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Polysystemic Approach to Risk Assessment

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1. Introduction

Health hazard from natural and anthropogenic sources has begun to be analyzed 2-3 decades ago. Until recently this analysis was primarily applied for human safety in incidents. However, due to stochastic features of risk assessment its applicability to the diagnosis, prevention, and protective or compensatory measures in some cases can be very limited.

The proposed concept combining approaches based on risk analysis and sanogenetic analysis is aimed at increasing the efficiency of both approaches in addressing issues of risk prediction.

There are several reasons necessitating integrated approach to risk analysis:

1. Identifying of risk groups in the population subjected to anthropogenic influences is associated with the formation of groups in which the frequency of fixation of certain pathological processes is dramatically increased. However, modern approaches of epidemiological analysis are based on statement of the accomplished fact (prevalence of cancer, hematological diseases, congenital and hereditary abnormalities, reduced life span, *etc.*). Most these consequences are long-lasting (sometimes for many years) and state the hazard rather than predict the risk.
2. In cases when anthropogenic influences are evaluated on the basis of detection of undesirable biological responses (e.g. chromosome aberrations, accumulation of lipid peroxidation products, changes in activity of some enzymes etc.) the recorded shifts usually ambiguously determine the expected consequences. Biological aftereffects of the detected shifts can to a certain degree reflect both desirable (development of resistance) and undesirable (fixation of certain pathologies) outcomes.
3. In most cases, the above-listed approaches are informative only in relatively high doses and concentrations of this or that anthropogenic factors and are almost irreproducible in case of low-dose and low-concentration influences.
4. Many effects (mortality, pathologies) as consequences of anthropogenic risk factors are unspecific, i.e. can be caused by other factors.
5. The data on the effect of some harmful factors suggest that the probability of this effect is proportional to the probability of spontaneous appearance of the same effect, caused by a complex of other factors of human life. We can speak about synergism of various factors.
6. Modern industrial conditions practically exclude single-factor exposure. Existing methods of epidemiological risk assessment practically cannot differentiate between

chemical, radiation, climatic, noise, vibration, and other impacts. Hence, they do not provide a reliable argument for choosing optimal preventive measures.

7. The method of documentation of pathological consequences rules out the possibility of early prophylactic protection of the population contacting with hazardous industries, which is a priori more efficient and less costly than treatment of the realized risks.

By the wide range of effects, all existing real dangers of radiation, physical, chemical, and biological nature can be divided into two categories: 1) risks in the deterministic range of doses and concentrations (doses and concentrations far surpassing than the established thresholds), 2) risks in a stochastic range of doses and concentrations (doses and concentration near the established thresholds). In the deterministic range of doses and concentrations, the biological effects strictly depend on doses and concentrations of anthropogenic factors and can be detected by existing methods of epidemiological analysis. In the stochastic range of doses and concentrations of anthropogenic factors, the consequences strictly depend on individual sensitivity of biological objects, including humans.

In the human body, many regulatory systems operating at different levels of organization provide sensitivity or resistance to both external and internal factors. On the results of analysis of functional adequacy of sanogenesis systems predicts the level of resistance or sensitivity to relatively tolerable doses and concentrations of anthropogenic influences. Hence, sanogenetic monitoring is more informative for the range of doses and concentrations that cause stochastic effects.

Due to individual variability of functioning of sanogenetic processes, the same anthropogenic factor in equal doses and concentrations will cause certain effects in some organisms (sensitive), will not cause in others, and will induce resistance in the third. This implies that at the population level three subpopulations should be determined at relatively low-dose and low-concentration exposures: sensitive, neutral, and super resistant. The ratio between these subpopulations can eventually serve as a criterion of population risk from this exposure.

Any stable fixation of the pathological trace is preceded by processes of dysregulation of the corresponding functions. The most probable pathological outcomes can be predicted on the basis of the results of polysystemic sanogenetic monitoring by detecting dysregulation in certain systems of the organism (cardiorespiratory, psychomotor, and metabolism systems). Monitoring is carried out using computerized measurement instrumentation and data processing systems, which provides the basis for strict quantitative assessment of the dynamics of risk for the studied populations. The risks assessment goes from the instrument of control to the rank of controlled processes, which is the basis for successful operation of potentially hazardous industries.

2. Methods

Hardware base of the sanogenetic monitoring complex includes three major appliances adapted to non-invasive screening survey:

- spiroarteriocardiorhythmograph for continuous non-invasive recording of blood pressure, expiration and inspiration air flows with a highly sensitive ultrasonic transducer, and electrocardiogram;

- computer-aided device for express-evaluation of psychomotor activity from motor tests;
- laser correlation spectrometer intended for identification of the pattern of regulation of metabolic and immune processes.

All tests were performed with strict adherence of general bioethical standards.

Individual *functional sufficiency of the cardiorespiratory system* was evaluated using a Spiroarteriocardiorhythmograph instrument complex (SACR, recommended by Ministry of Health Care and Social Development of the Russian Federation for clinical use; registration certificate #29/03020703/5869-04, St. Petersburg) allowing simultaneous recording of the heart, vascular, and respiratory rhythms. The method makes it possible to calculate the relative contribution of sympathetic and parasympathetic autonomic nervous system (ANS) into heart rate and BP regulation, integrated values of cardiogram intervals, parameters of lung ventilation, baroreflex parameters, etc.

Electrocardiogram (ECG) was recorded in standard lead I over 2 minutes. The time-amplitude parameters of PQRST complex and heart rhythm variability (HRV) were evaluated using statistic, geometric, and spectral parameters. HRV power in different frequency bands determined using Fourier-transform analysis characterizes ANS activity and the function of the central mechanisms of heart rate regulation. Three frequency bands can be distinguished in spectra: very low frequency (VLF, 0-0.04 Hz), low frequency (LF, 0.04-0.15 Hz), and high frequency (HF 0.15-0.4 Hz), which are measured in absolute values of power ($msec^2$). These values can also be presented in standardized units (LFn, HFn) calculated as the ratio of each spectral component to their sum. Index of autonomic balance (AB=LF/HF) and index of centralization (C=(VLF+LF)/HF) were calculated from HRV spectral parameters.

Peripheral systolic and diastolic blood pressure (SBP and DBP, respectively) and their variability were measured on middle phalanx using the method of Penaz. From the parameters of BP pulse wave, hemodynamic parameters, stroke volume, and cardiac output were calculated using phase analysis of cardiac cycle and BP. Spontaneous arterial baroreflex sensitivity ($BRS=LF_M/LF_{SBP}$) was also evaluated. From geometric parameters of HRV (mode, mode amplitude, amplitude of oscillations, etc.), autonomic balance index, parameter of adequacy of regulation processes, autonomic rhythm index, and regulatory system strain index (SI) were calculated.

For evaluation of functional reserves of the cardiovascular system, a functional test with increased “dead space" was used (Trukhanov et al., 2007). Reactivity of the cardiovascular system was evaluated by changes in the parameters describing its function (in %) during ECG recording in spirometric mask in comparison to ECG recorded without the mask. The time of inspiration and expiration, volume rate of inspiration and expiration, and respiratory volume of quiet breathing in an averaged cycle were evaluated. Parameters of forced expiration (vital capacity of the lungs and volume of forced expiration) were also measured.

The study of the *latent period of simple sensorimotor reaction and other psychomotor parameters* was performed using a specific instrument called "CMM" (computer movement meter), Registration Certificate # 29/03041202/5085-03. The accuracy of measuring the time of simple sensorimotor reaction was 1 ms. A subject was placed in a comfortable chair,

while his/her hand was placed on a special handle and lever, which, in turn, may revolve around a vertical axis. The fulcrum of the rotating segment was treated as the upper third of the forearm. In this position the forearm and wrist could commit abduction and adduction. Mechanical resistance to rotational movement of the forearm was insignificant and therefore ignored in the calculations. Initially, the lever with forearm and wrist resting on it was fixed on the zero position by electromagnetic stoppers. Participants were instructed to focus on the cross in the centre of the screen, and to adduct the handle of the lever with their forearm and wrist as quickly as possible in response to the visual signals started at random intervals (4–8 seconds). The visual signals (vivid light) were slightly peripheral to the central visual field, in order to potentially speed up sensor motor reaction. At the instance of switching on light the electromagnetic stoppers were simultaneously removed and the lever was able to move freely. The latent period of the motor response (reaction time – RT) was measured from the moment of switching on light until the angular displacement of the the handle with forearm at 1 degree was recorded by a computer. The subjects were not limited in the amplitude of translation of the lever. The study was performed for both dominant and subdominant hands. Each subject received 16 signals for reaction with each hand. Training before the experiment included reaction for 10 visual stimuli which were randomly distributed in time.

Subfractional composition of blood serum was analyzed using laser correlation spectrometer (LCS, certificate of Committee on New Medical Instrumentation, Ministry of Health Care and Social Development of the Russian Federation, RU.C. 39.003.A N 5381, St. Petersburg). The method is based on changes in spectral characteristics of monochromatic coherent helium-neon laser radiation due to light scatter in disperse system (blood serum, urine, and other biological fluids) (Karganov et al., 2011). The degree of this scatter is proportional to particle speed, which depends on its hydrodynamic radius. The spectra of blood serum samples (0.2 ml) were recorded and processed routinely (Karganov et al., 2011b).

Evaluation of individual sensitivity to ionizing radiation was carried out on mitogen-stimulated peripheral blood lymphocytes. The cells were cultured in glass flasks in a medium containing sterile embryonic calf serum ("Perbio-HyClone", USA), RPMI-1640 medium with 25 mM HEPES and sodium bicarbonate, phytohemagglutinin ("Sigma", USA), glutamine, and antibiotics.

The cells were irradiated on a "Luch-1" γ-apparatus (Medical Radiological Research Center, Russian Academy of Medical Science, Obninsk) at 0.25 Gy/min radiation power and 65 cm distance. The adapting dose (AdD) was 0.05 Gy and the damaging dose (DD) was 0.5 Gy. Three samples for each examinee were irradiated according to the following schemes: 1) AdD at G0 stage of the cell cycle without DD (for evaluation of the effect of AdD); 2) DD at G2 stage of the cell cycle 48 hours after AdD; 3) DD without AdD (for evaluation of DD). The cells were incubated and fixed using standard methods (Hungerford D.A., 1965). The preparations for routine analysis were stained with azure and eosin and examined under a microscope in transmitted light under oil immersion at ×1000. The following chromosome aberrations were counted: chromatid and isochromatid fragments and symmetrical and asymmetrical chromatid exchanges. For each donor, 100 metaphase plates per term were analyzed. The RAR coefficient at different irradiation doses was calculated by the ratio of the number of chromosome aberrations: RAR=control + DD/(AdD+DD); RAR≥1.5 indicates the presence of the adaptive response.

Statistical processing of experimental data was performed using nonparametric tests (Kruskal-Wallis, Mann-Whitney U test, Spearman correlation coefficient, and Fisher exact test), because empirical data did not conform normal distribution according to Kolmogorov-Smirnov test. The differences between the parameters within the group were evaluated using paired Wilcoxon T test. The data are presented as M±SEM. The significance level was 5%.

3. Results

3.1 Screening examination of workers of the nuclear fuel plant

Device complex and methodological approaches have been tested during screening examination of workers of the nuclear fuel plant.

Laser correlation spectroscopy was used for the analysis of blood serum from individuals exposed to repeated or single irradiation. Changes in spectral characteristics associated with shifts in the homeostatic system were revealed (Akleyev, Kisselyov, 2000). Further studies in this field showed that even single radiation exposure leads to metabolic shifts in the organism towards predominance of catabolic processes. Redistribution in the blood serum spectrum was also directed towards accumulation of the low-molecular fraction (Akleyev & Kisselyov, 2000).

We examined workers (n=328) employed at nuclear fuel cycle plant in Electrostal' town contacting with open (shop #1, uranium and its products, MPC level) and sealed (shop #2) sources of radiation, or exposed to combined influence of radiation (shop #3, uranium and its products, above MPC level), chemical, and other factors. Blood serum samples from workers not contacting with radioactive materials were used as the control (n=16).

Comparison of the percentage of metabolic shifts in different shops showed that the percentage of normological spectra was low in shop #1; the percentage of anabolic shifts in all shops was decreased in comparison with the control (Fig. 1).

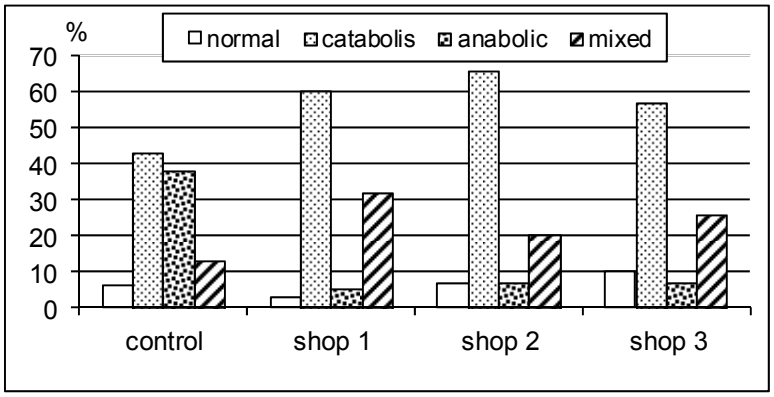

Fig. 1. Incidence of different types of metabolic shifts at nuclear fuel cycle plant of Electrostal' town.

High percentage of catabolic shifts is typical of all shops, which reflects similar life conditions and influence of similar environmental factors.

Detailed analysis of integral LC histograms with evaluation of the contribution of particles of a certain size into light scattering allows identification of the fraction determining maximum differences between the shops (Fig. 2). Significant differences of averaged LC histograms in different shops from summary LC histograms of blood serum in the control group were noted at various points.

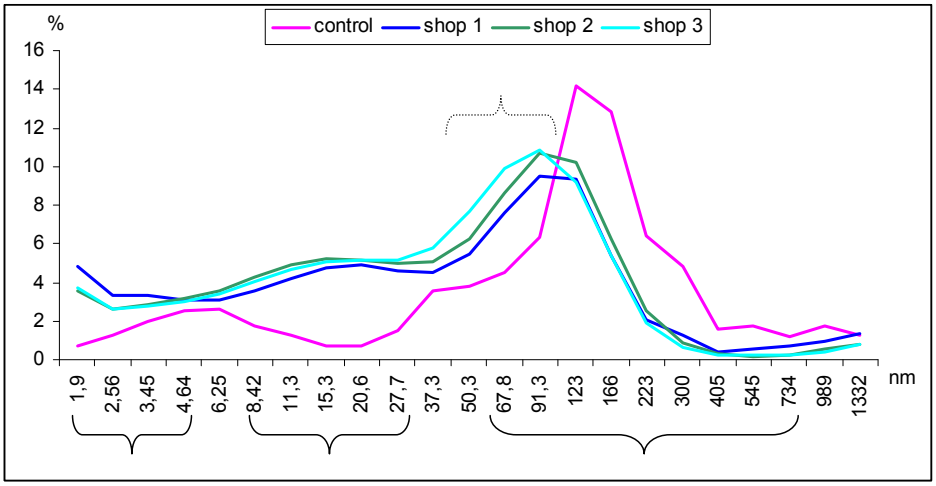

Fig. 2. Integral LC histogram of blood serum in different shops of nuclear fuel cycle plant of Electrostal' town. Points significantly differing from the control group are shown with solid bracket, dashed bracket shows difference of LC histogram of shop # 1 from that of shop # 2 and # 3; $p<0.05$.

This method of data presentation shows that despite the differences of all three shops from the control group, the subfraction composition of blood serum in shop # 1 workers significantly differs from that in two other shops by many points. This situation makes us to conclude that technological factors are the main cause determining different distribution of metabolic shifts in this sample.

One of the leading technological factors is ionizing radiation (primarily γ-radiation). According to the current sanitary and hygienic standards, occupational irradiation from artificial sources should not exceed 50mZv per year.

Shop #1 workers receive 2 times higher annual dose of irradiation than workers of other shops. Therefore, plant management is expected to improve medical monitoring and safety control for workers of this shop.

Twofold increase in the dose may cause changes in metabolic shifts; therefore it is necessary to analyze the dynamics of the ratio of predominant directions of metabolic shifts in 2002-2003 (Fig. 3).

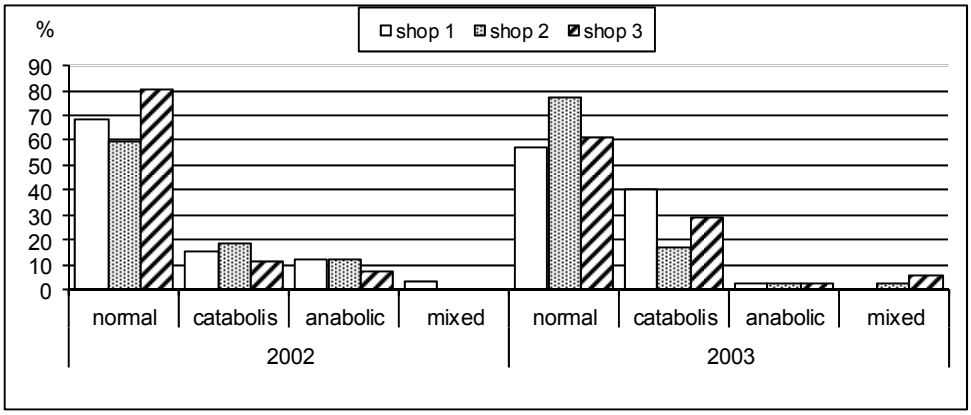

Fig. 3. Ratio of metabolic shifts in different shops

Evaluation of the dynamics of metabolic shifts of plasma homeostasis in different shops revealed the same character of differences: increased contribution of catabolic shifts. Questions arise whether this increase is related to accumulation of the irradiation dose and in which shop the strain of adaptive resources in workers was maximum. To this end, the ratio of metabolic shifts in the same workers after repeatedly evaluated after 12 months (Fig. 4).

Fig. 4. Changes in the ratio of metabolic shifts over 12 months. Ordinate: differences between the percentage of the spectra in 2002 and 2003.

It is obvious that more pronounced changes were found in shop #55 (work with sealed sources of radiation): significant increase in catabolic shifts after exposure to apparently less damaging influences.

The studied parameters were expressed in absolute values (msec, ml, mm Hg, %) and also scored. This method of analysis is based on comparison of the real parameter with averaged data for conventionally normal sample with consideration for age and gender.

Centile rating for the parameters used in our studies implies that the incidence of the most balanced values (within ±0.5 centiles) is about 50%, moderate strain (from -0.5 to -1.5 and from +0.5 to +1.5) is detected in 40% observations (of them 20% are shifted towards hypofunction and 20% towards hyperfunction) and more pronounced (pre-pathological) strain is detected in 10% observations (<-1.5 centiles for hypofunctional shifts and >+1.5 centiles for hyperfunctional shifts). Thus, each measured parameter can be attributed to one of the three above degrees of strain and the direction of functional strain can be designated with plus or minus sign.

Apart from parameter evaluation, we used integral systemic rating; to this end, modal values of all parameters of the system were summed up. If the system included 7 parameters (e.g. the system characterizing myocardial contractility includes the following parameters: HR and P, PQ, QR, QRS, QT, and ST intervals), the corresponding ratings of the integral strain of the system were determined as follows: 1, balanced, modal sum <4.5 centiles; 2, moderately strained, modal sum from 4.51 to 6.5 centiles; 3, strained, modal sum >6.51.

Apart from modal summation, algebraic sum reflecting predominant direction of functional shifts for the whole system was calculated: negative values attested to hypofunction and positive to hyperfunction.

According to general polysystemic monitoring concept, individual functional reserve of the organism to certain working conditions can be determined only on the basis of dynamic observation. Since in our study we performed only single functional scanning, interpretation of the results can be performed using centile distribution of the parameters in populations not burdened by verified pathologies (conventionally healthy individuals).

The examinees were primarily male individuals: from 93% (shop 3) to 61% (shop 2) and 63% (shop 1).

The analyzed groups differed by the age: individuals aged 40.1-50 years predominated, while the percentage of individuals of other age groups was similar (19 and 24% for the groups of 30.1-40 and over 50.1 years, respectively).

Analysis of variability of RR intervals of integral ECG showed that the incidence of balanced states (level 1) was maximum in shop 2 (70%) and minimum in shop 3 (54%). The percent of individuals with strained functional state (level 3) was similar in all shops (8-9%) and did not exceed the range of frequencies characteristic of healthy population (≤10%).

The latter suggests that the myocardial contractility regulation system is not the target of unfavorable factors.

It should be noted that the incidence of strained levels of autonomic regulation of the heart rate in shop 1 (17%) and shop 3 (15%) surpassed the level for healthy population by 1.5-1.7 times and the corresponding value in shop 2 by 2.5 times.

On the basis of age-dependent relationships between the incidences of different strain levels for autonomic regulation of the heart rhythm, we can conclude that the increase in the strain in the heart rhythm regulation system is determined by aging, rather than by technological processes.

Integral evaluation of the functional state of peripheral resistance regulation system (based on measurement of systolic and diastolic pressure strain in digital arteries and baroreflex levels by low- and high-frequency components of the arterial spectrum) showed that the incidence of strained states (rating level 3) in all three groups almost 2.5 times surpassed the level observed in healthy population (10%).

Hence, occupational risks can be expected to be realized in the system of vascular regulation. This assumption is also confirmed by more strained situation in shop 3. Moreover, the incidence of detection of strained parameters practically did not depend on the age of the examinees both in the whole group and in each shop separately.

Of the parameters constituting the integral rating of the whole system, the contribution of hypertensive strain of systolic and diastolic pressures (15 and 14%, respectively) was lower than the contribution of hyperfunctional baroreflex strain (by the low- and high-frequency components of blood pressure spectrum) observed in 23 and 19% cases, respectively. Baroreflex regulation is most tightly coupled with endogeneous mechanisms of tissue metabolism regulation, which was conformed by LCS analysis of the blood plasma, oropharyngeal washout fluid, and urine.

The levels of systolic and diastolic blood pressure are regulated by different mechanisms; the strain in these mechanisms can differ in different groups. The incidences of strained states by the individual components of systolic pressure regulation in the whole examined group considerably surpassed those observed for individual components of diastolic pressure regulation (16-27% *vs.* 10-18%). However, different picture was observed in some shops. For instance, in shop 2 strained ratings for systolic pressure regulation parameters were least incident (≤10%). For systolic pressure regulation, the worst picture was revealed in shop 3 (22-31% strained states, i.e. 2-3-fold more incident than in shop 2); while shop 1 was the worst by diastolic pressure regulation, especially by variability parameter. These differences most likely reflect different degree of adaptation of the blood pressure regulation systems to the working conditions, rather than are age dependent. Thus, predominant contact with sealed sources of radiation minimizes direct damage to the vascular bed, which enables optimization of diastolic pressure regulation processes (shop 2). Predominant contact with open radiation sources (shop 1) increases the risk of strain in the regulation of both systolic and diastolic pressure; this effect was most pronounced in case of combined exposure (shop 3).

All these findings suggest that processes of neuroendocrine regulation of peripheral circulation are most susceptible to the influence of occupational risk factors. Therefore, it was interesting to study in detail functional adequacy of the processes of psychomotor functions at various levels of neuromuscular regulation.

By functional characteristics of the respiratory system (on the basis of spirometry data) and constitution (anthropometry data), the studied populations by the majority of criteria did

not differ from the normological one. The third strain rating level by the vital capacity of the lungs was found in 15% cases and by airway conductance (Tiffeneau index) in 20%. In the latter case, hypofunctional shifts were detected in 57% cases and hyperfunctional in 43%. The differences between the groups were insignificant, which excludes age-related and occupational differentiation.

When studying the functional state of psychomotor regulation of employees, we revealed a fivefold increase in the incidence of strained states. In all cases, the strain had hyperfunctional direction (i.e. professional activity primarily mobilizes psychomotor functions) and its incidence was similar in all examined groups (i.e. the observed strain is not related to age).

Thus, working conditions selectively complicate neuromuscular regulation and first of all regulation of smoothness of motions and correction of motions. Both parameters are related to the central and subcortical levels of regulation. Hence, the observed strain in the regulation of locomotion is not associated with muscular fatigue, but is determined by constant need to accurately perform motor acts, which leads to mobilization of neuroendocrine regulatory mechanisms.

However, we revealed certain differentiation in the structure of psychomotor strain depending on the nature of technological process. For instance, in shop 2 (predominant contact with sealed sources of radiation) relative low incidence of strain in correction of movements and extremely high incidence of strain in smoothness of movements were observed. In shop 1 (primary contact with open radiation sources), an opposite situation was observed: maximum strain in error correction and relatively low strain in smoothness of movements. In shop 3 (combined exposure), the situation by the discussed criteria was intermediate.

These findings suggest that working conditions at the industrial plant primarily mobilize the processes of neuroendocrine regulation of psychomotor functions. It should be noted that working with sealed sources of radiation requires extreme smoothness of movements, while working with open sources requires precision of movements.

The tested approaches are aimed at detection of individual functional strain, which ensures targeted correctional and rehabilitation measures. This is the field where we the above-discussed basic methods of polysystemic monitoring can be practically applied for maintaining more stable functional reserve of the organism under the influence of technological factors.

3.2 Assessing the health of military pilots

In examination of a group of **military pilots**, radioadaptive response (RAR) of peripheral blood lymphocytes was additionally studied. The health status of flight personnel is one of the most important components of flight safety requiring realization of complex measures aimed at prevention of diseases, prolongation of career longevity, and improvement of tolerance of unfavorable flight factors. In modern aviation medicine and physiology, the primary attention is paid to the control of health reserves, rather than transition of health to disease. The focus is thus shifted to individual evaluation of the functioning of the main organs and systems in

pilots. Flight personnel is exposed to various potentially dangerous factors during flights. Evaluation of biological risks in modern aviation is complicated by the fact that these influences do not exceed or only slightly exceed the maximum permissible level. This is the range where organism's response to the studied factors is maximally individual.

Long-term predictions of the state of organism's functional reserves and evaluation of the probability of negative biological effects during flights should be based on the results of not only standard physiological tests, but also detection of pathological markers at the cellular and biochemical levels. Complex examination of passenger aircrafts crews carried out by Italian researchers revealed changes in a number of cytogenetic parameters of buccal epithelial cells and lymphocytes (e.g. elevated chromosome aberrations frequency, oxidative damages in DNA, etc.) (Cavallo et al., 2006). Changes in cell functioning lead to activation of DNA reparation, on the one hand, and induce apoptosis of damaged cells, on the other, thus promoting adaptation of the organism to stressors. However, chronic exposure to minor influences leads to exhaustion of adaptive reserves, which creates prerequisites for the development of organism hypersensitivity.

When discussing delayed consequences of the effect of occupational factors on morbidity in aviators, ionizing radiation is considered to be the most important genotoxic factor (Cavallo et al., 2006). The compliance of radioactivity situation during flights with modern radiation safety standards is now intensively discussed. It was suggested to consider pilots as professionals working with ionizing radiation sources (Bhatti et al., 2010; Yong et al., 2009). During flights the pilots are exposed to low-dose radiation; therefore the individual reactions of the organism at the cellular and systemic levels are difficult to predict. The capability of a biological object to respond to ionizing radiation in this or that way, i.e. individual radiosensitivity, is determined by genetic and environmental factors and depends on various processes such as postradiation reparation capacity, intensity of metabolism, rate of redox, physicochemical, and biochemical reactions in cells, etc.

Irradiation of peripheral blood lymphocytes is a convenient tool for evaluation of individual radiosensititvity. Low doses of radiation trigger nonspecific defense mechanisms, i.e. the results can be extrapolated to the effects of other damaging agents. One of the above-mentioned effects of low-dose radiation is radioadaptive response (RAR): cells exposed to low-dose radiation become more resistant to ionizing radiation (or other agents) in high doses (Pelevina et al., 2003). RAR can be used as a measure of individual radiosensitivity.

Our aim was to study the correlation between individual radiosensetivity and changes in molecular composition of blood serum and function of the cardiorespiratory system in pilots with different flight time.

The examinees were divided into three groups: control group A included people who had no flight hours, but their work is related to aviation (flight operations officer, technical staff, and parachutists; n=9, mean age 23.1±2.6); group B comprised pilots with flight time <1000 hours (n=17, mean age 33.1±1.5); group C includes pilots with flight time >1000 hours (n=12, mean age 39.7±1.1).

All examined pilots were in one age group by the level of chromosome aberrations. The incidence of RAR of PHA-stimulated lymphocytes decreased proportionally to the flight time: 78%, 59% and 33% in groups A, B and C, respectively ($p<0.05$ for groups A and C, Fisher's

exact test). A negative correlation was revealed in the examined cohort between the flight time and RAR coefficients (r=-0.37, p=0.021). RAR coefficient decreased with increasing flight time: 1.68±0.15, 1.08±0.10, and 0.93±0.17 in groups A, B. and C, respectively ($H(2, n=36)=6.32$, $p<0.05$, ANOVA Kruskal-Wallis test). Post-hoc analysis revealed significant differences in group C in comparison with group A ($p<0.05$; U test). Previous studies on lymphocytes isolated from animals and healthy donors showed that RAR develops in not all individuals and is not detected in 30-60% cases. The intensity of RAR depends on genetic predisposition and physiological status of the organism (Weissenbok et al., 2000).

Comparison of averaged LC spectra using the method of trapezoidal integration revealed a pronounced tendency towards a decrease in the contribution of small particles (hydrodynamic radius <11 nm) into light scatter in group B in comparison with the control ($p=0.073$, U test). In group C this decrease attained the level of statistical significance. The contribution of large particles (>165 nm) into light scatter increased in both groups. The increase in the relative number of 300-400-nm particles in group B and 500-900-nm particles in group C in comparison with the control is worthy of note (Fig. 5, A, B). Since the observed

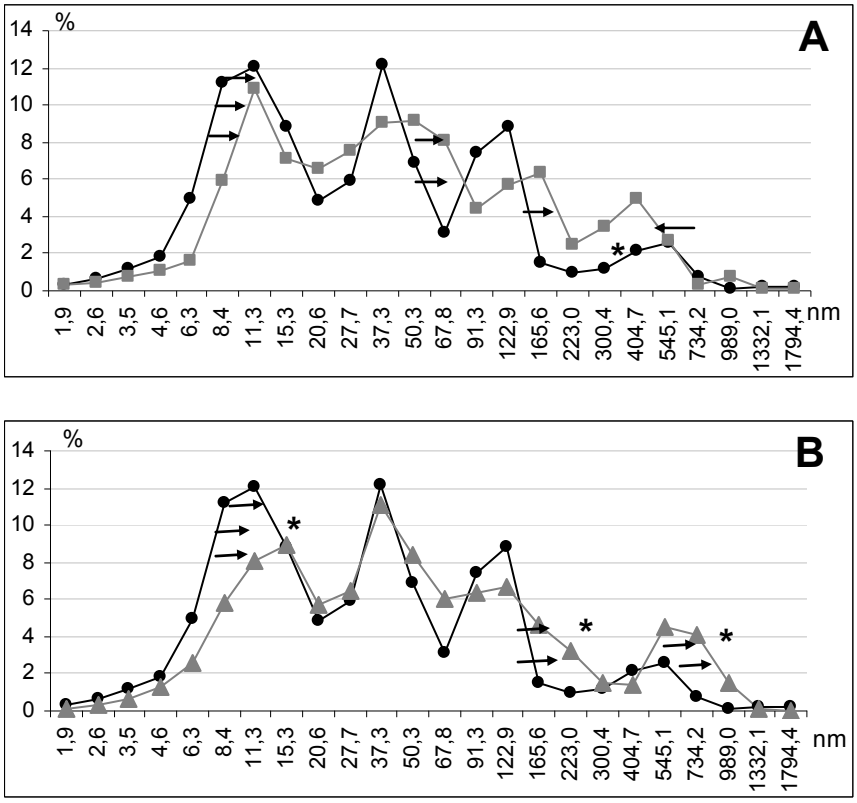

Fig. 5. Changes in the LC spectra of blood serum of pilots with a touch of up to 1000 hours (A) and more than 1000 hours (B). Abscissa - particle size (nm), the vertical axis - the contribution of particles of different hydrodynamic radius of the light scattering (%).

differences were detected in healthy individuals undergoing regular medical examinations, it can be hypothesized that they are determined by the action of flight factors (including radiation) and various mechanisms of adaptation. Low radiation doses are considered to produce an activating effect on the immune system, stimulate phagocytosis and antibody production, increase lysozyme activity, and improve general immunological status.

According to the data obtained on a large sampling of conventionally healthy individuals, the total light scatter spectrum for blood serum can be divided into 5 discrete informative zones. Zone I (0-10 nm) corresponds to monomer albumins and glycolipid complexes; zone II (11-30 nm) contains globular proteins and low-molecular-weight lipoprotein complexes; zone III (31-70 nm) includes high-molecular-weight lipoprotein complexes and low-molecular-weight immune complexes; zone IV (71-150 nm) comprises medium-size immune complexes, ribonucleoproteins (RNP), and deoxyribonucleoproteins (DNP). Larger particles (>150 nm, zone V) appear in cases of induction of immunopoiesis with the formation of high-molecular-weight complexes; this process usually accompanies allergization and autoimmune sensitization of the organism. Depending on the increase (or decrease) in the percent contribution of particles of this or that fraction into light scatter, we can speak about the direction of shifts in homeostasis and humoral immunity.

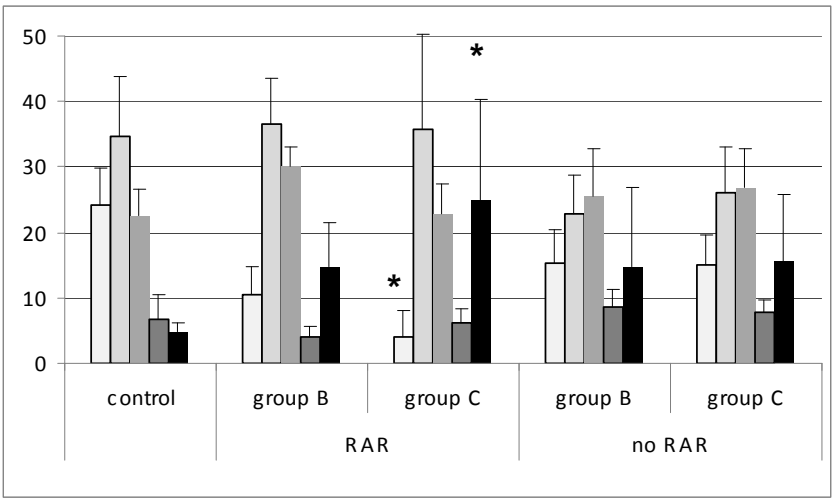

Fig. 6. The distribution of light-scattering particles in the standard zones in the serum of pilots with a flight time up to 1000 h (group B) and 1000 hours (group C), depending on the presence or absence of RAR.

Analysis of LC spectrometry data with consideration for the presence or absence of RAR showed that in pilots with RAR the contribution of small particles (<10 nm) into light scatter decreased and the contribution of large particles (>150 nm) increased in comparison with the control values (Fig. 6). In pilots without RAR, no significant differences in subfraction composition of blood serum from the control were revealed. Apart from the tendency towards a decrease in the contribution of zone I particles and the increase in the contribution of zone V particles into light scatter, a decrease in the

contribution of zone II particles was noted, which led to changes in the general pattern of spectra (Fig. 6). It should be noted that we failed to collect a representative control subgroup without RAR in this study, therefore pilots with and without RAR were compared only with controls with RAR. The observed differences for the subgroups with RAR can be explained by epigenetic influences of flight factors on metabolic processes in the organism. At the same time, changes in subfraction composition of blood serum in pilots without RAR in comparison with that in pilots with RAR in groups B and C can be genetically determined.

The development of RAR is probably related to changes in cell metabolism. Comparison of LC spectra of lymphocytes-conditioned culture medium showed that the contribution of particles with a radius of 91.3–223.0 nm was lower in samples conditioned by cells with RAR in comparison with samples conditioned by cells without RAR ($p<0.05$, U test). It can be hypothesized that irradiation in the absence of RAR leads to accumulation of a factor with a size of about 100 nm in the medium, e.g. RNP and DNP particles (80-100 nm).

Induction of RAR in peripheral blood lymphocytes is a loading test that allows evaluation of the adaptation reserves at the cellular level by complex parameters. Here we evaluated the quality and the intensity of reparation systems. Irradiation of interphase cells with low AdD induces stimulation of reparative mechanisms in cells. DD was applied after the cells passed the main reparation checkpoints (pre- and postreplicative reparation). It can be hypothesized that prolongation of the exposure to flight factors produces a negative effect on the adaptation capacities of the organism at the cellular level.

The cell response to stimulation aimed at conservation of genetic material is a particular case of stress reaction development. The function of the genome determines the relative role of mechanisms of cell reparation, proliferation, and death; these processes, in turn, determine the function of enzymes, cells, tissues, organs, and systems responsible for adaptation of human organism to the environment. The efficiency of this process is also determined by the intensity of the influence, duration of exposure, and adaptation capacity of the organism depending on functional resources and the corresponding strain of regulatory mechanisms. Thus, the rate and quality of adaptation depend on both the state of the genetic apparatus and physiological characteristics of organism's systems. The state and function of the cardiorespiratory system can be an indicator of adaptive reactions of the organism (Baevsky et al., 2004).

In our study, the parameters of PQRST complex, heart rate, SBP and DBP at rest and during functional tests were similar in all groups and did not exceed the normal limits for male individuals of the corresponding age. However, stability of the functioning of the cardiovascular system in groups was provided by different mechanisms.

The total power (TP) of HRV spectrum was different in groups A, B, and C at rest ($H (2, N=30)=6.944$ $p=0.031$) and during functional tests ($H (2, N=29)=8.740$ $p=0.013$). In both cases, this parameter in groups B and C was considerably lower than in the control (Table 1). This drop of TP was due to a decrease in absolute values of frequency components in all frequency bands in groups B and C. SI was maximum in group B, but the results of analysis of variance showed that pair-wise comparison of groups cannot be performed ($H (2, N=29)=5,360$ $p=0.067$).

Resting state (without mask)														
	HR (1/m)	**TP (ms2)**	**VLF**	**VLF, %**	**LF**	**LF %**	**HF**	**HF %**	**LFn, n.u**	**HFn, n.u**	**AB**	**C**	**SI**	**BRS**
A	77.1 ± 2.9	6126.4 ± 1620.5	1311.7 ± 398.2	22.9 ± 5.7	2413.1 ± 1004.1	33.8 ± 7.7	2401.6 ± 741.8	43.4 ± 8.5	44.2 ± 9.6	55.8 ± 9.6	1.2 ± 0.45	2.0 ± 0.6	155.0 ± 101.7	13.1 ± 4.8
B	81.1 ± 3.1	2507.6 ± 514.2*	486.0 ± 143.9 *	21.7 ± 4.0	1224.4 ± 319.2^	44.3 ± 4.1	797.1 ± 184.3^	34.0 ± 4.5	57.0 ± 5.0	43.0 ± 5.00	1.9 ± 0.54	2.9 ± 0.8	183.8 ± 41.0	8.6 ± 0.9
C	78.4 ± 4.3	1745.8 ± 484.5*	428.5 ± 120.6^	32.9 ± 4.9	943.1 ± 449.1^	41.5 ± 6.7	374.2 ± 93.7*	25.6 ± 5.3	61.4 ± 6.9	38.6 ± 6.90	3.6 ± 1.60	5.4 ± 1.6	513.7 ± 196.9	7.5 ± 1.5
Functional test with hypoxic stress (in mask)														
	HR2	**TP2**	**VLF2**	**VLF%2**	**LF2**	**LF%2**	**HF2**	**HF%2**	**LFn2**	**HFn2**	**AB2**	**C2**	**SI2**	**BRS2**
A	77.1 ± 2.4	8201.1 ± 2584.9+	1412.0 ± 238.9	20.9 ± 5.5	4872.3 ± 1875.1	58.7 ± 7.4+	1916.8 ± 1082.1	20.3 ± 6.8+	74.4 ± 7.4+	25.6 ± 7.4+	5.1 ± 1.50+	3.8 ± 1.3+	76.6 ± 40.1	15.9 ± 2.7
B	82.5 ± 3.8	1707.3 ± 272.9*	342.0 ± 489.1*	25.3 ± 4.2	915.7 ± 199.2*+	49.0 ± 5.4	449.6 ± 122.7^+	25.6 ± 4.8	65.4 ± 5.7	34.6 ± 5.80	5.1 ± 2.30	3.2 ± 0.9	212.9 ± 65.0	9.1 ± 0.9*
C	79.8 ± 4.3	2105.5 ± 763.9*	641.8 ± 192.2^	33.2 ± 3.6	1041.6 ± 462.9*	46.9 ± 3.9	422.2 ± 153.6^	19.9 ± 4.3	70.8 ± 4.9	29.2 ± 4.9	3.6 ± 0.90	5.2 ± 1.2	412.7 ± 88.5*	8.3 ± 1.4*

*p < 0.05; ^p = 0.07 - compared with the value of the indicator in the control group (Mann - Whitney); + p <0.05 - compared to the value of the index at rest in the appropriate group (Wilcoxon test).

Table 1. Indices of heart rate variability in pilots with different number of flying hours

Functional tests revealed no significant changes in TP and absolute values of power in individual frequency bands in comparison with the corresponding values at rest in all the groups. However, we observed an increase in the percent contribution of low frequencies (LF%) and a decrease in the contribution of high frequencies (HF%) in control group A, which led to a shift in AB and CI towards a decrease ($p<0.05$, Wilcoxon test). In groups B and C, the contribution of very low frequencies increased in comparison with that at rest (VLF%). AB and SI in these groups remained practically unchanged. Moreover, we revealed negative correlations between the total number of flight hours and the total power of HRV

spectrum (r=-0.55, p=0.006) and powers of low-frequency (LF) and high-frequency (HF) components of the spectrum (r=-0.62, p=0.001; r=-0.48, p=0.021, respectively). SI after functional load increased with increasing the total flight time (r=0.64, p=0.001) and in groups C this parameter significantly differed from the control (H (2, N=21)=6.019, p=0.049). BRS in the control groups was higher than in groups B and C (tendency at rest and significant differences during functional tests H (2, N=27)=6.142, p=0.046).

The total power of SBP rhythm variability spectrum in the control group remained unchanged during functional test (in mask) in comparison with that at rest, while the power of VLF band significantly decreased (Fig. 7) and LF component of the spectrum increased (pronounced tendency p=0.087 Wilcoxon test) under these conditions. In group B, the total spectral power decreased during functional test due to reduced contribution of VLF and HF bands. In group C, no significant changes in SBP rhythm variability were revealed. Groups A, B, and C did not differ by DBP parameters and parameters characterizing the state and function of the respiratory system at rest and during functional tests.

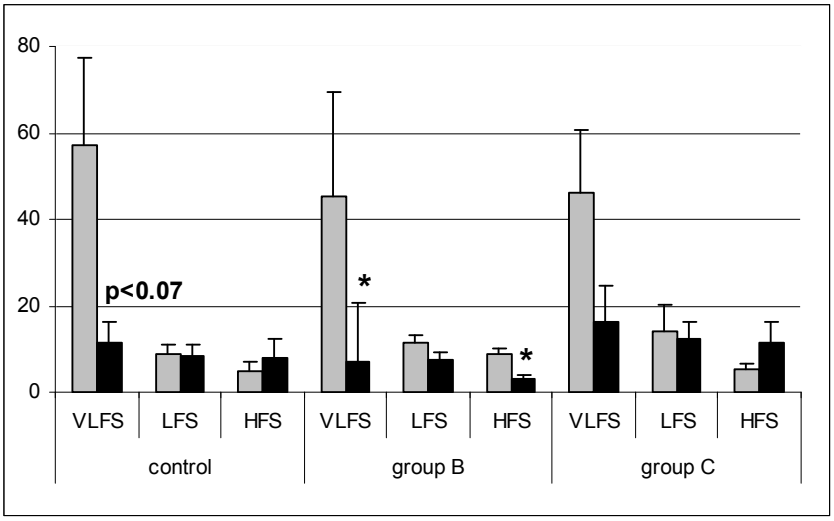

Fig. 7. Frequency ranges of systolic blood pressure variability changes when performing a functional test in pilots with different total time of the raid. Gray columns - measurement at rest, black columns - functional test (spirometric measurements in a mask) (hypoxic stress). * p <0.05 compared with the value of the index at rest (Mann - Whitney)

Comparison of the parameters characterizing functional activity of the cardiorespiratory system in pilots with and without RAR revealed no significant differences between these groups at rest by all analyzed characteristics. After functional test, peripheral SBP and DBP and total and specific peripheral resistance increased in the group without RAR in comparison with the corresponding values at rest (Table 2). In individuals with RAR these parameters little changed. HRV and SBPV also underwent different changes in groups with and without RAR during functional tests. In pilots without RAR, the total spectral power of SBPV and the contribution of very low frequency band considerably decreased in

comparison with the corresponding values at rest. In the group with RAR, the total spectral power of SBP did not change, but the relative contribution of low frequencies into HRV and SBPV increased and the contribution of high frequencies decreased under these conditions, which led to an increase in their ratio and CI in comparison with those at rest.

Intergroup comparison showed that in the group with RAR, the contribution of low frequencies into HRV was greater and the contribution of high frequencies was lower than in the group without RAR. The relation between these values and CI were also higher, while SBP was lower in the group with RAR in comparison with the corresponding values in the group without RAR (Table 2). Parameters of regulation of the respiratory system after functional test were similar in these groups.

Resting state (without mask)														
	LFn, %	HFn, %	AB	C	SBP, mmHg	DBP, mmHg	TPS, ms^2	VLFS, ms^2	LFS, ms^2	HFS, ms^2	LFSn, %.	HFSn, %	ABS	TPVR
No RAR	56.1 ± 6.2	43.9 ± 6.2	2.7 ± 1.2	4.0 ± 1.3	126.6 ± 6.6	79.7 ± 4.5	66.1 ± 15.27	44.2 ± 11.5	13.8 ± 4.8	8.1 ± 1.7	59.6 ± 5.5	40.4 ± 5.5	3.0 ± 1.1	1327.1 ± 84.8
RAR	55.0 ± 5.2	45.0 ± 5.2	1.9 ± 0.4	3.0 ± 0.6	132.2 ± 5.9	88.3 ± 5.6	68.3 ± 21.0	53.0 ± 19.7	10.0 ± 1.4	5.4 ± 1.1	67.7 ± 2.6	32.3 ± 2.6	2.5 ± 0.3	1576.0 ± 130.3
Functional test (the mask)														
	LFn	HFn	AB	C	SBS	DBD	TPS	VLFS	LFS	HFS	LFSn	HFSn	ABS	TPVR 2
No RAR	61.4 ± 5.0	38.6 ± 5.0	2.4 ± 0.6	2.8 ± 0.5	143.4 ± 8.9+	88.6 ± 6.2+	40.8 ± 10.7+	19.6 ± 6.3 +	9.6 ± 3.2	11.6 ± 4.2	60.7 ± 6.5	43.7 ± 7.1	1.8 ± 0.4	1581.7 ± 160.0 +
RAR	75.1 ± 4.1 +	24.9 ± 4.1 *+	6.1 ± 1.6 *+	5.2 ± 1.0 *+	132.9 ± 4.6 *	87.1 ± 6.0	33.2 ± 7.3	20.5 ± 6.2	9.5 ± 1.6	3.2 ± 0.7	76.0 ± 2.6 *	24.0 ± 2.6 *	3.7 ± 0.5*	1542.3 ± 116.1

*p < 0.05 - compared with the value of the index in the group without RAR (Mann - Whitney); + p <0.05 - compared to the value of the index at rest in the appropriate group (Wilcoxon test)

Table 2. Indices of heart rate variability and peripheral blood pressure to the presence and absence of lymphocyte radioadaptive response in pilots

The total power of HRV spectrum and its constituents were reduced in groups with individuals with flying practice. In contrast, SI increased with increasing flight time; the

difference from the control attained the level of statistical significance in the group with long flight time. It is currently accepted that the total power of HRV spectrum reflects the influence of the humoral system on myocardial functions. The decrease in HRV is associated with exhaustion of functional reserves of the organism (Baevsky et al., 2004). It can be hypothesized that flight factors produce a negative impact on the humoral component of the cardiovascular system regulation. The data of LC spectrometry confirm this assumption.

In the control group, the contribution of LF component into the total spectral power of HRV and SBPV increased in response to functional test. Calculated parameters AB and SI also increased under these conditions. In pilots, functional test elevated the percent contribution of VLF band into HRV and increased SI. According to averaged data obtained on adult individuals not employed in aviation, a decrease in relative LF power and an increase in relative HF power were observed during testing in spirometric mask, the total spectral power being unchanged. Hence, activation of the respiratory system leads to relative strengthening of the influences of the autonomic regulatory contour. Reduced HF power and/or increased LF power together with the increase in HF/LF ratio are related to activation of the sympathetic ANS, which is considered to be an adaptive response to stress load. Increased contribution of VLF band into HRV spectrum in pilots can attest to both metabolic changes and psychoemotional factors. Elevated SI attest to mobilization of functional reserves leading to their exhaustion with increasing the degree of sympathetic activation. An unfamiliar test included into aviation medical expertise can act as significant stress factor and the response of pilots and examinees of the control group to this stress can differ.

Functional test led to an increase in peripheral vascular resistance and BP in examinees without RAR. In this subgroup, no changes in HRV spectra and no significant differences in subfraction composition of blood serum from the control were revealed. In examinees with RAR, BP and peripheral vascular resistance remained unchanged during the functional test. However, a redistribution of the HRV and SBP spectral power was observed: increase in LF power and decrease in HF power. In pilots with RAR, pronounced metabolic shifts in blood serum in comparison with the control were detected.

It is now proven that activation of signal pathways (cytokines, protein kinase C, etc.) plays an important role in induction of RAR. Adaptation in cell culture is associated with an increase in the content of reactive oxygen species and NO, which can act as the key signal molecules (Coates et al., 2004). According to the hypothesis of V. N. Titov, in cell pools regulated by paracrine mechanisms, stress induces metabolic changes accompanied by weakening of the dilatation effect of NO, functional shutdown of peripheral peristaltic pumps (muscular arteries), and increase in total peripheral vascular resistance (TPVR). The compensatory reaction consists in participation of the myocardium as the central pump in the regulation of homeostasis and increase in systemic BP.

4. Conclusion

Our findings suggest that in individuals with and without RAR, different regulatory mechanisms are involved into adaptation to varying environmental conditions, due to

which they can respond to physical and psychoemotional loads in different ways at both the organism level and metabolic level. The pattern of systemic responses for humans with and without RAR are apparently genetically determined. The intensity of RAR, in turn, decreases under the action of flight factors. It can be hypothesized that individual radiosensitivity reflects general resistance of the organism to negative environmental influences.

Authors are grateful to M. M. Antoshchina, Chief Researcher of Medical Radiological Research Center, Ministry of Health Care and Social Development of the Russian Federation, Obninsk, for her help in the experiments.

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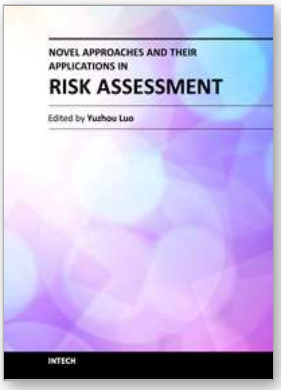

Novel Approaches and Their Applications in Risk Assessment

Edited by Dr. Yuzhou Luo

ISBN 978-953-51-0519-0
Hard cover, 344 pages
Publisher InTech
Published online 20, April, 2012
Published in print edition April, 2012

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Mikhail Karganov, Irina Alchinova and Nadezhda Khlebnikova (2012). Polysystemic Approach to Risk Assessment, Novel Approaches and Their Applications in Risk Assessment, Dr. Yuzhou Luo (Ed.), ISBN: 978-953-51-0519-0, InTech, Available from: http://www.intechopen.com/books/novel-approaches-and-their-applications-in-risk-assessment/polysystemic-approach-to-risk-assessment

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