includes constitutive medium-molecular-weight immune complexes; the fifth interval is filled in case of immunopoiesis activation with the formation of high-molecular-weight immune complexes (usually associated with allergisation or autoimmune sensitization).

The same method is applied for the analysis of urine samples and oropharyngeal washout fluid (OPWF), but in these cases other informative intervals are used. For OPWF the spectrum is divided into 4 intervals: I - 0-50 nm; II - 51-400 nm; III - 400-2000 nm; IV - >2000 nm; for urine: I - <75 nm; II - 76-220 nm; III - 221-1500 nm; IV - >1500 nm. According to data obtained during studying of various pathological states, these intervals contain molecular components of cells: from polypeptide fragments to high-molecular weight protein complexes. It is assumed that increased areas in low- and medium-molecular-weight intervals of LC spectra reflect predominance of biosubstrate degradation processes, while increased areas in high- and very-high-molecular-weight intervals indicate predominance of biosubstrate polymerization processes.

Basing on the increase (or decrease) in the percent contribution of particles of a certain fraction into light scattering, a semiotic classification of LCS spectra was proposed including identification of 8 types of shifts in homeostasis and humoral immunity.
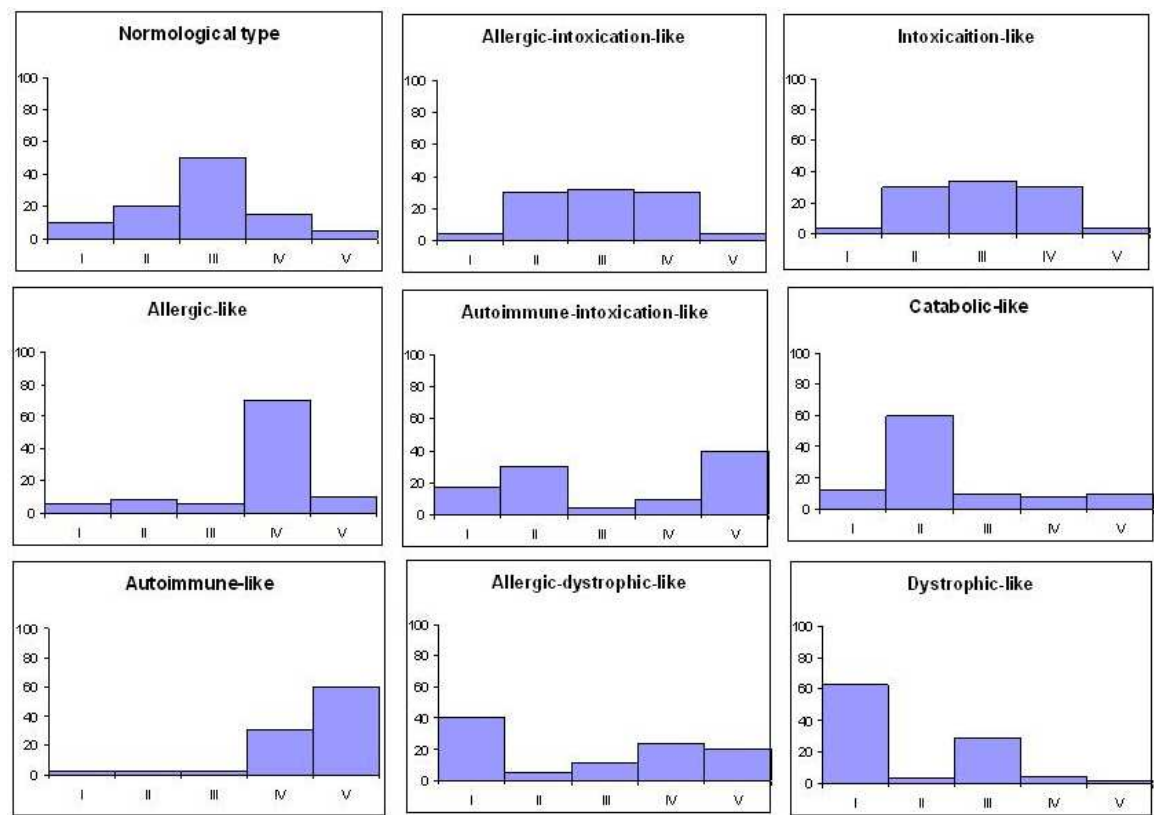
Fig. 3. Scheme of metabolic shifts, used in semiotic classifier. Ordinate – contribution of particles of the corresponding zone into light scattering (%).

Several gradations reflecting the degree of the above-listed spectral shifts correspond to each symptom-complex: initial, moderate, and pronounced.

4. Sample preparation and storage

For laser correlation spectroscopy, any biological fluid (blood serum/plasma, urine, oropharyngeal washout fluid, lachrymal fluid) can be used.

The oropharyngeal washout fluid is a multicomponent medium containing saliva and cell elements (epithelial cells and leukocytes). Some of the cells are partially or completely destroyed. The saliva is characterized by high proteolytic activity and its structural components (glyco- and lipoprotein complexes, immunoglobulins, etc.) are in a degraded state. Rapid and considerable dilution of this highly enzymatically active fluid sharply inhibits proteolytic activity. Simultaneously, structural components of the saliva are diluted to low concentrations making very difficult their detection by routine biochemical and physicochemical methods.

For preparing urine samples, medium portion of morning urine is used. The material (not less than 1 ml) is centrifuged for 30 min at 5000 rpm. The supernatant is collected in 1.5 ml disposable plastic tubes.

Blood is taken routinely: 200 µl of whole blood is transferred to plastic tubes with 800 µl physiological saline. The samples should be left at room temperature for 0.5-2 h and then centrifuged at 5000 rpm for 15 min. The supernatant is collected in new plastic tubes.

The samples can be frozen immediately after preparation and stored until analysis. Freezing is indispensable for transportation and long-term storage of the obtained biological material. Freezing and storage of samples at -25°C and below is an optimal regimen. Rapid freezing to -10°C/-15°C in the freezing compartment of domestic refrigerator is acceptable, but the samples should not be stored at this temperature for more than 3 months. Even single defrosting of the biological samples during storage is inadmissible.

Express LCS analysis of oropharyngeal washout fluids allowed us to perform screening of students of general and special schools in different regions of Russia influenced by various factors.

5. Ecological factors

For evaluation of the effect of recreation activities on functional state of children, we examined schoolchildren in a summer camp (Al'met'evsk, Tatarstan). This stationary country camp is located in ecologically benign region (pine forest) and meets recommendations for children recreation and nutrition in accordance with physiological and age-specific requirements. During examination, the peculiarities of homeostasis and humoral immunity were analyzed by laser correlation spectroscopy of oropharyngeal washout fluid.

The results of the first and second tests were compared in pairs. Shifts in the magnitude of the revealed changes along the axis "pronounced—moderate—initial" or transition from a shifted state to normal metabolism were taken as positive dynamics. Opposite shifts by degree of changes or transition from normal metabolism to predominance of catabolic or anabolic processes were taken as negative dynamics. Unchanged degree of metabolic shifts was regarded as the absence of dynamics.

A total of 62 schoolchildren (age 13±2 years) were examined. At the beginning of the session, schoolchildren with intoxication-like and dystrophy-like homeostatic shifts predominated (38 and 30%, respectively); pronounced shifts constituted 49%, initial and moderate shifts constituted 51%. On the whole, catabolic shifts predominated (79%) over anabolic (10%) and normological (11%) (Fig.4).

At the end of the session, we observed a decrease in the incidence of intoxication-like (from 38 to 22%) and dystrophy-like (from 30% to 14%) homeostatic shifts in tissues of the upper airways of the examined schoolchildren; the contribution of allergy-like shifts increased from 3 to 35, while the contribution of normological shifts remained unchanged.

By the end of the session, the metabolic shifts were classified as initial and moderate. The ratio of catabolic to anabolic shifts was 44% to 39%. Normological spectra constituted 17%.

Thus, using the LCS method we revealed differences in the direction of metabolic shifts in children at the beginning and after 1-month stay in the recreation camp. After this period, positive changes in metabolic status were observed in almost a half of children; in 22% examinees no changes were revealed and 31% children demonstrated negative dynamics.

By their magnitude (irrespective of the direction), 45.5% shifts at the beginning of the session were classified as pronounced and 54.5% as initial and moderate. At the end of the session, initial and moderate shifts (74%) predominated over pronounced ones (26%).
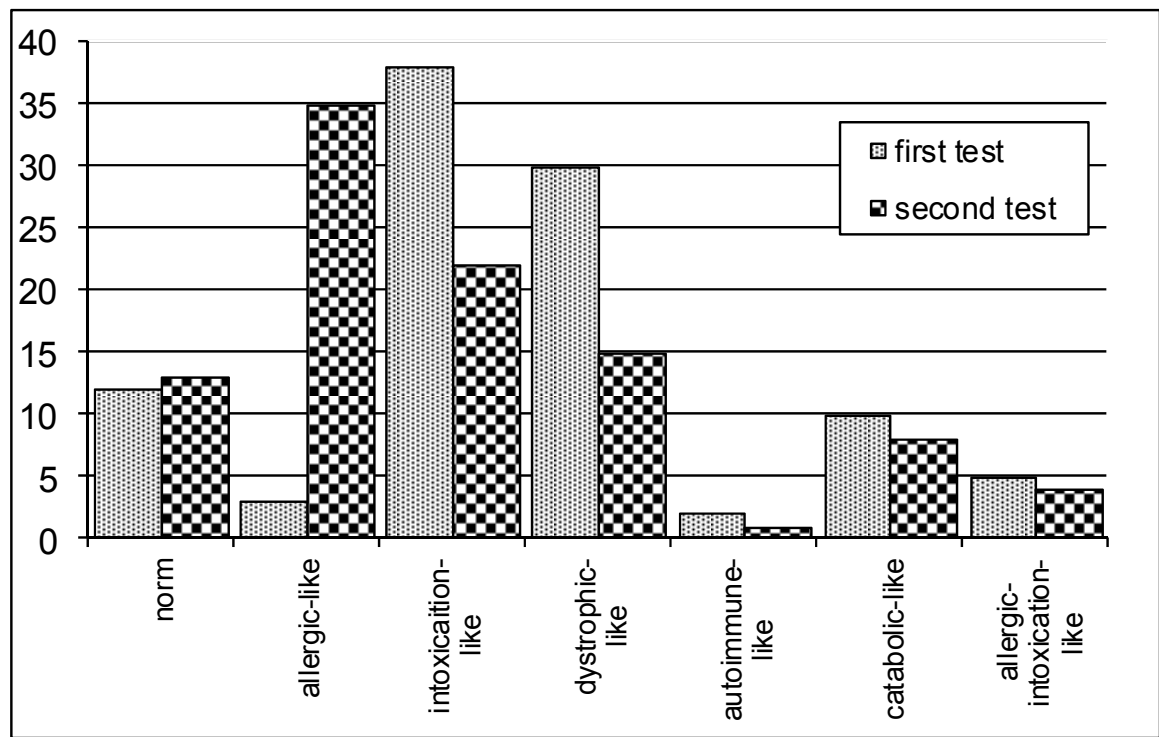
Fig. 4. Prevailing types of metabolic shifts in children in a summer camp. Ordinate - % of children with corresponding metabolic shift.

6. Nutrition factors (drinking regimen)

Various environmental factors act on human organism; for evaluation of the effect of a single factor, e.g. drinking water, on population health, copy-pair studies should be carried out.

Within the program for drinking regimen arrangement in Moscow schools (North-western and North-eastern districts) 240 pupils were examined.

Experimental groups consisted of preschool and primary school children attending schools with special water supply regimen. The control groups included children living in the same districts and attending schools with usual water supply conditions. Children in experimental schools of the North-western district drank "Moskoviya" mineral water over a period from September to May; the same water was used for cooking. In the North-eastern district, children drank "Troitsa" mineral water containing iodine, calcium, fluorine, magnesium, and sodium, and consumed "Zolotoi shar" vitaminized drink (30% of the dose recommended by the manufacturer). "Zolotoi shar" vitaminized drink contains vitamins C, A, E, D, B1, B2, B6, PP, biotin, folic acid, and pectin. The children received vitamins in dosage constituting 10-15% of daily physiological requirement. "Troitsa" and "Moskoviya" light-mineralization hydrocarbonate-calcium-magnesium waters (high-quality mineral waters according to Sanitary Regulations and Norms 2.1.4.1116-02) are intended for children's and dietetic nutrition. The children drank 300-400 ml per day.

Analysis of LC spectra showed that the initial distribution of predominant metabolic shifts (groups of catabolic and anabolic LC spectra) in children differed insignificantly in the two districts. Repeated examination was performed 6 months after the start of drinking bottled water.

Results of the change in drinking regimen were evaluated by individual dynamics of metabolic shifts. It was found that incidence of negative dynamics was significantly lower in children drinking specially prepared water (irrespective of its type). The percent of children with stable parameters of metabolism and with positive shifts increased under these conditions.

It was interesting to compare the effects of special drinking regimens. To this end we analyzed individual dynamics of metabolic shifts in children drinking "Moskoviya" water (North-western district) and "Troitsa" water with "Zolotoi shar" additive (North-eastern district).

It was found that special drinking regimen increased the percent of children with normological character of metabolism in both districts. However, the incidence of allergy-like metabolic shifts significantly increased in children of Northeastern district compared to the control. In children of the Northwestern district receiving water without additives, the incidence of anabolic (allergy-like) shifts was lower than in the control group. (Fig. 5).
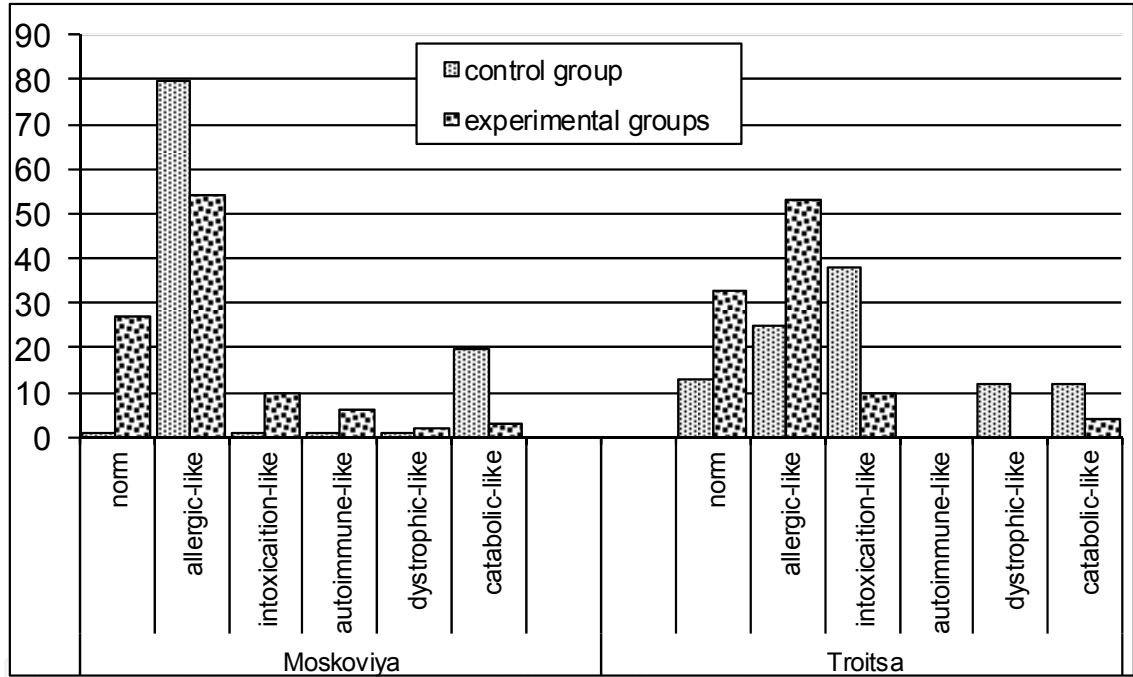

Fig. 5. Prevailing types of metabolic shifts in children with different drinking regimen. Ordinate - % of children with corresponding metabolic shift.

Moreover, drinking "Troitsa" water with "Zolotoi shar" additive led to an increase in the incidence of negative dynamics and a decrease in the incidence of neutral and positive dynamics. Taking into account the time of examination, this result can be explained as follows. The second examination was performed in May when the children are sensitized by natural allergens, and therefore we probably observed a combined effect of these factors and some components of "Zolotoi shar" additive.

Thus, complex examination of children consumed water with improved trace element composition and supplemented with vitamins over 6 months revealed a considerable decrease in the incidence of negative dynamics of metabolic processes in tissues of upper airways and gastrointestinal tract and an increase in the percent of children with normological type of metabolism.

7. Intoxication factors

Laboratory diagnostics allow not only obtaining specific results for each individual, but also combining basically different methods for more precise evaluation of the functional state of various body systems. This approach implies evaluation of combination and reciprocal influence of various parameters, their relationships with clinical symptoms and correlations with other indexes, rather than simple accumulation of laboratory data.

We present data obtained during combined use of standard trace element assay protocol in individuals with different content of some chemical elements (Hg, As, Mn, etc.) and laser correlation spectroscopy of blood serum, urine and OPWF. We analyzed biological samples obtained from 18 individuals conceivably exposed to toxicants: Hg and As.

Examination of biomaterials obtained from individuals exposed to mercury and arsenic showed that the incidence of some shifts significantly correlated with the content of these elements.

Biological samples for the analysis of trace elements were obtained twice with a 3-day interval; samples for LCS were taken once (during the second examination).

The content of chemical elements in biosubstrates was measured by inductively coupled plasma mass spectrometry (ICP-MS). The method of ICP-MS is characterized by high sensitivity and allows detection of a complex of trace and ultra-trace elements (Li, Be, B, V, Co, As, Se, Rb, Cd, Sn, Cs, Hg, Tl, Cr, Mn, Ni, Cu, Sr, Ba, Pb etc.) in samples.

ICP-MS measurements showed that the percent of individuals with normal mercury content considerably increased by the second measurement, which attests to toxicant elimination from the body. Similar tendency was observed during the analysis of arsenic content in the urine (Fig. 6).

The LCS analysis of serum revealed predominance of spectra, where small particles make the major contribution into light scatter. Intoxication-like and catabolic shifts prevailed, while dystrophic shifts were infrequent, which was a result of predominance of hydrolysis and catabolism.

LC-histograms of the urine and oropharyngeal washout fluids reflect the processes of epithelium destruction. In this sample, primarily normological and anabolic shifts were observed in the gastrointestinal system and upper airways, whereas in the excretory system normological, catabolic, and anabolic shifts were detected. Although we cannot prove the fact of intoxication or determine its degree, we revealed significant correlations between the results of LCS study and standard toxicological examination (Fig.7).

Analysis also revealed a negative correlation between the shifts in OPWF and arsenic concentration in the urine: the higher was the urinary concentration of metals, the greater was the contribution of small particles into light scatter.

The lower was the arsenic concentration in urine, the higher was the contribution of large particles into light scattering in OPWF (r=-0.75, p<0.05). We revealed two tendencies related to mercury concentration: in the urine (r=0.06, p<0.05) and in hair samples (r=0.074, p<0.05), which indicated that in samples with low metal concentrations the contribution of large particles into light scattering was higher.
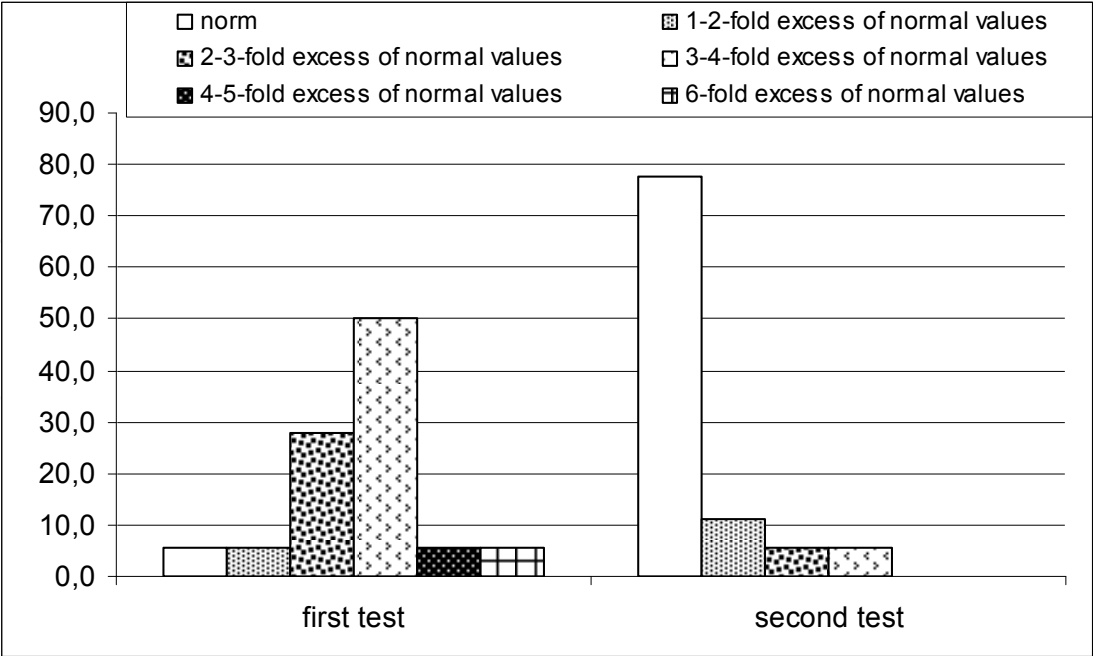
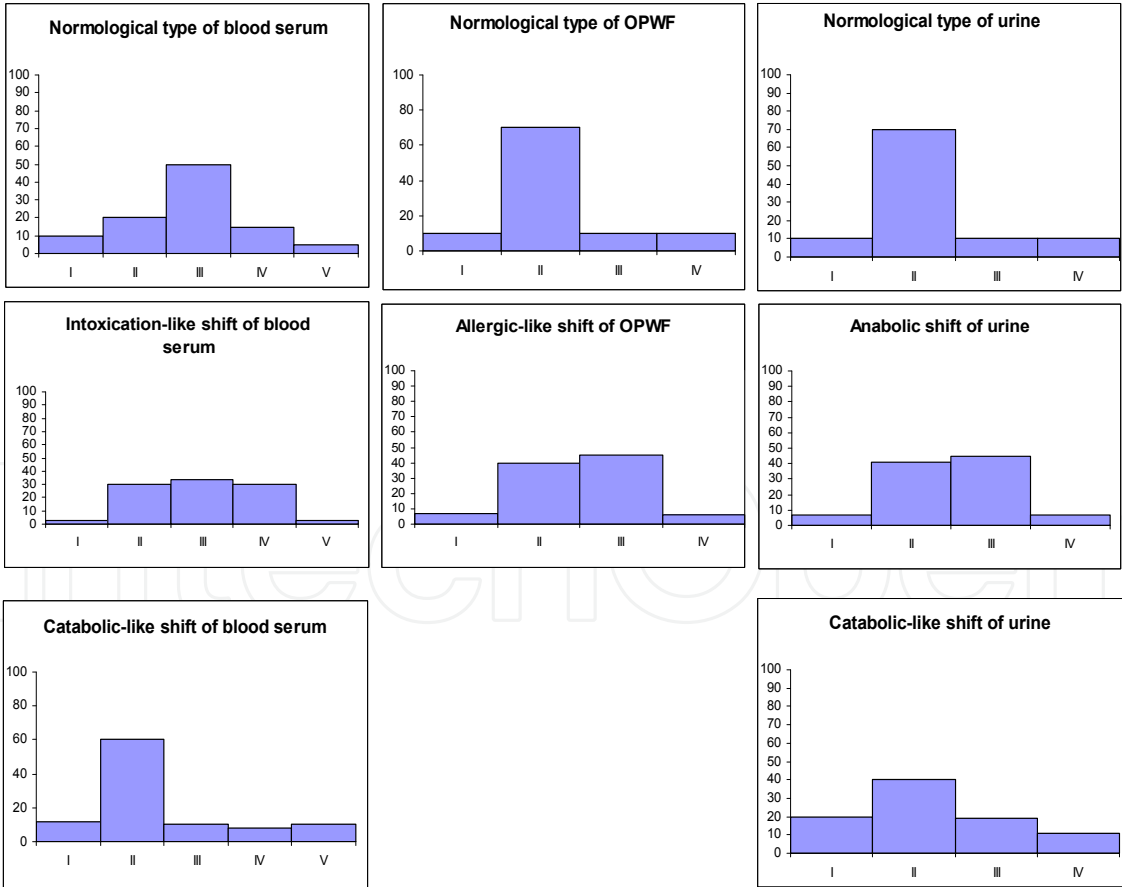Fig. 6. Percent of individuals with normal and enhanced arsenic content.

Fig. 7. Histograms of size distribution of particles in different biological fluids corresponding to different types of metabolic processes. Abscissa - informative zones of the spectrum, ordinate – contribution of particles of the corresponding zone into light scattering (%).

Significant correlations between shifts in the blood serum and metal concentration in the urine were found for samples taken during the second examination: mercury ($r=0.50$, $p<0.05$) and arsenic ($r=0.82$, $p<0.05$)

Analysis of the relationship between metal concentration and the contribution of certain particles into light scattering revealed a correlation between the presence of arsenic in urine and accumulation of the first-zone particles (0-10 nm) ($r=0.53$, $p<0.05$) in blood serum. An increase in arsenic content in the urine correlated with an increase in the contribution of second-zone particles (51-400 nm) ($r=0.76$, $p<0.05$) and a decrease in the contribution of the third-zone particles (401-2000 nm) ($r=-0.79$, $p<0.05$) in OPWF samples; the increase in arsenic content in hair samples correlated ($r=0,58$, $p<0,05$) with an increase in the contribution of first-zone particles (0-50 nm).

Thus, LCS analysis of biological fluids from individuals with elevated metal content revealed increased contribution of small particles into light scatter.

All these changes in the content of toxic elements did not exceed the biologically permissible levels and should not induce pathology development. It is known that elevated content of toxic elements by itself does not indicate the development of pathology. Moreover, individual peculiarities of sanogenetic systems of the organism determine various consequences for health in individuals exposed to equal low doses of potentially harmful factors (Kryzhanovsky, 2004). Nevertheless, their correlation with the contributions of particles of a certain size in biological fluids to light scatter suggests that changes in trace element composition are related to metabolic shifts.

It is known that mercury in low concentrations activates lipid peroxidation (LPO) and initially increases, but then decreases activity of antioxidant enzymes. In this case, accumulation of LPO products (malonic dialdehyde and β-microglobulin, protein in the urine) can be expected. Indeed, positive correlations were revealed between the content of toxic elements and relative content of small particles in the examined fluids. Moreover, the contribution of the fraction containing immunoglobulins into light scatter in the serum tended to decrease with increasing arsenic concentration in the urine. Previous studies showed that the intensity of light scatter by this fraction correlates with immunoglobulin content determined by routine laboratory methods.

The maximum number of correlations was found for shifts in the urine and oropharyngeal fluid. This is most likely related to initial stages of renal dysfunction and presumably peroral intoxication route.

The observed changes in the direction of metabolic shifts are stochastic and reflect either adaptive or disadaptive responses of the organism to low doses of toxic compounds. More numerous sample and broader range of effective concentrations are required for deciphering of the real significance of the detected relationships (Karganov et al., 2011).

Metabolic deviations, measured by LCS, are due to different external influences including effects of such typical ecopollutants as mercury and arsenic. Thus we can suppose the LCS can be an integrative method, useful for experimental and clinical bioelementological research, what was demonstrated in the experimental study described below.

8. Rat spleen genome DNA stability in experimental model of folate-induced hyperhomocysteinemia

Hyperhomocysteinemia is a result of disturbed methionine/cysteine metabolism and vitamin deficiency (B_6, B_{12}, folate). Homocysteine (HC), a product of natural degradation of the essential amino acid methionine, exhibits some toxic properties. High plasma content of HC is an independent risk factor for atherosclerosis, venous thrombosis, and other cardiovascular diseases (Thompson, Kim, 2004), as well as neurodegenerative disorders accompanying Alzheimer's (Miller, 1999) and Parkinson's diseases (Duan, 2002).

We have studied the influence of homocysteine level in blood plasma (control – 10.4±2.3, experiment – 33.2+6.3 μmol/l) on the structure of genomic DNA from the spleen of six-month rats under conditions of experimental folate-induced hyperhomocysteinemia. The specimens of DNA were kindly granted by Prof. Renat Zhdanov.

Particle size was different in the control and experimental samples (Fig. 8). In the control samples, particles with a diameter of 300-400 nm and 60-12 nm predominated (57 and 37%, respectively); in the experimental samples the percentage of 300-400-nm particles was the same (56%), while the content of 90-120-nm particles decreased to 26%. The experimental samples contained also medium-sized particles (165-220 nm, 11%) and large particles (545 nm), which were absent in the control (Fig. 8).

Measurements were performed also for DNA samples treated with DNaseI for 30 and 120 min (Fig. 8). The first distribution (30 min) showed that the content of 300-400-nm particles in the control samples decreased by 15%, but new particles with a diameter of 165 and 220 nm appeared (10 and 13%, respectively). In the control samples, particles with a diameter of 90-120 nm predominated (40% of all particles, Fig. 8).

In the experimental samples, large particles (300, 405, and 545 nm) practically completely disappeared (only 4.6% particles with a diameter of 300 nm were present). The amount of medium-sized (165 and 220 nm) and small (90, 120 nm) particles increased by more than 15% and by 10%, respectively. Thus, particles of all sizes, from 90 to 220 nm, were present in the experimental samples in equal amounts (about 23, 5%).

Repeated measurements were performed 120 min after DNaseI treatment. In the control, the proportion between the numbers of particles remained practically unchanged (changes did not exceed 2.5%, Fig. 8). At the same time, in the experimental samples the content of medium-sized and small particles differed considerably from the results of previous measurement. The content of medium-sized particles (165 and 220 nm) increased to 31 and 42%, (vs. 20 and 27%, respectively). The content of small particles (90 and 120 nm) decreased from 21 to 6% and from 24 to 13%, respectively. Particles with a diameter of 220 nm predominated (42% of total number of particles, vs. 5-7% in the control).

So, the presented data are good illustrations of the necessity of complex biophysical and biochemical investigation in life sciences, because biosphere is an assembly of bioelements and living organisms, existing under permanent regulatory influence of physic-chemical factors (Skalny, 2011).
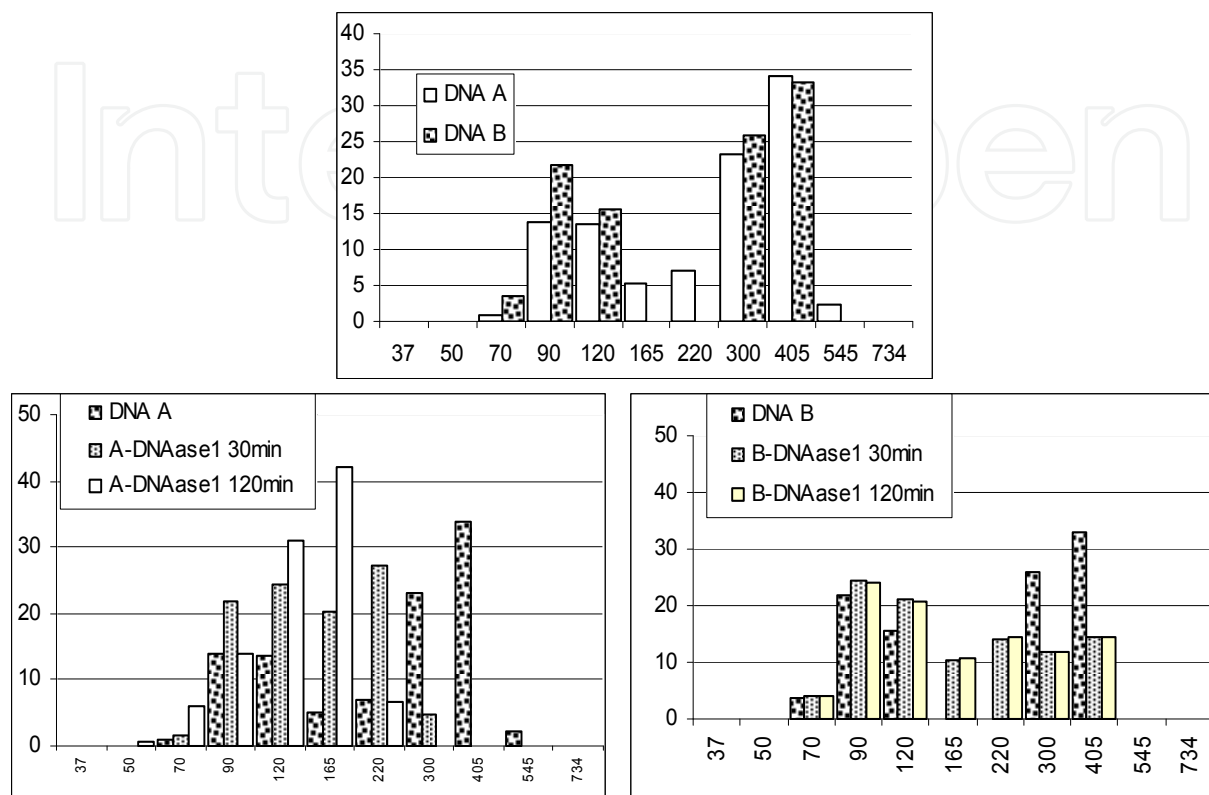
Fig. 8. Stability of rat spleen genome DNA in experimental model of folate-induced hyperhomocysteinemia. Abscissa – particle size (nm). Ordinate – contribution of particles of the corresponding zone into light scattering (%).

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Edited by Dr. Prof. Dr. A.N. Misra

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