MODERN PROBLEMS OF GENETICS, RADIOBIOLOGY, RADIOECOLOGY AND EVOLUTION

Abstracts
Papers by Young Scientists

Joint Institute for Nuclear Research

MODERN PROBLEMS OF GENETICS, RADIOBIOLOGY, RADIOECOLOGY AND EVOLUTION

The Second International Conference
dedicated to the 105th anniversary of the birth
of N. W. Timofeeff-Ressovsky and the 70th anniversary
of the paper «On the Nature
of Gene Mutations and Gene Structure»
by N. W. Timofeeff-Ressovsky, K. G. Zimmer,
and M. Delbrück

Yerevan, September 8–11, 2005

ABSTRACTS, PAPERS BY YOUNG SCIENTISTS

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Modern Problems of Genetics, Radiobiology, Radioecology and Evolution: Second
М78 Intern. Conf. dedicated to the 105th anniversary of the birth of N. W. Timofeeff-Ressovsky and the 70th anniversary of the paper «On the Nature of Gene Mutations and Gene Structure» by N. W. Timofeeff-Ressovsky, K. G. Zimmer, and M. Delbrück (Yerevan, September 8–11, 2005): Abstr., Papers by Young Scientists. — Dubna: JINR, 2005. — 318 p.

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The collection contains theses of the reports and competition papers by young scientists presented at the conference. The theses and young scientists' papers are published in the authors' wording.

Современные проблемы генетики, радиобиологии, радиоэкологии и эволюции: Вторая междунар. конф., посвященная 105-й годовщине со дня рождения Н. В. Тимофеева-Ресовского и 70-летию публикации статьи Н. В. Тимофеева-Ресовского, К. Циммера и М. Дельбрюка «О природе генных мутаций и структуре гена» (Ереван, 8–11 сентября 2005 г.): Аннот. докл. и статьи молодых ученых. — Дубна: ОИЯИ, 2005. — 318 с.

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Сборник содержит аннотации докладов, представленных на конференцию, а также короткие исследовательские статьи, включенные в конкурс молодых ученых в рамках конференции.

Издание представляет интерес для специалистов в области генетики, радиобиологии, радиоэкологии и эволюции.

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PROGRAMME OF THE CONFERENCE

Genetics

Mutation processes in gene and chromosomes

Mutations in natural populations

Problems of medical genetics

Radiobiology

The genetic concept of biological effects of ionising irradiation

The hit principal and untargeted effects

Radiation biology of polluted areas

Radiation biophysics

Radioecology

Ecosystems and their sensitivity to contamination

Radiation equi-dosimetry in populations and communities

Methods for contamination assessment of large areas

Adaptive Evolution

Mechanisms of evolution

When can evolution occur?

Impact of stress on DNA transactions

Biospherology

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N.W. Timofeeff-Ressovsky and his outlook;

Communication on INTAS programmes and the new scientific policy (INTAS representative)

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PRINCIPLES AND RESULTS OF GENETIC MONITORING OF CHEMICAL MUTAGENS AND RADIATION IN ARMENIA

Aroutiounian Rouben

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In this lecture the main approaches to detect effects of environmental pollution in Armenia will be presented. Most of them are in action at Yerevan State University in cooperation with other institutions in Armenia, Germany, France and USA. Basic techniques for the detection of genetic damages and results of appropriate investigations of different groups of species in Armenia include: Analysis of chromosomal aberrations. The routine analyses in groups of genetic risk can give data only about nonspecific action of environmental pollutants. The detection of genotoxic effects became more differentiated after studying of clastogenic factors (ultrtrafiltrates of patients' blood plasma). Last years the research was fulfilled by methods of molecular cytogenetics. FISH analysis was used in groups of patients with leukemia (more than 100 patients) and inborn defects (50 patients). We applied Comet Assay earlier to study DNA damage and repair in leukocytes of Chernobyl accident liquidators and patients with familial Mediterranean fever. The results shown an increased sensitivity or changed repair capacity of DNA of their cells exposed to UV-C. Now we realize the Comet-FISH approach to detect not only total DNA damage, but also its levels in selected loci. Analysis of micronuclei (MN) induction in exfoliated cells revealed the significant increase of chromosomal damage and their nondisjunction in the groups exposed to mutagens. MN assay can be very useful for population monitoring. Our research group is responsible for the genetic monitoring of the Armenian nuclear power plant and its environment. This monitoring of genotoxic effects is a multidimensional investigation. The results of chromosomal and point mutations monitoring in model plants will be presented in comparison with dosimetry data. For the investigation of groups of genetic risk in populations we apply programs with different combination of presented methods. Genetic programs are usually combined with epidemiological data. The essential part of the research presented is supported by the ISTC Grants No A-301.2 and A-773.

GENOME RECONSTITUTION AFTER EXTENSIVE DNA DAMAGE IN *DEINOCOCCUS RADIODURANS*

John R. Battista[1] *and Michael M. Cox*[2]

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The bacterium *Deinococcus radiodurans* can withstand extraordinary levels of ionizing radiation, reflecting an equally extraordinary capacity for DNA repair. This bacterium is 200 times more radiation resistant than *E. coli*. *D. radiodurans* is multiploid, maintaining 4-10 copies of each of its four chromosomes at all times. After treatment with 5000Gy of ionizing radiation, *Deinococcus radiodurans* suffers about 180 DNA double strand breaks per cell. Over the next six hours, new chromosomes are reconstructed from overlapping fragments. This reconstitution of the bacterial genome is both highly accurate and efficient.

There are at least four distinct mechanisms contributing to genome reconstitution. First, the genome is organized in the form of a tightly structured toroid which may contribute to genome reconstitution in a manner not yet defined (Englander et al. (2004) J. Bact. 186, 5973). In addition, there are at least three repair processes involved. Five novel *Deinococcus* genes involved in genome restoration (*ddrA*, *ddrB*, *ddrC*, *ddrD*, and *pprA*) define three epistasis groups. The first two involve repair processes that are RecA-independent. One, defined by the *ddrA* gene and protein, is a DNA end-protection system that prevents nuclease-mediated degradation of chromosomal DNA fragments. The second, defined by the *ddrB* gene, promotes a degree of homology-dependent genome reconstitution and may be a single-strand annealing pathway. The *pprA* gene product participate in a third, RecA-dependent process during recovery from radiation damage.

The RecA, SSB, DdrA, and PprA proteins of *Deinococcus radiodurans* have been purified and characterized *in vitro*. Each offers a novel perspective on the mechanisms for chromosomal repair in this bacterium. These *in vivo* and *in vitro* characterizations demonstrate that novel mechanisms contribute to the ionizing radiation resistance in *D. radiodurans*.

DOSE-EFFECT DEPENDENCE FOR LOW-LEVEL EXPOSURES TO CHEMICAL AND PHYSICAL AGENTS: RISK EVALUATION

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The question of existence or absence of a threshold in the action of ionizing radiation is of interest from both the theoretical and practical points of view..

In radiobiology, the concept about existence of a threshold has been dominating for many years; all governmental decrees about liquidation of consequences of the accident are based on this concept.

There is the opinion that one should proceed from the humane considerations when applying the concept of linear dose-effect dependence and assess the risk of development of malignant tumors associated with irradiation of population.

On the basis of our experimental studies and available published data, we describe the dose dependence by a nonlinear non-monotonic polymodal dependence of effect on dose.

This kind of function makes it possible to describe not only damaging but also stimulating effects (in particular, hormesis) of irradiation. Depending on the values of parameters *a, b, c, d*, the function may be transformed into various dose-effect that are used at present.

We showed how these parameters and the shape of the function vary with changes in the irradiation dose-rate, distribution of the dose received by population, relationship between functions of systems responsible for damage and repair, etc.

It is important that there is, at least, two points of intersection of the curves with the abscissa, which is evidence of existence of thresholds, which are different in value and basic mechanisms. Similar dependences were discovered in the studies on effects of chemical agents in a wide range of concentrations. Note the presence of the so-called "dead zone" i.e., the zone of absence or a drastic decrease in the value of effect in a certain range of concentrations. We put forward the concept about the impossibility the risk assessment for the whole dose-effect dependence curve and the necessity of a separate calculation of risk for separate dose ranges and dose-rates of low-level factors of the chemical and physical nature.

In the lecture, explanations to the effects observed and recommendations on decreasing them will be given.

RADIOECOLOGICAL ASSESSMENT OF THE CHNPP ACCIDENT IN THE WESTERN EUROPE AND ADJACENT AREA, WITH SPECIAL REFERENCE TO THE MODERN PROBLEMS OF RADIOECOLOGY IN THE MEDITERRANEAN

Arrigo A. Cigna

The impact on Western Europe of the Chernobyl accidents is assessed. In particular an evaluation of the contamination of air, soil, seawater and food with special reference to geographical and orographical situations.

A simple and reliable method to evaluate the levels of Cs134, Cs137 and Sr90 in green vegetables, hay and milk on the basis of the soil deposition only is described and experimental results are given. The model appears to be rather conservative and to overestimate the contamination.

The situation prior to Chernobyl with respect to regulations of radiation protection against the consequences of a major accident is considered. The development of the recommendations and regulations issued by the Commission of the European Communities for the Maximum Permitted Levels of different groups of radionuclides in foodstuffs is reviewed.

The different reactions to the accident are examined and some data on the average individual effective dose equivalents estimated in a number of countries are also reported. Also the consequences of the countermeasures are discussed. Some main problems concerning the information of the public and the preparedness for possible future accidents are summarised.

Finally the present status of radioecology in the Mediterranean Area is described pointing out the unjustified reduction of the efforts on the monitoring and research with a special reference to the radiation protection problems in emergency situations.

MUTATION AND DNA REPAIR: FROM THE GREEN PAMPHLETE TO 2005

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The "Green Pamphlet" tried to understand the gene by studying its mutations. The authors, one of whom mentored me in the distant past, were seriously constrained: almost nothing was known about the gene in the mid-1930s, much of what was believed turned out to be wrong, and Max Delbrück was too confidant. Fortunately, Timofeeff-Ressovsky knew about as much real genetics then as anyone in the world. The attempts of physicists and radiologists to describe the gene as a target, with a volume (but not a shape) that could be measured, instantly excited biologists, and target theory became very respectable for several decades. However, another quarter of a century revealed not only the fundamental structure of the gene, but the almost completely unanticipated complication of DNA repair and, later, damage circumvention.

Now it is impossible to separate DNA repair and mutagenesis. Consider "replication repair", a term invented in the mid-1970s by a group working with cultured mammalian cells. Their idea that a blocked primer strand could briefly use the other daughter strand as a template is often invoked, but only recently were we able to solve this problem using a bacteriophage T4 system, perhaps in part because this phage conducts many DNA transactions in a more mammalian than bacterial manner. Now we have explored the role of template switching in a bizarre mutational process in which the primer strand switches to the wrong template, that is, to the other parental strand. When this occurs in the context of an imperfect reverse repeat, complex mutations can be generated that consist of either short inversions or clusters of small mutations.

Another kind of complexity, also not at all anticipated in the mid-1930s, is the non-randomness of mutation. It became clear in the 1950s that point mutations are strikingly non-random in their distribution within a gene. It has recently become clear that mutations are non-randomly assorted among mutants. In particular, a minority of mutants contain two or more mutations that appear to have arisen in bursts as a result of some kind of transient hypermutability. I will describe several aspects of this process.

THE LIVING MATTER AND BIOSPHEROLOGY

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Without any question biospherology is primarily a Russian discipline. Its appearance is closely related to such Russian scientists as V. V. Dokuchaev, V. I. Vernandsky, N. V. Sukachev and N. V. Timoffeev-Ressovsky. In its creation is especially great the merit V. I.Vernadsky, the student of the founder of genetic pedology - V. V. Dokuchayev.

V. I. Vernandsky discovered the phenomenon of the living matter which forms the biosphere.

He was the first in the history of science with his theory of living matter to place life in its proper position in the general picture of the universe and discovered fundamental laws which control geochemical acitivities of living matter in the biosphere.

In the first half of the previous century V.I.Vernadsky laid the basis for a new vitally important for the future science which we call biospherology (Gegamyan, 1980, 1981).

N.W.Timoffeef-Ressovsky, even as a young scientist, rightly considered the importance of V.Vernadsky's ideas on biospherology. His studies in the late 1920s in Germany on radiobiology with radium and x-rays, later, radioecological research continued in the 1950s in the Urals (according to the terminology by Nikolai Vladimirovich, "radiation-biogeoceniligical") as he stressed, were much inspirited by ideas of V.Vernadsky

Timoffeev-Ressovsky was the first scientist who re-established Vernandski's forgotten discipline about the biosphere. He gave us a short but very perspective program with his report called "Biosphere and the humanity". It now can be included in any national or international program for "sustainable progress".

FROM THE MUTATION THEORY TO THE THEORY OF MUTATION PROCESS

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Contemporary concept of mutagenesis originates from Korzhinsky - de Vries (1899-1901) mutation theory of evolution, based upon study of discrete inherent phenotypic variations. Since that time development of the theory of the gene (Johanssen, 1909, Morgan 1926) and discovery of radiation mutagenesis (Muller, 1927) led to the deeper understanding of mechanisms of mutational process.

Three men's paper (Timofeeff-Ressovsky, Zimmer, Delbruck, 1935) launched the modern paradigm in genetics and presented the first radiobiological study of the gene as a macromolecule. The theory developed, though a bit formalistic, nevertheless served as a stimulus for the further study of the physiology of the gene and of its' mutational transitions, the same as the molecular biology in general. More specific description of mutagenesis started with the physiological hypothesis of mutational process, initiated by Lobashev (1946), who put together "mutation" and "repair". He did it more than 20 years before von Borstel, who connected mutations and mistakes of 3R (replication, recombination and repair) late 60-ies of the XX c. The forthcoming general theory of mutational process now is connected with the dynamics of genetic material underlying its evolutionary conservation. Contemporary view of variability connects mutations with the template principle in biology founded by Koltsov and Timofeeff-Ressovsky (convariant reduplication principle) in the late 30-ies of the XX c.

The theory of mutational process today is still contradictory putting together a set of events heterogeneous by their mechanisms: true gene mutations in one hand and so-called chromosome and genome mutations in the other hand. It is evident that chromosome rearrangements and transpositions are connected rather with recombinations than with real mutations. Genome mutations are also cannot be considered the same way as mutations being connected with cytosceleton abnormalities. This is only a part of wider problems encountered now by the general theory of variability destined to embrace inherent and non-inherent variations such as modifications, ontogenetic variability and epigenetic variations (and inheritance). The widely accepted classification of variability deserves a revision, basing rather upon mechanisms than on phenomenology of the processes under investigation.

GENE NETWORKS AND COMPLEXITY OF BIOLOGICAL ORGANIZATION

N.A. Kolchanov, V.V. Suslov, D.P. Furman

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Emergence of new organisms and their correlation with the evolution of ecosystems is an important problem. Paleontologic data suggest three global evolutionary trends, namely, increases in (1) complexity of organisms, (2) number of structural types of ecosystems and degree of their isolation (however, a complete isolation has never been achieved), and (3) taxonomic diversity in ecosystems. Dynamics of these trends are phasic—a geologically short "evolutionary explosion" is followed by a long evolutionary stasis (although molecular genetic data demonstrate that the evolution does not stop during the stasis). No distinct correlations between these changes and external (cosmic) factors have been found, forcing researchers to search for the reason underlying the dynamics of trends in organization of the living systems per se. The common features of pro- and eukaryotic gene networks (GN)—block-based hierarchical structure and overlapping of subnetworks (blocks)—suggest certain common mechanisms of rapid evolution. These mechanisms involve rearrangement of relations in GN due to mutations of few genes, central regulators. Negative feedback loops (NFL) may "neutralize" such mutations, thereby slowing the evolution. NFL destruction due to a change in the selection vector may expose the previously "neutralized" mutations and lead to the explosive evolution. Abundance of NFL of various natures at all the levels of life organization (from molecular genetic to ecosystemic) implies a similar principle in evolution of ecosystems, which complies with ecological data. Genomic sequencing demonstrated the correlation between complexity of prokaryotes and size of their genomes. However, no such correlation exists in eukaryotes. Molecular genetics and mathematical modeling discovered the limitations on increase in prokaryotic genomes, reflecting the features of their organization (small gene regulatory regions), evolution (horizontal transfer of genes), and ecology (bacterial mats). The haplodiplophase cycle, removing these limitations, allowed eukaryotes to complicate their gene regulation through enlarging their regulatory regions, while the mechanisms of block–module evolution (crossover, splicing, sex, etc.) permitted a radical increase in the complexity independently of the genome size.

RADIATION-GENETIC RESEARCH AT JINR ACCELERATORS

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Joint Institute for Nuclear Research

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The unique accelerators of charged particles of high energy in Joint Institute for Nuclear Research (JINR) allow solving a wide range of radiobiological problems. The main task of scientific research at Laboratory of Radiation Biology (LRB) of JINR is connected with investigation of genetic effects of heavy ions with different energies. LRB is the leading laboratory in Russia and other JINR Member States of JINR in the field of the study of genetic effects produced by ionizing radiation with different physical characteristics. Using accelerated heavy ions with low energy, the following investigations were performed: the molecular mechanisms of genetic and structure mutations in prokaryotic cells were studied; the influence of repair systems on the mutagenic processes after irradiation in a wide range of linear energy transfer (LET) was investigated; the first SOS lux-test system was designed and employed in experiments with beams of heavy charged particles for the study of the mutagenic genes' expression. A mathematical models were developed to describe the genetic regulation of the SOS system in prokaryotic cells; the regularities of genetic mutation inductions in low eukaryotic cells under action of ionizing radiation with different LET were investigated; the regularities of stable chromosomal aberrations (translocations) in human cells under action of ionizing radiation with wide LET range were studied; the mutagenic (HPRT gene) effects were investigated in mammalian cells in culture after heavy charged particle irradiation; a new effect — chromosomal instability in HPRT-mutant clones of mammalian cells — was found and investigated; a new phenomenon of cell hypersensitivity at very low doses of radiation and increase of radioresistance at higher doses was found in human and mammalian cells and studied with the help of cytogenetic analysis.

The radiation-genetic studies now carried out at the ion beams with high energy generated by the Nuclotron — a new JINR accelerator. This accelerator allows solving a series of completely new problems in the field of radiogenetics and radiobiology and gives the possibilities to hold the research on the world level. The LRB programme involves the most vital tasks of modern radiation genetics, related to the JINR basic facilities, such as: the study of the regularities and mechanisms of stable and unstable chromosome aberrations induction in human cells under irradiation with high-energy heavy charged particles. This region of cytogenetics is poorly investigated and such information is very important for the radiation protection during

long space flights, development of the biology dosimetry technique, etc.; the investigations of low doses' effects on the chromosomal apparatus of cells that are continued on the basis of original approaches developed in LRB. The work concerned with the study of influence of inducible repair processes of DNA lesions on the chromosome lesions; the investigation of mechanisms of mutagenic action of different types of radiation on the prokaryotic and low eukaryotic cells, molecular mechanisms of inducible repair processes of DNA lesions, genetic control of check-point regulation in low eukaryotic cells. These investigations are important for the determination of mechanisms of genetic and structure mutations in different cells and for the search of their repair ways.

GEOMICROBIOLOGY OF URANIUM DEPOSITS - SURVIVAL UNDER RADIATION STRESS

Wolfgang E. Krumbein and Anna A. Gorbushina

Geomicrobiology, ICBM, Carl von Ossietzky Universitaet, Oldenburg (Germany) and BIOGEMA (Edewecht, Germany) E-Mail: wek@biogema.de

Natural reactors are known for several places in Africa (Gabun events) and other places in Precambrian deposits. Further most Uranium and Radium deposits exhibit plenty traces of extinct lfe forms. This way it can be clearly concluded, that from the early periods of onset of life until today many microorganism types, among which bacteria and fungi predominantly are capable of surviving extremely high doses of radiation. In addition it has been convincingly shown by experiments, that bacteria, cyanobacteria and fungi are capable of enriching radioactive elements to levels of concentrations which may initiate natural nuclear events such as the nuclear reactors of Gabun. The ore deposit of Menzenschwand in the black forest of Germany is loaded with microbial fossils indicating that one of the major pathways of ore enrichment is microbial accumulation of radioactive elements by way of metabolic processes and cell wall catchment of heavy elements. The same has been shown for iridium, platinum and gold. V. I. Vernadsky was one of the fore-runners of ideas concerning radiation biology as also N.V. Timofeev-Ressovsky. In Ukrainia some mycologists have also studied the survival potential of black yeast like fungi after the radioactivity release event of the Tschernobyl reactors. N. N. Lyalikova of the INMI, Moskwa as well as B.D. Dyer of BU, Boston have worked on the enrichment of radioactive elements and of iridium from atomic waste deposits in Russia. From their and our findings we derive, that not only prokaryotes, but also fungi have a high capability of not only surviving high doses of radioactivity but also to enrich radioactive elements in the cell vicinity and hereby helping to create radioactive ore deposits by ways of metabolic activity.

RADIOCAPACITY – CHARACTERISTIC OF STABILITY AND REALIBILITY OF A BIOTA IN ECOSYSTEMS

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In models of radiocapacity for a miscellaneous type of ecosystems (slope's, water ecosystems and etc.) the rather small levels of primary contamination of ecosystems are established, which one are capable were subject the laws of reallocating of radionuclides, to result in to concentrating in a biota of critical levels of the contents of radionuclides (for water ecosystems it is a biota of bottom sediments) and formation of noticeable radiation doses.On a number of estimations doses rate on a biota of (0.4 Gy/year for animal and 4 Gy/year for plants) can notably oppress growth and condition of a biota in ecosystems. If to accept ecological risk, arising at it, for unit, it is possible to define concentrations of radionuclides in a biomass of a biota which are capable to form similar radiation doses. It makes on Cs-137 - 100 kBq/kg for animal and 1000 kBq/kg for plants.

Outgoing from a hypothesis about linear increase of ecological risk for a biota from 0 up to 1 with growth of a level radionuclide's contamination of ecosystems for each of concentrations the general ecological risk from miscellaneous radionuclides fallen in a concrete ecosystem can be estimated.Thus for each type of ecosystems (water, terraneous) with the help of models of radiocapacity, depending on dynamics of reallocating of radionuclides it is possible to establish marginal levels primary of radionuclid1s contamination ecosystems. Is rotined, that these primary levels of radionuclide's contamination can be very rigid.We for the first time design a method of an ecological standardization of miscellaneous ecotoxicants-pollutants on the basis of the theory both models of ecological capacity and radiocapacity of a miscellaneous type of ecosystems.

The analysis of dynamics of distribution and reallocating of tracer (Cs-137) in model ecosystems - aquacultures of plants is conducted. This analysis has allowed us to establish, that in reply to operating of the stressful factors, the biota of ecosystems is capable sharply to change Tf (Transfer factors) and by that to call sharp reallocating of tracer at the expense of its let from a biomass of a biota in environment.

THE MINIMUM DETECTABLE DOSE BY BIODOSIMETRY IN A RADIATION OVEREXPOSURE

D.C. Lloyd, A.A. Edwards and M. Szłuińska

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Chromosomal aberration analysis is the most sensitive biological method to indicate exposure to ionising radiation. This paper will distinguish between the *detection* and the *measurement* of low doses by aberration analysis and show how to quantify their limits. Worked examples will be presented using the lymphocyte dicentric assay and data typical of Co-60 gamma rays. The principles illustrated can be applied to other aberration types and other radiation qualities. Two situations will be considered: conventional by eye scoring of 1,000 metaphases from a suspected low exposure patient and scoring more metaphases with computer assisted microscopy. Low dose quantification is ultimately limited by the uncertainty on the assumed background level of dicentrics. With conventional scoring the Poisson uncertainty on the patient's observed dicentric frequency is the major component to the uncertainty on low dose estimates. With increased scoring, assisted by computer, this is reduced but the standard error on the linear calibration coefficient becomes more important. The optimum is reached where both components contribute equally to the overall uncertainty. A dose estimate may be considered as a *measurement* when its lower 95% confidence limit falls above zero. A dose can be regarded as having been *detected* when the dicentric frequency is above an assumed background but the lower 95% confidence limit includes zero. Conventional scoring of 1,000 metaphases will permit a measurement lower limit of about 100mGy of gamma radiation. This can be reduced by scoring many more cells (~10,000) to about 70mGy. Further improvement is unlikely due to the background 'noise' in the assay.

ADAPTIVE RESPONSE AND BYSTANDER EFFECTS IN HUMAN AND NON-HUMAN BIOTA

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Recent advances in our understanding of effects of radiation on living cells suggests that fundamentally different mechanisms are operating at low doses compared with high doses. Also, acute low doses appear to involve different response mechanisms compared with chronic low doses. Both genomic instability and so called "bystander effects" show many similarities with well known cellular responses to oxidative stress. These predominate following low dose exposures and are maximally expressed at doses as low as 5mGy. At the biological level this is not surprising. Chemical toxicity has been known for many years to show these patterns of dose response. Cell signaling and coordinated stress mechanisms appear to dominate acute low dose exposure to chemicals. Adaptation to chemical exposures is also well documented although mechanisms of adaptive responses are less clear. In the radiation field adaptive responses also become important when low doses are protracted or fractionated. Recent data from our group concerning bystander effects following multiple low dose exposures suggest that adaptive responses can be induced in cells which only receive signals from irradiated neighbours. We have determined using genetically distinct mice, with different radiosensitivities, that bystander effects occur in vivo and vary according to genetic background. We also have data showing delayed and bystander effects in fish and in prawns following in vitro irradiation of haematopoietic tissue. These data have implications for environmental radiation protection of human and non human species alike. Simple extrapolations from high to low dose exposure may need to be re evaluated. This presentation will review and discuss our knowledge about these low dose radiobiological effects in both human and non-human biota.

SELF-ORGANIZED CRITICALITY IN IMMUNE SYSTEMS

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We propose a new model of the co-evolution of a virus population and an adaptive immune system. We show that, under some natural assumptions, both probability distribution of the virus population and the distribution of activity of the immune system tend during the evolution to a self-organized critical state.

MICROBEAM STUDIES OF LOW DOSE RESPONSE IN TARGETED CELLS

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The development of sophisticated ionising radiation-based microbeams has been a major advance in radiation biology. They allow individual tracks of radiation to be controlled in time and space to allow biological responses to radiation exposure to be followed on a cell by cell basis. Microbeams involved the coupling of sophisticated imaging, cell positioning and radiation detection technologies with individual cell assays. For low dose studies, they allow the effects of single radiation tracks to be determined without the complication of the Poisson distribution inherent in conventional exposures.

Our own studies at the Gray Cancer Institute have used both a charged particle microbeam, producing protons and helium ions and a soft X-ray microprobe, delivering focused carbon-K, aluminium-K and titanium-K soft X-rays. Using these tools we can produce highly localised DNA damage within individual cell nuclei and follow recruitment of repair processes to these sites. Sites outside the nucleus can also be exposed to follow the effects of localised production of reactive oxygen species. Much of the use of microbeams, however is currently in studying signalling from irradiated to non-irradiated cells by a bystander effect. We are building a comprehensive picture of the underlying differences between bystander responses and direct effects in cell and tissue-like models. What is now clear is that bystander dose-response relationships, the underlying mechanisms of action and the targets involved are not the same as those observed for direct irradiation of DNA in the nucleus. Our recent studies have shown bystander responses even when radiation is deposited away from the nucleus in cytoplasmic targets. This adds new evidence to the emerging hypothesis that effects after low dose exposure may not be driven by the standard DNA damage dependent model.

MUTATION AS A STRESS RESPONSE

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In starving cells of the bacterium *Escherichia coli*, a special mutagenic program is induced that increases mutations, some of which can allow survival of the stress (adaptive mutations). Both polymerase-error-style point mutations (small frameshift mutations and base substitutions) and gene amplifications are induced. The point-mutagenesis requires at least three stress responses: the SOS DNA damage response, the RpoS- (sigma S)-controlled general stress response to starvation, stationary phase, oxidative, osmotic, cold-shock and acid stresses, and an *E. coli* heat-shock/protein-stress response. We suggest that such multilayered control by stress responses severely limits the dangerous process of global mutagenesis to times of stress, when organisms are poorly adapted to their environments: that stress responses are the signalers of poor adaptation that initiate genetic change mechanisms that are often deleterious, but sometimes result in an adaptive mutation. A second level of control is spatial: both the point mutations and the amplifications are also induced by DNA double-strand breaks (DSBs). We show that stress-induced point mutagenesis is caused by a switch from high-fidelity to error-prone DSB repair controlled by RpoS, using the special error-prone DNA polymerase, DinB/Pol IV. DNA near a DSB is mutated but another molecule is not. We suggest that coupling of stress-induced mutagenesis to DSB-repair further regulates and limits mutagenesis by restricting it to potentially small fractions of the genome, those in which DSBs are being repaired. This could be a regulatory strategy that both reduces deleterious mutations in cells that acquire a rare adaptive mutation, and potentially facilitates concerted evolution of genes and gene clusters. The themes of control by stress responses, and coupling to DSB-repair suggest a strongly limited and regulated mechanism that, though random, minimizes damage and maximizes potential benefit of increasing mutation rate in response to stress.

CANALIZATION AND EVOLVABILITY: TEMPERING THE EFFECTS OF MUTATION IN A CHANGING ENVIRONMENT

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The biological consequences of new mutation depend on the mapping between genotype and phenotype, which is modified by developmental and physiological processes including genetic buffering by the Hsp90 protein chaperone. Hsp90 assists many developmental processes, allowing them to occur normally. During stress, Hsp90 buffering is reduced, the expressed mutation rate is suddenly increased and dramatic morphological changes occur in previously invariable traits. I will discuss the regulation of canalization and evolvability by Hsp90. The release of sudden morphological novelty by Hsp90 during stress plausibly explains episodic bursts of morphological radiation, but whether Hsp90 dependent changes could be adaptive was not previously tested. To test the 'Hsp90 capacitor hypothesis' under alternative models of adaptive evolution, we measured the fitness costs of a large Hsp90-buffered transition and deconstructed smaller changes in natural quantitative traits using Drosophila differing only by loss-of-function or wildtype alleles of Hsp90. Random differences in left-right symmetry, trait values among isogenic flies and genetic effects on trait means across wild backgrounds were used to parse developmental noise, microenvironmental and genetic variation. Under low Hsp90, the most invariant traits regained variability and their predicted response to selection sharply increased. A sudden change in eye morphology was selected without correlated fitness effects but unconditionally deleterious variation remained tightly repressed independent of Hsp90. Its central position in the topology of developmental networks predicts Hsp90 control over canalization, modularity and evolvability.

LOW DOSE RADIATION EFFECTS IN THE ENVIRONMENT: A SOURCE OF IRRATIONAL FEAR?

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Initially radioprotection was anthropocentric, there being two main rationales for this. The first was that man was the only thing in the environment worth protecting. The second was that if man were adequately protected, then everything else in the environment would be too.

Attitudes have now changed, to the extent that man should now be regarded as part of his environment, and if the environment fails so does man. Depletion of the ozone layer and accumulation of greenhouse gases are contributing to accelerating climate change. There is concern that loss of environment may equate with loss of man.

As a result there is now a growing lobby that feels that the environment itself should be protected. This approach is fraught with difficulty, as what is the environment that is being protected ? Generally man is already managing the environment, through agriculture and industry. There is also more overt control through, for example mosquito spraying. Ethically man seems to have no problems with trying to completely eliminate bacteria and viruses that are pathogenic to man.

This paper will discuss whether the desire to protect the environment from radiation is a logical step in environmental protection, or whether adequate protection already exists, and concern for the environment is primarily driven by another form of expression of fear of radiation.

N.V. TIMOFEEV-RESOVSKY AND RADIATION GENETICS

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Researches into the radiation genetics of drosophila, performed by N.V. Timofeev-Resovsky in 20-30's, became the basis of modern radiation genetics. He pioneered in the detailed study of the dose dependence of genetic effects and in phenotype description of radiation-induced mutations. These experiments gave rise to the Hit principle and Target theory, which played a large role in creation of the methodology of genetic risk assessment after irradiation. Nikolai Vladimirovich, using the results of his own experiments on drosophila, first calculated the doubling dose for the frequency of spontaneous mutations in humans. The extrapolation method he used for calculations was subsequently applied for estimation of genetic risks after exposures of humans to radiation in all the UNSCEAR reports, the first one being issued in 1956. In a series of his papers, dedicated to analysis of congenital and acquired hereditary diseases, N.V. Timofeev-Resovsky emphasized that radiation-induced mutations and, therefore, hereditary diseases can be provoked by quite low radiation doses.

The author of the present paper had invaluable experience of being in contact with Nikolai Vladimirovich to consider the problems of radiation genetics of natural algae populations inhabiting the South-Ural radiation track area. N.V. Timofeev-Resovsky made a number of experiments on the effects of uranium nuclear fission products (NFP) in algae, and on the determination of the coefficients of accumulation of some radionuclides, NFP mixture components, by various algae types. Nikolai Vladimirovich always took a keen interest in experimental data, obtained in our laboratory, which were reported on his seminar, and, subsequently, he actively supported such studies during conferences and dissertation discussions.

OBNINSK PERIOD IN N.V. TIMOFEEFF-RESSOVSKY'S SCIENTIFIC RESEARCH

A.F. Tsyb

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N.V. Timofeeff-Ressovsky worked in Obninsk during 1964-1969 being a Head of radiobiological and genetic department in Research Institute of Medical Radiology of RAMS (now Medical Radiological Research Center). It was a very fruitful period of his scientific work. In 1965 he was awarded the Mendel prize and medal of Czechoslovak Academy of Sciences, the Kimber prize for genetic research (USA). In 1966 he has been elected an honorary member of the British Genetic Society in Leeds. During Obninsk period of N.V. Timofeeff-Ressovsky's scientific research a great attention was devoted to the mechanism of cell radiation effects, regulatory systems, cell recovery from damages inflicted by UV light and ionizing radiation. He was a leadership on the basic problems of radiation biology, radiation genetics, population genetics, experimental and theoretical ecology. In Obninsk N.V. Timofeeff-Ressovsky got back to the classical problems that have been developed by him earlier - theory of evolution and population biology. As a result, more modern presentations of these problems were established which have been described in several books: one of them was published together with N.N. Vorontzov and A.V. Yablokov, another with A.V. Yablokov and N.V. Glotov. The development of genetic and radiobiological scientific directions has been reflected in the book "Application of Hit Principle in Radiobiology" published together with V.I. Ivanov and V.I. Korogodin as well as in the book "Introduction in Molecular Radiobiology" published together with A.V. Savich and M.I. Shalnov. The principal place in N.V. Timofeeff-Ressovsky's personal scientific research in Obninsk was occupied by the global problem "Biosphere and mankind", his thoughts related with the future fate of the live and inert matter of our planet and with the place and perspectives of mankind on the Earth. His conclusion was close to V.I. Vernadsky's idea about the role of living matter in the migration of chemical elements in the earth. Other important aspects of this problem were the analysis of biological effects produced by radioactive nuclides on ecological systems and development of different means for purification of land and water areas contaminated with radioactive materials. These ideas have been used practically for reducing the consequences of radiation accident in Chernobyl.

SAFEGUARDS OF NUCLEAR MATERIALS AND INTERNATIONAL TERRORISM

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Since the beginning of nuclear industry, nuclear material accountancy was performed at national level in counties with significant nuclear activities. With the entry into force of the Non-Proliferation-Treaty (NPT) in 1970, every country signatory of the treaty undertook the obligation to establish a State System of Accountancy and Control and to submit all nuclear activities and materials under their jurisdiction to international control as administered by the International Atomic Energy Agency (IAEA). In order to fulfill this task, the IAEA developed techniques which became more and more sophisticated as the nuclear technology advanced. The objective was to deter a potential diverter from the removal of a significant quantity of nuclear material from peaceful use and to detect it if this removal occurred. This system in the course of decades of its implementation provided good assurance of compliance to the states signatory of the NPT. In 1991, however, the discovery of a parallel and undeclared nuclear program in Iraq and subsequently the problems associated with the verification of the nuclear activities in the Democratic People's republic of Korea, urged a revision of the existing approach with the aim of strengthening the safeguards system in order to increase the Agency effectiveness in detecting undeclared nuclear material and activities. These new measures, which were identified and then gradually implemented, consist, among others, in a broader access to information, use of advanced analytical techniques and enhanced physical access to locations. The new system is designed to give results even in area where the cooperation is lacking or insufficient. All this results to be a powerful tool available to the international community to identify areas of concern where clandestine and illegal activities are suspected and to prevent the possibility of acts of nuclear terrorism. The IAEA is also providing support to strengthen the states ability to combat terrorism through better physical protection, improved nuclear material control and control of other radioactive materials.

* Dr. Giancarlo Zuccaro Labellarte is a retired senior officer of the Department of Safeguards of the International Atomic Energy Agency (IAEA).

GENETICS

CYTOGENETICS INVESTIGATION OF PATIENTS WITH LEUKEMIA IN ARMENIA

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Cytogenetics findings have been demonstrated to be powerful indicators in predicting clinical course and outcome in leukemia patients and in guiding their management. DNA probes library has been created at our Department for FISH (Fluorescence *In Situ* Hybridization) diagnostics. This technique of molecular cytogenetics plays an important role in the detection of chromosomal loci containing genes involved in leukemogenesis. Conventional and molecular cytogenetic investigations of more than 400 patients with leukemia were performed. The most of them were patients with CML (chronic myeloid leukemia). In the majority of patients with CML Philadelphia chromosome was identified and FISH was applied to monitor the response to therapy. In some CML cases complex translocations and additional aberrations (by application of whole chromosome painting probes) were observed. In 19 patients with acute myeloid leukemia numerical abnormalities were revealed with the aim of centromere specific probes. These abnormalities included various aneuplodies and near-tetraploidy. FISH results were compared with data obtained by conventional cytogenetic analysis. The presented research is a part of conventional practice of leukaemia diagnostics in Armenia.

THE INFLUENCE OF THE SOLAR ACTIVITY AND SOME OTHER AGENTS ON THE FREQUENCY OF MICRONUCLEI IN CHILDREN BUCCAL MUCOSA

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Now the factors causing genomic instability are being researched actively including such a factor as the solar activity for it can provoke seasonal changes of genomic stability. That is why we have tried to examine the level of micronuclei (MN) in children buccal mucosa (BM) during a year. The MN test was chosen due to its relative simplicity to be performed its informative character and also because the BM is a kind of mirror reflecting the processes of an organism. The MN test in BM of 6-7 year old children was done in Stary Oskol city, Belgorod region in different season of the year. The material was collected for a year among the children attending the same group in a kindergarten. The microscopic preparation were made and analysed according to the methods described by Arutyunyan et al., 1987. The 3-way ANOVA allowed to reveal the influence of the month ($P<0,001$), the sex ($P<0,01$), the parents smoking ($P<0,05$) with the united factor of the month and the sex in addition on the MN level of children oral mucosa. The maximum number of MN was in August (0,22±0,09%) and October (0,24±0,07%), the minimum number – in May (0,08±0,03%). In summer and autumn the quantity of MN was approximately the same (0,18±0,06%) and (0,18±0,02%). These figures differed significantly from those in spring (0,12±0,03%) and winter (0,15±0,02%). Such fluctuations of MN quantity in children BM correlated with changes of the solar activity ($r_s=0,580$, $P<0,05$). The girls MN level (0,18±0,02%) in comparison with the boys level (0,13±0,02%) was revealed to be higher ($P<0,01$). The burst of girls cells with MN was noticed in summer (0,22±0,11%) and autumn (0,21±0,04%), in August and October. The boys MN level was approximately the same in autumn, winter and summer and only in spring it was lower regarding those three seasons. The main picks took place in December and June. Girls appeared to be more sensitive to tobacco smoke than boys: the girls whose parents are smokers, had the bigger number of MN (0,18±0,03%) compared with the boys of the same group (0,14±0,02%) ($P<0,05$). Thus the number of MN in children BM depends on season, sex and inhaling of tobacco smoke. The results of our research will help to evaluate more correctly the data of MN tests done in different seasons. This study was supported by the Grant of President of Russian Federation for young Russian Scientist (Grant MK-2587.2004.4).

COMPARATIVE SPECTROSCOPIC AND MICROCALORIMETRIC ANALYSIS OF THE INTERACTION ANTICANCER DRUGS WITH DOUBLE-STRANDED NUCLEIC ACIDS

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Investigated of thermodynamics, base specificity and the possible interaction mechanism of anthracene derivatives mitoxantrone and its congeners with double-stranded nucleic acids by means of spectroscopic (UV-visible adsorption, circular dichroism and fluorescence) and microcalorimetric techniques at different ionic strength and temperatures.

Such agents when interact with double-stranded nucleic acids may intercalate, as well as interact electrostatically by the side chains with the phosphates of nucleic acids. Hence it is expected that the character of their interaction will depend on the nucleic acids conformation. Investigated of interaction of mitoxantrone and ametantrone with right-handed (B-DNA and A-RNA) and left-handed (Z) helices. Microcalorimetric determinated of enthalpy (ΔH) changes at binding mitoxantrone with B-DNA and the dependence of ΔH on different binding ratios. It was shown that the ΔH of mitoxantrone with DNA a RNA increases linearly and reaches $-(3.0\pm0.5)$ ccal per 1 mol mitoxantrone. The calculations show that the saturation stoichiometry is one mitoxantrone molecule per $2\div3$ base pairs DNA and $6\div8$ base pairs RNA. The dependence of binding constant from GC-content is observed. In the low ionic strength (0.01M NaCl) the dependence of the melting point (Tm) on the concentration of mitoxantrone passes through a minimum, and Tm falls in the region where there is one mitoxantrone molecule for about 100 DNA base pairs. In this concentration region of mitoxantrone the enthalpy of melting of the complexes increases linearly as the mitoxantrone concentration increases. The phenomenon is explained qualitatively by increase in the entropy of coiled state of the DNA-mitoxantrone complex due to the additional freedom of rotation of the mitoxantrone.

A MODEL OF THE COMPLEXES ETHIDIUM BROMIDE WITH SINGLE-STRANDED POLY(DA) AND POLY(DT)

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A general description of drug-nucleic acid interaction requires not only specification of the binding constant of the ligand to an isolated binding site but also a description of the mechanism of binding preferences to particular base sequences at the certain groove side of DNA.

In this study we suggest a model of ss-polynucleotides - Ethidium Bromide (EtBr) complexes determined by comparing the thermodynamic properties of the ligand with ss- and ds-DNAs. Earlier we showed that EtBr may form at least two types of complexes with ss-DNA, one of which is "strong" and other is "week" [1]. Obtained in this study data point to the independence of the binding parameters (binding constant Ki and binding site size rI) on the ionic strength for both types of interactions. On the other hand calculated Van't-Hoff free energy ($\Delta G°$), enthalpy ($\Delta H°$) and enthropy ($\Delta S°$) of the complexes indicate the enthropy character of the interaction of EtBr with ss-poly(dA) and ss-poly(dT).The differences of $\Delta G°$ between "strong" and "weak" interactions of EtBr with ss-poly(dA) and ss-poly(dT) and that of between "strong" interactions of ss- and ds-polynucleotides (in the both case the differences is ~1kcal/mol) allowed us to suggest an hemintercalative model, according to which EtBr binds to ss-poly(dA) more strongly than to poly(dT).

EtBr chromophore is stacked between the A-A and T-T bases . Due to the weak stacking interaction and high flexibility of poly(dT) the numbers of nucleotides corresponding to a bending site (binding site size) two times as smaller as for poly(dA).

1. Karapetian et. all. *J. Biomol. Struct. Dyn.*, 14,275 (1996).

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GENETIC POLYMORPHISMS OF GLUTATHIONE S-TRANSFERASES AND CHROMOSOMAL DAMAGE: EFFECT ON MICRONUCLEI FREQUENCY IN LUNG CELLS OF LUNG CANCER PATIENTS

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At present the solution of early diagnostics problems and prediction of a cancer course is closely related to molecular-genetic research. Carcinogenesis is conceptually associated with reactive metabolite formation in reactions of xenobiotic biotransformation and damage of "critical genes" by them. The analysis of numerous data on relation of inherited functional deficiency of glutathione S-transferases (GSTM1 and GSTT1) with genetic damages typical for lung cancer, verifies direct involvement of this enzyme in the metabolic ways providing cell protection against chemical and radioactive carcinogens inducing DNA damages. The study of cytogenetic manifestation on various genotypes and the opportunity to identify on this basis people with high risk of lung cancer development is of great interest. In this connection the interrelation between GSTM1 and GSTT1 polymorphisms and the level of micronuclei in lung tissue of lung cancer patients was analyzed.

The frequencies polymorphic alleles of GSTM1 and GSTT1 genes were investigated by PCR analysis in squamous lung cancer patients. DNA samples for the analysis were obtained by the standard procedure from lung tissue. Homozygotes and heterozygotes for normal alleles for GSTM1 and GSTT1 genes were determined on electrophoregrams by the presence of amplification product 215 bp in size (GSTM1) and 480 bp (GSTT1). Amplification of albumin gene fragment was used as the internal control. At the same time the micronuclear analysis was made in lung cells on postoperative smear-prints from tumoral and normal tissue of lung cancer patients.

Preliminary results have shown the possible relation between the GST genotype and the level of micronuclei in normal tissue of lung cancer patients. Obviously, such effects can be explained by the unequal ability to biotransformation in people with various GST genotype, their metabolic specificity and, hence, different expression of enzymes, involved in repair of DNA-damages.

INTERACTION OF NEW PORPHYRIN DRUGS WITH DNA

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Porphyrins comprise an important class of compounds whose chemical and photochemical properties are widely exploited in both medical and biological applications. Due to their ability to accumulate in malignant cells, porphyrins have found utility as diagnostic agents for determination of the tumor design. The understanding of the interactions will lead porphyrins and their metal derivatives to medical applications to the inhibition of AIDS virus, HIV-1, and photodynamic therapy of tumors.

By methods of UV/visible spectrophotometric melting, circular dichroism and fluorescence the binding of **new** *meso-tetra*-(4N-alylpyridyl)porphyrin (TAlPyP(4)) and its Ag metallocomplex (AgTAlPyP(4)) with DNA has been investigated. It has been shown that:

1. These porphyrins essentially increase the melting temperature of DNA (T_m) and so it becomes impossible to register the melting curve. This increase is proportional to porphyrin concentration.

2. The binding of AgTAlPyP(4) with DNA and polyribonucleotides brings to the quenching of porphyrin fluorescence, while for TAlPyP(4) the changes of fluorescence spectra have more complicated character. The stochiometry (r_{sat}) of saturated DNA+porphyrin complexes has been determined from the dependence of fluorescence curves on relative concentration of porphyrin.

3. The analysis of induced CD spectra of these DNA+porphyrin complexes shows that these porphyrins prefer to interact with DNA mainly via intercalation. However, at high relative concentrations of porphyrins the external mode of binding has taken place, too. The comparative analysis of DNA CD spectra changes in the presence of these porphyrins shows that at the same relative concentrations of porphyrins (r), there are more changes in CD spectra caused by AgTAlPy(4) than in the case of TAlPyP(4).

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A NEW TYPE CELLS WITH MULTIPLE CHROMOSOME REARRANGEMENTS

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The term "multiaberrant cells" (MAC) is used by radiation cytogeneticists for cells in which 5 and more breaks of the chromosomal type are detected with standard staining by Giemsa.

Using FISH analysis of stable chromosome aberrations in the culture of peripheral blood lymphocytes of the liquidators of the ChNPP accident we could observe cells with multiple exchange aberrations of another type. No considerable disturbances in the structure of chromosomes were noted, but there were multiple (up to eight) rearrangements of the stained chromosomal material present in three studied chromosome pairs. Cells with such rearrangements, without polycentrics and rings, cannot be registered as multiaberrant with the routine cytogenetic procedure. Disturbances of such kind can be indirectly indicated only by the appearance of atypical monocentrics. Nevertheless the discovered cells may be referred with good reason to the category of multiaberrant ones. It should be stressed that they were found in a group of subjects in whom classic MAC were already discovered earlier in the routine cytogenetic study. Only in one case, a worker of a radiochemical enterprise, presumably a carrier of incorporated plutonium, no MAC were revealed. Thus, a new type of MAC was discovered in the course of cytogenetic analysis with the use of more delicate methods already widely employed in radiation cytogenetics. It can be assumed that classical MAC revealed by the routine cytogenetic procedure are a visible part of an iceberg of multiple chromosome and gene rearrangements occurring as a result of high-energy radiation. To detect a full spectrum of induced chromosome rearrangements, it is necessary to use actively the potentialities of modern molecular-genetic methods of research.

SECONDARY LEUKEMIAS: SIGNIFICANCE OF FOREGOING THERAPY, IRRADIATION AND FACTORS LINKED WITH INHERENT DEFECTS OF REPARATION SYSTEM

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Malignant therapy progress and full recovery achieved by using methods of cytostatic therapy and irradiation leads in a number of cases of new malignances and new leukemias in particular. These malignances and leukemias are called secondary bearing a relation to primary malignances treated by chemotherapy or/and radiation therapy. Secondary leukemias appear in months or years after the treatment in 6-10% of patients recovered from primary malignances. Frequency of secondary leukemia occurance depends on type of chemotherapy and radiation therapy courses used.

We observed 54 patients who developed secondary leukemias after primary malignances treated by different cytostatic agents (alkilating agents, antracyclines, topoisomerase inhibitors, irradiation) and their combinations. Detected chromosome aberrations in secondary leukemic clone in bone marrow correlated with the type of chemotherapy and irradiation used for treatment of the primary malignance. We detected monosomy 7 and/or 7q deletions in patients treated by irradiation only; abnormalities in 11q23 locus (containing MLL gene) were typical for patients treated by topoisomerase inhibitors; in patients treated by alkilating agents +8,+9,11q-,12p-,-18,-19, 20q-,+21, t(2;11) and complex karyotypes were detected.

We revealed aggressive chemotherapy courses frequently leading to development of secondary malignances and milder ones. So, local fractional irradiation under 20 Gy used in lymphogranulomatose treatment didn't lead to secondary malignances. On the contrary complex therapy by irradiation and MOPP courses is most aggressive especially repeated more than 6 times or in combination with lomustin, bleomycin or with BEACOPP courses. Most aggressive chemotherapy courses caused secondary leukemias in 10% of patients. We suppose these patients to have a inherent defects of reparation system in particular as a result of polymorphism in genes NAD(P)H quinone oxidoreductase, glutation S-transferase P1 and some others.

HOMOLOGOUS RECOMBINATIONS IN TRANSGENIC MICE WITH GENE OF NEOMYCINPHOSPHORYBOSYLTRANSFERASE

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The concept of genome stability is commonly accepted; due to this stability, hereditability of signs is performed. However, recently, an increasing attention of many geneticists has been paid to instability of genome. This problem is very important, as it is essential to know forms and amount of such instability as well as its effect on physiological processes and, of course, on heredity. At present, point mutations are established to take place in genome more frequently than it was thought earlier. Recently it has become evident that the majority of point mutations are neutral for life because either their effect on cells is not significant or the cells-mutants are eliminated from an organism by apoptosis. A great attention is paid to the role of micro- and mini-satellites, of mobile gene elements in the genome stability, and of homologous DNA recombinations in the normal cell functioning, in development of pathology, particularly of malignant growth, and in evolution. To elucidate effects of the homologous DNA recombination on the animal organism, we used transgenic mice carrying gene of neomycinphosphoribosyltransferase (*neo*) with two deletions in the 3'- and 5'-sites of gene. Homologous recombination was revealed in all 8 examined transgenic mice *neo*. The set of tissues, in which the homologous DNA recombination of the gene neo was found, differed in mice from different parents. At the same time, the set of such tissues in F_1, F_2 mice — offsprings of one parent — coincided to a significant degree, which indicates heredity of such recombination. This means that either efficiency of the homologous DNA recombination of gene *neo* differed in different tissues or this recombination occurred at different moments of tissue differentiation. Recombination of the *neo* gene most likely took place at the period of embryogenesis and the subsequent growth of organs. It is to be noted that the homologous recombination neo did not affect the mice phenotype. This allows the conclusion to be made about the possible neutral character of recombination processes in genome in nature. The fact that recombinations occurred in different mice in different organs means that this process has a purely stochastic character. The recombinations able to play a role in development of pathology and in evolution.

IONIZING IRRADIATION AND MODIFICATION OF MUTATION SPECTRA

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The mice of laboratory lines BALB/c, C57BL/6j and CC57W/Mv were investigated in two age groups (2-3 month and 12-18 month) in control conditions (vivarium of Institute in Kiev, Ukraine) and their sibs in experimental vivarium near Chernobyl's NPP (near 0,6 Sv,). Representatives of a *Microtus oeconomus, Clethrionomys glareolus* and *Microtus arvalis* were trapped in places of zone alienation of Chernobyl's NPP, distinguished by radio nuclide pollution. Any constitutive mutations in investigated species, surprisingly, including those, trapped in the Red Forest (near 1000 Ci/km^2) was not revealed.

In exposed mice only those types of cytogenetic anomalies were increased which were spontaneously highly variable in an age- or season-dependent manner in the same mice lines not subjected to radiation. Moreover, in the group of exposed "old" mice under influence of ionizing irradiation, some cytogenetic anomalies were less frequent, than in the mice of the same age in the control group. This corroborated with the increased rates of cell division in Chernobyl's animal populations in comparison with control group. Our results indicate that ionizing radiation does not induce new anomalies in mice lines, but strengthens realization of inherently unstable line-specific cytogenetic characteristics. The differences of the contributions of individual chromosomes in cytogenetic anomalies in various species of mice and voles were observed. So, for example, in *M. oeconomus* the tendency to more often involving in aneuploidy and asynchronous centromere divisions of sub- and acrocentric chromosomes 10 and 14 was revealed. The preferable aneuploidy on chromosomes of the intermediate size and increased frequency of chromatide gaps on centromere regions in *M. arvales* were observed. More often asynchronous divisions of centromere regions in group of small-sized chromosomes in *Clethrionomys glareolus* were marked.

Thus, our researches allowed to suppose, that the increase of ionizing radiation did not induce directly the "new" genetic damages, but more often lead to modify the systems which controlled of their reparation and elimination of cells, carrying them.

THE INVESTIGATION OF DIAGNOSTIC POSSIBILITIES OF THE PRENATAL KARYOTYPING IN THE FIRST AND SECOND TRIMESTER

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First-trimester chorionic villus sampling (CVS) and second-trimester amniocentesis are widely used methods for the prenatal detection of chromosome abnormalities. We investigated a difference in the diagnostic performances of cytogenetic investigation in amniotic fluid (AF) cells and CVS. It was measured as success rate, maternal cell contamination (MCC), mosaicism. During the years 1996-2004, we received 10990 AF-samples, 1987 first- and 644 second-trimester CVS in our department for prenatal cytogenetic investigation. Amniocentesis and CVS were performed under continuous ultrasound guidance by transabdominal aspiration. The aspirated villi samples were carefully washed and estimated under an inverted microscope. Short term culture (STC) and long term culture (LTC) were available in 1145 CVS of first-trimester and the remaining CVS were karyotyping only by STC. Karyotyping was routinely performed by G-banding using the Trypsin-Giemsa staining technique. A karyotype was considered normal in cases of 46,XX or 46,XY±inv(9)(p11;q13) and/or±any one-cell abnormality. Mosaicism was considered when two or more cells were abnormal. Confined placental mosaicism (CPM) was defined as CPM I, II and III, respectively, when a (mosaic) abnormal karyotype in STC-, LTC- or both was accompanied by a normal karyotype in the fetus or amniotic fluid cells. Cytogenetic results were fulfilled in 10963 AF-samples (99,75%), 1943 first- (97,79%) and in 522 second-trimester CVS (81,06%). Mosaicism was detected in 26 AF-samples (0,24%), 23 STC- (0,95%) and 8 LTC- (0,68%) CVS. CPM was 1,30%: I - 0,70%, II - 0,52%, III - 0,08%. True fetal mosaicism was present in 1,34% CVS. Four false-negative and one false-positive results were recorded, all after STC CVS. Frequency of MCC was 0,16% in AF-samples and 2,36% in LTC CVS. We didn't find any differences in success rate between AF-samples and CVS. Mosaicism and MCC were more often in CVS. The simultaneous analysis of STC- and LTC-villi preparations minimize the proportion of both false-negative and false-positive results.

INVESTIGATION OF DNA DAMAGE AND TELOMERE FRAGILITY BY COMET-FISH TECHNIQUE IN DIFFERENT CELL LINES

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The purpose of present study was the detection of the involvement of telomeric repeats in DNA damage induced by cytostatic bleomycin (BLM). The investigation was performed with Comet-FISH, combination of Comet-assay (single cell gel electrophoresis) with FISH (Fluorescent *In Situ* Hybridization) technique. This approach permits to detect in the individual cell simultaneously the total DNA damage and specific DNA sequences. To visualise telomer damages telomere-specific PNA probes were used. BLM effect was studied on three cell lines: HT 1080 (derived from a human fibrosarcoma), CCRF-CEM (derived from a human T-cell acute lymphocytic leukemia) and CHO (Chinese hamster ovary cells). The number of telomere signals and the localisation of the signals (at comet head or tail) were recorded. Comet image analysis and Comet-FISH evaluation was performed by the Komet 4 software package (Kinetic imaging, UK. It was shown that the level of DNA migration is increased in all cell types in dependence on BLM dose. CHO cells were shown to be most sensitive to the agent. The same cell line demonstrated the highest level of telomeric signals in the comet tail. The relation between amount of DNA and the number of telomeric signals outside the nucleus varied between the examined cell types. In CCRF-CEM cells this relation was equal to 2.62±0.55, in CHO cells - 2.06±0.33, and in HT1080 cells - 0.73±0.28. Nuclei of intact cells shown mainly produced slightly damaged comets with a few telomeres outside of the head. Thus, the results suggest applicability of Comet-FISH technique to estimate the sensitivity in malignant cells to chemoterapeutics and to reveal the involvement of specific genomic regions in the genetic damage. This study was supported by ISTC grant A-301.2.

DNA DAMAGE INDUCED BY NEW PORPHYRINS OF DIFFERENT CHEMICAL STRUCTURE

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Some porphyrins are known to be applied in cancer treatment and, especially, in photodynamic therapy of cancer. The new cationic meso-substituted N-quarternized 4-pyridylporphyrins and their metal derivatives were synthesized to discover and develop novel chemoterapeutics. The levels of DNA damage induced by porphyrins were estimated by Comet-assay (single cell gel electrophoresis) in human leukocytes. This technique allowing to quantify DNA fragmentation is commonly used in pharmaceutical industry as a standard tool to assess the safety of new preparations. The genotoxicity of porphyrins TOEt4PyP, TOBut4PyP, TOEt4PyPMn and TOBut4PyPMn was estimated and its dependence on compound chemical structure was analysed. Casp software was used to measure comet images. The percent of DNA in tail of comet was applied as an index of DNA damage. The investigated porphyrins may be distributed by their genotoxic activity in the following range: TOEt4PyP < TOEt4PyPMn <TOBut4PyP< TOBut4PyPMn. Thus, i) the genotoxicity of Mn-derivatives of TOEt4PyP and TOBut4PyP is higher than original porphyrins and ii) the genotoxicity of TOEt4PyP and TOEt4PyPMn is increased after substitution of ethyl radical with butyl one. The applied Comet-assay permits to reveal the dependence of DNA damage induction on chemical structure of porphyrins. This study was supported by ISTC grant A-301.2

COMPARATIVE INVESTIGATION OF GENOTOXIC ACTIVITY OF NEW PORPHYRIN ON THE LEVELS OF PURIFIED DNA, CELL AND ACELLULAR EXPERIMENTAL SYSTEMS

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Porphyrins complexes are applied in the cancer therapy. In this work the interaction of the newly synthesised porphyrin TOET4PyPCo with DNA was investigated at three different levels: purified DNA, cell and acellular systems. In experiments with purified DNA the specificity of the interaction of TOET4PyPCo (10^{-4}-10^{-6}M) with DNA was studied via determination of thermodynamic parameters (melting curves of DNAs with various GC content). TOET4PyPCo was shown to form reversible outside binding with DNA at the AT-rich sequences (0.4-0.5 molecules of porphyrin per one nucleotide pair). Experimental cell and acellular systems used were based on Comet-assay. Intact (cell system) or lysed (acellular system) human blood leukocytes were treated with porphyrin. Nucleoids formed after cell lysis are known to contain non-nucleosomal but still supercoiled DNA. The level of DNA breaks was estimated as percents of DNA in the comet tail by Casp software.In acellular system porphyrin at the concentration 10^{-5}M destroyed the majority of cells, at 10^{-7} and 10^{-6}M induced 8.0±5.23 and 10.6±8.56 % of DNA in the tail versus 2.38± 2.18 % in the control. In cellular system porphyrin at concentrations 10^{-7}M, 10^{-6} M and 10^{-5} M induced, respectively, 2.38±2.95, 7.54 ±6.49 and 20.2±9.64 % DNA in comet tail versus 1.54±1.56 % in the control. The differences in the sensitivity of three models presented are though to reflect the different level of their organisation. The approach applied may be recommended both for the estimation of genotoxic effects of different agents and comparative analysis of role of cellular mechanisms in DNA damage. This study is supported by ISTC grant A-301.2.

ON THE ORGANIZATION OF MEDICAL AND GENETIC CONSULTATION IN CASE OF RADIATION INJURIES

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After the accident at the Chernobyl Nuclear Power Plant (Chernobyl NPP) in the Republic of Armenia it was established rather great "risk group" of liquidators of the accident effects at the Chernobyl NPP (more than 3000 individuals) which from the point of view of medical and genetic consultation (MGC) requires more intent attention. One of the earliest indices in case of the radiation injuries are chromosome changes in somatic and generative cells that can bring to a) the initiation of carcinogenic effects in the irradiated organism and b) the increase of the frequency of inborn vices of development (IVD) of irradiated individuals' descendants. The organization of MGC in case of radiation injuries has an individual, as well as a population character. In this aspect, the MGC while the action of mutagens of external media turns on/rests on a carrying out of a monitoring. It is obvious, that the monitoring must be focused on the population of a man. That's why on the basis of registration of mentioned changes during the MGC both preventive and medical measures on a possible elimination of radiation injuries will be used. Due to the 19-years observation of the condition of the chromosome apparatus of lymphocytes of the Armenian liquidators, now we are able to organize the MGC service, as well as to conduct in a compulsory form trials pointing out the radiation injury: 1) cytogenetic trial in case of irradiated individuals; 2) spermatological trial in case of irradiated individuals; 3)gynecological trial, aimed at registration of spontaneous abortions; 4) cytogenetic trial of a descendants of irradiated individuals; 5) clastogenic factors trial in the plasma of irradiated individuals.

Basing on the mentioned trials, the radiation injury of the organism is fixed; after which begins the MGC on future descendants and possible long-term effects of the irradiated organism itself. The consultation will include: 1) computation of risk (at definite hereditary or chromosome disease); 2) councils on terms of conception and medical observation during pregnancy; 3) strict control of the clastogenic factor level and, if necessary, antioxidant and other treatment; 4) compulsory regular cytogenetic trial of irradiated individuals (once during 6 months period).

POLYMORPHISM OF CYTOGENETIC CHARACTERISTICS OF BIRCH SEED PROGENY ON ECOLOGICAL CLEAN AND POLLUTED TERRITORIES

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The study of genotype composition of population and genetical mechanism, responsible for typicalness and reproduction of population, has allowed to reveal and to select the resistant forms of woody plants for use in planting trees in towns. In this connection we studied the structure of populations of birch (Betula pendula Roth) using cytogenetic characteristics (the mitotic and nucleolar activity, frequency and spectrum of pathological mitosis (PM)). The study was conducted in populations of the birch, growing on ecologically clean and polluted territories. From each trial territory we studied 40 plantlets. Statistical analyses of the data were made using the cluster analysis, which has allowed to distinguish in each of studied populations 4 clusters of plantlets: 1) "Sensitive" cluster of plantlets characterized by raised frequency of PM (7,5-14,7%) and large surface area of single nucleoli (100,7-141,2 mkm^2) in interphase cells, and the high level of persistent nucleoli on stage of meta-telophase of mitosis (8,8-18,4%). There is a lot of lagging chromosomes and their fragments (25,0-41,7%) in spectrum of PM. This fact indicates the low intensity of reparative processes in these plantlets. 2) "Resistant" cluster of plantlets is characterized by the least amount of PM (3,2-8,4%), prevalence in spectrum of nucleolus types the high-actively "bark-core" nucleoli (69,2-93,0%), which have the smallest sizes (87,8- 111,7 mkm^2). This cluster has relatively low amount of persistent nucleoli in mitosis (8,9-12,5%). Thus, these cytogenetic characteristics can be considered as the criteria of stability of birch seed progeny to unfavorable conditions of the environment. This cluster of seedling is characterized also by high frequency of bridges (58,8-66,7%) in spectrum of PM, that is indicates the active functioning of the reparation system. 3) Two remained clusters of plantlets on each of examined territories are characterized by intermediate cytogenetic characteristics between "sensitive" and "resistant" clusters. There is the prevalence of plantlets from the "sensitive" cluster on ecologically clean territories (25-40%) and the plantlets from the "resistant" cluster on contaminated territories (27-30%). These data indicate the reduction of gamete and zygote selection on ecological clean territory, where the specimens from the "sensitive" cluster survive, and on polluted territories the specimens from the "resistant" cluster have advantages in survival possess. The results of our studies can be used for selection of maternal tree producing seed progeny, which has the resistant to unfavourable conditions of the environment for using in planting of trees in towns. This study was supported by the Grant of President of Russian Federation for young Russian Scientist (Grant MK-2587.2004.4)

IMMUNOFLUORESCENT DETECTION OF 8-OXOGUANINE DNA DAMAGE IN AGING CELLS OF OXYS RATS WITH INHERITED OVERGENERATION OF FREE RADICALS

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An important biomarker of oxidative DNA damage is 8-oxoguanine (8-oxoG) formed by exogenous and endogenous ROS attacking nucleic acids. Formation of 8-oxoG in cellular DNA was found to be associated with such important biological processes as mutagenesis, carcinogenesis, aging, and several age-related diseases, and may even be causative for cancers and neurodegeneration. The available evidence indicate that 8-oxoG is present in all growing cells, but the conditions that may contribute to the formation of this DNA modification *in vivo* are still poorly understood. An inbred rat strain (OXYS) was derived from Wistar rat (Albino, *Rattus norvegicus)* stock by selection for the susceptibility to a cataractogenic effect of a galactose-rich diet and siblings mating of highly susceptible animals. The main characteristic of the OXYS strain is the inherited overgeneration of free radicals, lipid peroxidation, protein oxidation, and pathological conditions paralleling several human degenerative diseases. Thus, the OXYS strain may constitute a good model to study human and animal degenerative diseases as well as oxidative DNA damage and DNA repair in mammals. We have developed the quantitative version of ELISA to determine the relative levels of 8-oxoguanine in the samples of DNA. The levels of 8-oxoguanine in DNA from different tissues (liver, lung, heart, kidney, spleen) of OXYS and Wistar rats of different age were estimated. It was shown that the lungs are the first target for oxidative stress. We have used monoclonal anti-8-oxoG antibodies in combination with indirect immunofluorescence microscopy and image analysis to follow the relative amounts, distribution, and age-dependent dynamics affecting 8-oxoG levels in genomic DNA from OXYS and Wistar rat liver cells. Wistar rat liver tissue was used as control. We have shown that 8-oxoG increases with age in both strains of rats, with OXYS rats always displaying higher levels of oxidative DNA damage than Wistar rats.

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ANALYSIS OF THE CYTOMEGALOVIRUS INFECTION IN HUMAN PROSTATE AND PROSTATE CARCINOMA TISSUES

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The interaction of the human cytomegalovirus (HCMV) with the infected cell remains to keep many questions. Recent investigations have revealed a high frequency of HCMV in tumor cells but failed to show that HCMV can transform human cells to malignancy. At the same time, it has been shown that HCMV increase resistance to apoptosis in some cancer cell lines. So, a special term oncomodulation has been introduced to express CMV ability to modify tumor cell biology.

We aimed to study specificity of CMV infection vs. other members of Herpesvirus family in prostate tissue of patients with prostate cancer.

Pairs of tumor and adjacent normal prostate tissue specimens from 16 patients were collected in a course of radical prostatectomy at the Department of Urology of the CRIRR. Viral DNA of CMV, human herpes virus 8 (HHV8) was detected by PCR. Our analysis has revealed positive CMV signal in 11 specimens from 16 patients. For 5 patients, positive CMV signal was detected both in tumor tissue and normal tissues. For 3 patients, positive CMV signal was found only in normal tissue. For remaining 3 patients, positive CMV signal was found only in tumor specimens. Only one specimen of 16 tested was HH8-positive. All examined tissues were HHV1-, HHV2-, and EBV-negative. Additionaly prostate cancer and normal tissues of extra 3 patients were examined simultaniously with there blood specimens. Only one specimen of tumor revealed HCMV DNA. All blood specimens were CMV-negative.

These results reveal high frequency (specificity) of CMV infection of prostate tissue of prostate cancer patients with nearly absence of other investigated herpesviruses.

Association of CMV with tumor or/and normal prostate cells will be further investigated employing immunohistochemistry and *in situ* hybridization techniques. The connection of infection of prostate tissue by HCMV with genotype of patients and the success of treatment will be examined.

THE ROLE OF THE YEAST *HSM3* GENE IN UV-INDUCED MUTAGENESIS

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Mutation in the *HSM3* gene, controlling one of the mismatch repair pathways and acting in slowly dividing cells, increased the level of both spontaneous and UV-induced mutations in the yeast *Saccharomyces cerevisiae*, but this mutation did not alter the sensitivity to the lethal action of UV-irradiation. Some mutations in the genes controlling three principal pathways of damaged DNA repair can alter the level of UV-induced mutations, and consequently, it is concluded that the products of these genes take part in UV-induced mutagenesis. To clarify the possible role of the *HSM3* gene in the control of UV-induced mutagenesis, we studied the interaction of the mutation *hsm3-1* with mutations blocking excision repair (*rad1, rad2, rad4* and *rad14*), error - prone repair (*rev3, srs2*), mismatch repair (pms1) and recombination repair (*rad54*). For this purpose the frequency of appearance of UV-induced Ade$^-$ mutations in 5 *ADE* loci was measured in the double mutants. UV-induced mutagenesis was completely blocked in the *hsm3 rev3* strain, showing epistasis between *rev3* and *hsm3* mutations. The double *hsm3srs2* mutant did not show UV-induced mutagenesis, although single srs2 showed the normal level of induced mutagenesis. Triple *hsm3srs2pms1* mutant is characterized by wild type level of UV-induced mutagenesis. These data show that mismatch repair take part in regulation of induced mutagenesis. The *hsm3* mutation epistatically interacts with *rad54*. These data show that error-prone and recombination repair pathways supply substrates for the repair controlled by the *HSM3* gene. The double mutants *rad1 hsm3* and *rad2 hsm3* are characterized by very high levels of induced mutagenesis. To a lesser degree this effect is observed for the single *rad* mutants and double mutants *rad4 hsm3* and *rad14 hsm3*. On the basis of the data obtained, we developed a model for the appearance of mismatched bases in the process of the repair of UV-induced DNA damage and determined the role of the *HSM3* gene in UV-induced mutagenesis.

CHARACTERIZATION OF ISOZYME COMPOSITION OF THE *PICHIA METHANOLICA* MUTANTS WITH DECREASED ALCOHOL OXIDASE ACTIVITY

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Methylotrophic yeasts *Pichia methanolica* are the traditional object for studies on the enzymes of primary metabolism of methanol, ethanol and glycerol. Promoters of peroxisomal enzymes are widely used for heterological expression of hybrid proteins. In *P.methanolica*, the synthesis of AO being regulated by constitutive and inducible genes, AUG1 and AUG2, respectively. This leads to formation of nine AO isoforms with different affinity to methanol (Km) and regulatory pattern of their expression.

We have characterized izozyme composition of *P. methanolica* mutants with lowered activity of AO being resistant to allyl alcohol. Based on complementation and recombination analyses these mutants were preliminarily divided into 8 groups. Also, on the basis of ABTS qualitative test on AO activity the mutants were divided into two major groups (I and II) including one and seven groups of complementation, respectively. In wild type (WT) strain, the activity of AO was found only during growth on methanol. The mutants of group I were differed by the absence of the AO activity when grown on methanol, ethanol or glycerol or their mixture. Alternatively, in the mutants of the group II the AO activity was found during growth on all these carbon sources and was accompanied by decline of catabolic repression induced by ethanol. To understand the genetic control of regulation of the AUG1 and AUG2 expression the analysis of the AO isozymes was performed in 22 segregants, prototrophs and ade1 and ade2 auxotrophs of the mutants representing all 8 groups of complementation. The differences in AO isozymes composition of the mutants of I and II groups were revealed. Based on these results a system for genetic control of activity of two AO genes is presented by the only gene for constitutive AUG1 and at least by 7 genes for inducible AUG2. Our data imply that these mutants are deficient in genes responsive for positive regulation of AO synthesis that is first described.

GENETIC STRUCTURE AND CLONAL DIVERSITY OF TULIP ON THE URALS

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Tulipa riparia was separated from *T. biebersteiniana* (Knyasev et al, 2001) on the South Urals on the basis of various characteres. Predominance of pink flowered plants, triploid level and sterility were among them. We have done preliminary estimation of ploidy and male-female fertility in different populations of *T. riparia* (Kutlunina et al, 2003). In some populations two different level of ploidy was shown: 2n=24 – diploid and 2n=36 – triploid, other populations were only triploide. The formers demonstrated considerable variability in male-female fertility, while the latter had rather low values. We supposed two different hypotheses to explain this phenomenon. According to the first, populations with mixed ploidy and flower color include two different species: *T. riparia* with pink flower and *T. biebersteiniana* with yellow flower. Otherwise *T. riparia* consists of triploid and diploid individuals. In this study we examined genetic structure of one population of *T. riparia* with pink and yellow flowers on Kuryak River by analyzing a pattern of allozyme variation. Allozyme diversity was determined via vertical PAAG electrophoresis. Gels were stained for 10 enzyme systems. A behavior of allozymes was typical of that observed for triploid organisms. But two clearly different clones with pink and yellow flowers was separated. Some of pink flowered plants from the same clone grow in 100 m one from another and are isolated with bushes. It suggests the great role of vegetative propagation in *T. riparia* and the main role of the river in spreading of bulbs. In addition chromosomal analysis was used to determine level of ploidy. All samples of this population with both pink and yellow flowers are triploids (2n=36). Karyotype can be divided into three sets in 12 chromosomes, 24 chromosomes from 36 form paires, the rest differ from them significantly. Differential staining of chromosomes using GC-specific fluorochrome Chromomycin A_3 and AT - specific fluorochrome DAPI was made to confirm or disprove autopolyploid nature of *T. riparia*. The first and second sets have similar banding patterns wile the third differs greatly. So banding pattern and specific morphology show gybrid nature of *T. riparia* that differs with data of other authors (Bochantseva, 1962, Danelia, 1989) who assume that autopolyploidy is the reason of polyploidy in the genus *Tulipa*.

MOLECULAR BASIS OF HYPER-RECOMBINOGENIC ACTIVITY OF HOMOLOGOUS RECOMBINASE RecA IN BACTERIA

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The activity of RecA family recombinases is regulated at multiple levels. One level of regulation involves a programmed autoregulation of recombinase function. RecA family recombinases have a robust DNA pairing activity, but this activity appears to be suppressed to different extents in different species. In *Escherichia coli*, a relatively low frequency of recombination exchanges (FRE) is predetermined by the activity of RecA protein, as modulated by autoregulation as well as other factors including RecF, RecO, RecR, RecX and DinI proteins. Under stress conditions, resulting in SOS function induction, the FRE activity of RecAEc can be temporarily increased up to 17 times. Moreover, a temporary maladjustment of RecAEc activity is able to achieve such an abnormal level of hyper-recombination as a 50-fold FRE increase in an SOS-independent manner.

The RecA protein from pathogen *Pseudomonas aeruginosa* (RecAPa), being evolved in a cellular environment subject to higher levels of DNA damage, exhibits a more robust recombinase activity than its *E. coli* counterpart. Low level expression of RecAPa in *Escherichia coli* cells results in constitutive hyper-recombination (a 5-fold increase of FRE) even in the presence of RecAEc. Biochemical and structure-functional characteristics of RecAPa relative to RecAEc are in a logical agreement with the in vivo properties of these proteins. These and other data indicate that a 5-6-fold FRE increase is a threshold value in the alteration of RecAEc native characteristics. Overcoming this value results in accumulation of genome mutations to suppress the excess of constitutive hyper-recombination.

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INSTABILITY OF MICROSATELLITE LOCI IN PARTHENOGENETIC LIZARD *DAREVSKIA ARMENIACA* (LACERTIDAE)

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In this study, multilocus DNA fingerprinting was used for analysis of germline and somatic microsatellite instability in families and tissues of parthenogenetic Caucasian rock lizards, *Darevskia armeniaca*. DNA fingerprinting of 43 parthenogenetic families (overall 131 siblings), from different isolated populations of Armenia, with the use of $(GACA)_4$, $(GGCA)_4$, $(GATA)_4$ and $(CAC)_5$ oligonucleotides as hybridization probes, revealed mutant fingerprinting phenotypes in siblings. In three cases with the use of $(GATA)_4$, $(GACA)_4$ and $(GGCA)_4$ probes, mutation frequency was 12.98 %, 14.5 (%) and 17.56 % per sibling respectively. The mutant fingerprint phenotypes detected in siblings were also present in population DNA samples. In the most cases, mutant DNA fingerprints of all the siblings in the each brood are the same and differ from their mother ones. The observation of mutant fingerprint phenotypes in lizard families of *D.armeniaca* resulting from cooperative changes of several restriction fragments for all siblings of a single brood suggests a germline rather than somatic origin of mutations. Surprisingly, the same mutation at $(CAC)_n$ locus was detected in most of all lizard's progeny studied. In this case, somatic origin of the mutation was demonstrated in the course of different tissues examination of adult lizards. The results of this study provide the first evidence for existing unstable microsatellite loci in genome of parthenogenetic lizards *D.armeniaca*. These findings directly indicate that germline and somatic mutations of unstable microsatellite loci comprise an important source of genetic variation in the parthenogenetic populations of *D.armeniaca*.

APPLICATION OF MOLECULAR-CYTOGENETIC METHOD FISH (FLUORESCENCE *IN SITU* HYBRIDIZATION) IN PRE- AND POSTNATAL DIAGNOSTICS

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Introduction of molecular-cytogenetic method FISH (fluorescence *in situ* hybridization) in the practice of pre- and postnatal diagnostics in Armenia allowed to increase essentially the quality and reliability of cytogenetic analysis. Application of this sensitive technique permits to realize additional retrospective investigations of the patients, whose karyotypes, previously analyzed by the methods of conventional cytogenetics, continued to remain unspecified. On the base of conventional cytogenetic analysis of 168 patients of Armenian population, directed to the medical-genetic consultation at Research Center of Maternal and Child Health Protection in the period 2000-2004, 38 cases were selected for molecular-cytogenetic analysis of complex chromosomal anomalies. Analysis of 26 cases was performed in collaboration with Institute of Medical Genetics, University of Zurich. 8 cases are included in European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations (ECARUCA). In the quarter of cases cytogenetic diagnosis, performed on the base of conventional cytogenetics, needed in specification. Results of FISH-analysis confirm the results, obtained on the base of conventional methods, only in the half of cases. In the other cases cytogenetic diagnosis was in need of specification or review. In the complex cases of chromosomal anomalies for the improvement of cytogenetic diagnosis it is necessary to combine conventional cytogenetic methods with FISH, taking into account also data, received from clinical investigations. On the base of molecular-cytogenetic analysis data the recommendations for the medical-genetic practice are presented. They include algorithms of DNA-probes application for different types of chromosomal anomalies, recommendations for analysis by FISH and algorithm of consecutive molecular-cytogenetic analysis of cells, allowing to differentiate mosaic and non-mosaic forms of analyzed cases.

INFLUENCE OF ANTITUMOR PREPARATION ON DNA OF SARCOMA 45

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Antitumor preparation Netropsin specifically interacts with AT-steams of DNA by non-intercalation method placing in narrow groove [1]. As the primary and possibly the secondary structure of DNA is changed at tumor transformation of the cell, the influence of Netripsin on DNA extracted from tumor of Sarcoma 45 and liver of healthy animals was experimentally investigated on the animals. Netropsin and Deksametazone were intraperitoneal injected into the organism in isotonic solution of NaCl in optimal therapeutic dozes. Netropsin of "Farmitalia" Company was used. About possible changes in the structure of DNA of Sarcoma 45 and liver under the influence of Netripsin we can judge by melting curves of DNA. The melting curves were obtained with spectrometer Unicam SP-8-100 at continuous heating of DNA solutions at a speed of $0{,}25^0$C/min.

The experimental data show that under the influence of Netropsin the shape of melting curve and melting parameters of DNA of Sarkoma 45 undergo certain changes. Without getting deeper in the essence of observed changes in the melting parameters and the shape of melting curve of DNA tumor and liver of healthy rats, the influence of Netropsin on the structure of DNA was investigated based on the character of changes of the investigated parameters. As it was expected, Netropsin is toxic. Besides tumor it has influence on the other organs as well. About toxicity we can judge by the influence of DNA liver. According to the character of changes of melting parameters of tumor DNA we can conclude that Netropsin is less toxic in combination with Deksametazone and "better restores" the characteristics of DNA structure.

CLONAL DIVERSITY AND GENOME INSTABILITY IN PARTHENOGENETIC LIZARDS OF GENUS DAREVSKIA

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Caucasian rock lizards of genus Darevskia *(D.unisexualis, D.dahli, D.armeniaca, D.rostombekovi, D.bendimahiensis and D.saphirina)* are truly parthenogenetic, all-female, meiotic, diploid species arisen by hybridization between different bisexual parental species. As unisexual lineages, parthenogenetic lizards are characterized by some level of clonal diversity of allozyme loci and exceptionally low mt-DNA sequence diversity. Multilocus DNA fingerprinting was used to study genetic variation of some mini- and microsatellite DNA markers in parthenogenetic populations of the species *D.unisexualis, D.dahli, D.armeniaca* and *D.rostombekovi* from Armenia. It was shown that parthenogenetic Darevskia lizards possess species-specific DNA fingerprinting patterns revealed with all used mini- and microsatellite probes, but they also shows some level of intraspecific variation, which was detected with microsatellite probe of different types ($(GATA)_n$, $(TCC)_n$, $(GACA)_n$, $(TCT)_n$). DNA fingerprinting in parthenogenetic families has shown that mutations arising as DNA replication and/or DNA repair errors, are responsible for microsatellite variability. To understand the molecular basis of microsatellite variability it is important to know detailed structure of microsatellite alleles and their possible turn over. Genomic library of *D.unisexualis* was constructed and clones, containing di-, tri- and tetranucleotide microsatellite motives, were screened, sequenced and analyzed by PCR amplification. PCR products produced from polymorphic microsatellite loci were also sequenced and allelic variants of these loci were revealed. We found that allelic differences of these loci were caused by variation in a number of tandem repeats in microsatellites clusters and point mutations in the flanking regions. This information is significant for understanding of genetic variability in parthenogenetic lizard species and for study of phylogenetic relationship in unisexual and related bisexual species of genus Darevskia.

MONITORING OF GENOTOXICITY OF WATERS FROM RIVERS OF ARMENIA BY TRAD-SHM AND TRAD-MCN TESTS

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The genotoxicity of water specimens from some rivers of Armenia were investigated on the plant Tradescantia (clone 02) model. The specimens of waters from 25 locations of rivers Razdan, Getar and Marmarik (large inflows of river Razdan) and Sevjur (the left iflow of river Araks) flowing through the densely populated and intensively polluted areas of Armenia were studied. Tests of somatic mutations in stamen-hairs (Trad-SHM) and clastogenicity by micronucleus induction (Trad-MCN) were applied. To estimate the genotoxic action of water samples the cuttings of Tradescantia plants with flower buds were plated in chambers with tested water samples for 24 hours (18/6 hours day time/night cycle). After the seven-day regenerative period the pink mutational events were detected by standard technique. For application of Trad-MCN test after processing of cuttings, without the period of repair, flower buds were immediately fixed in acetoalcohol, stained by acetocarmine, the slides were prepared and the number of micronuclei was counted. The correlation analysis between mutagenic activity and the contents of heavy metals in river waters was carried out. The genetic monitoring of water samples in rivers mentioned performed for three years (2000-2002) revealed the variations of mutagenic and clastogenic activity of analyzed waters are caused by non-uniform distribution of the population, different level of technogenic pollution and seasonal variations.

ANALYSIS OF PIGMENTATION OF MUTANTS ADE1 AND ADE2 WITH LOWERED ALCOHOL OXIDASE ACTIVITY OF METHYLOTROPHIC YEASTS *PICHIA METHANOLICA*

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Collection of mutants methylotrophic yeasts *Pichia methanolica* with lowered alcohol oxidase activity were obtained early. Alcohol oxidase (AO) is a key enzyme of peroxisomes carrying out first stage of methanol metabolism. Mutants were divided into 8 groups (genes) after complementation and recombination analyses.

In this study we have investigated accumulation of red pigment from mutants with lowered alcohol oxidase activity from 8 complementation groups. In all were obtained 19 auxotrofic segregants ade1 (pur6) and ade2 (pur7) from mutants representing all of 8 groups.

Accumulations of red pigment were observed to mutants from all groups and strains of wild type AO on the medium with glucose in concentration 2%. Reduction glucose concentration to 0.4% in medium with adenine leads to loss or slacken pigmentation at ade1 and ade2 segregants from 7 groups. Wild type on AO strains accumulated red pigment on this glucose concentration.

Mutants from 4 groups have unusual "dotted" phenotype after incubation at least 2 weeks on medium with 0.4% glucose. Dotted phenotype characterized as appeared red dots on the surface of white colony. Groups are different in develop of this sign, since all colonies or part of they have dotted phenotype. In the same time segregants from red "dot" have also stable dotted phenotype.

Our data suggest that investigated mutants probably have blocks in regulatory genes which are common for processes formation, transport, folding AO in peroxisomes and formation of red pigment (product of polymerizations AIR and CAIR) in vacuoles.

THE STUDY OF PHENOMEN OF FASCIACIA ON THE ARABIDOPSIS THALIANA

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The study of fasciacia might reveal how meristem structure and function are established and maintained in normal plants. The study phenomenon of fasciacia also supports elaboration the methods rise crop. I crossed mutant 90 (clavata) with green-yellow mutants. I analyzed F_2 generation. (Tab) The fasciacia has had the hybrids mutants 90 (clavata) with yellow-green mutants. The phenomen fasciacia of stem was observed among class plants green with clavata pods. The plants with phenomen fasciacia of stem have been positively correlate between fasciacia of stem and the quantity pods and quantity of seeds in the pods. I examined stable of phenomenon of fasciacia during ten generations. I have five lines with different expressivity and penetrance.

Tab.

Results of screened for fasciated plants on F_2.

Line	Total	Wild type	Green, cla	Yellow, normal	Yellow, cla	X^2	P 9:3:3:1
90x12.6.15	156	105	31(23)	15	5	12,74	>0,05
90xV-76	70	49	14(6)	11	0	7,33	<0,05
90x568/5	116	91	19(10)	1	5	43,33	>0,05
90x58/15	130	93	30(12)	4	3	27,65	>0,05
90x130	126	80	27(3)	12	7	7,21	<0,05

Note: in blacket ( ) number fascinated plants

ESTIMATION OF MUTAGENIC ACTIVITY OF WATERS FROM ARTESIAN WELLS OF ARMENIA

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We studied the genotoxicity of underground waters from nine artesian wells of Armenia from different locations: Aknalich-1, Djrashen, Samahar, Aknalich-2, Djrarat, Zvartnoc, Apaga, Ararat and Surenavan (the depth of artesian wells varies from 40 to 350 m). Tradescantia (clone 02) stamen-hair–mutation (Trad-SHM) and Tradescantia micronuclei (Trad-MCN) tests were applied. It was revealed that all samples of underground waters studied increased the frequency of recessive (pink mutational events - PMEs) and genetically uncertain somatic mutations in Trad-SHM test. The highest level of PMEs (15 times higher than the control level) was induced by water from location Ararat (the depth of artesian well - 60 m), and the lowest – by water from Djrarat (150-160 m) - 1,71 times increase above the control level. With the increase of artesian wells depth the frequency of PMEs is decreased. Except point mutations also different types of morphological changes, including the most widespread - dwarfish hairs, have been registered. Application of Trad-MCN test had also revealed the increase of artesian wells waters genotoxicity, although the increase was much lower. The obtained results suggest that Trad-SHM seems to be more correct and precise approach to evaluate the mutagenic activity of waters than the determination of clastogenicity by Trad-MCN.

STUDY OF REGULARITY OF "TIME EFFECT" CHROMOSOME INSTABILITY IN THE IRRADIATED HUMAN POPULATION

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During 2004 it was studied the cytogenetic status of blood lymphocytes of 23 liquidators of Chernobyl NPP accident after-effects, 18 participants of the accident at "Mayak" Production Association in 1957. The control group consisted of 16 individuals. The participants of Liquidation of Chernobyl NPP accident after-effects were examined in 18 years, and the workers of "Mayak" Production Association were examined 46 years later after the accident. The control group under the age qualification corresponded to the group examined at "Mayak" Production Association. The investigations were carried out according to methods given in the IAEA Technical Report (Vienna, IAEA, 2001, Techn. Reports. Ser.№ 405, 126 pp). The data given in the table signifies that the status of irradiated individuals' caryotype depends on prescription of an irradiation.

Indices / Group	Chromosome aberrations (for 100 cells)	Proliferative activity (number of per 1.2 ml of blood)	Tetraploid cells (for 100 metaphases)
Chernobyl NPP	11,13 ±1,64*	157,6±40,6	0,48±0,16
Mayak	17,73±1,6*	109,5±17,7**	0,33±0,08
Control group	1,8±0,4	221,2±26,6	

where * $p<0.001$, ** $p< 0.05$

The frequency of chromosome aberrations of the "Mayak" P/A liquidators authentically exceeds the frequency of Chernobyl NPP liquidators, and proliferative activity of PHA-stimulated lymphocytes is reducing more than in 2 times, which specifies about the direct dependence of lymphocytes destruction/death on accumulation of chromosomal aberrations.

This phenomenon specifies that, first, the chromosome aberrations in the irradiated organism may be formed de novo (many years later), and, second, on a background of reduction of proliferative activity essentially suffers the cellular immunity that can lead to easing of the control over the process of tumor forming.

INVESTIGATION OF THE ORIGIN OF SMALL SUPERNUMERARY MARKER CHROMOSOMES IN CONSTITUTIONAL KARYOTYPE USING MOLECULAR CYTOGENETIC APPROACHES OF MULTICOLOUR FLUORESCENT IN SITU HYBRIDIZATION (FISH)

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Marker chromosome is structurally abnormal chromosome in which no part can be identified (ISCN, 1995). Small supernumerary marker chromosomes (sSMC) differ depending on chromosomal origin, euchromatic DNA-content, degree of mosaicism, possible of uniparental disomy presence. sSMC occur in about 0.045% of the human population (72/161536 newborn infants) (Liehr, 2004). sSMC characterization is the important task of prenatal and postnatal cytogenetic diagnostics and genetic counseling. In general, 45 cases with sSMC in constitutional karyotype were registered in Belarusian Registry of Chromosomal Abnormalities among the individuals who were cytogenetically examined during 1983-2004 years. Comprehensive molecular cytogenetic methods were used for diagnostics of all available cases: M-FISH, cenM-FISH, microdissection of sSMC with subsequent reverse painting, acro-cenM-FISH, subcentromeric BAC probe using, etc. Data of the origin, structure and mosaicism status of 20 prenatal/postnatal sSMC cases are presented. The great variability of clinical symptoms in our patients is the result of the difference in the genetic content of markers. Six phenotypically normal patients had reproductive failure. In 40% of cases marker is originated from chromosome 15. Inverted duplication is the prevalent mechanism of marker formation (65%). We present also derivative chromosome as the result of reciprocal translocations in parental karyotypes. Recently the phenomenon of neocentromere formation was discovered in human chromosomes. Neocentromere is new analphoid centromere that appear in chromosome localization other than that on the original centromere. We described the extraordinary case of non-mosaic neocentromeric microchromosome 12 in a girl with partial Pallister-Killian stigmata. Two cases of 15q11-13 tetrasomy (SNRPN region) were discovered. The "silent" marker in adult healthy male with extra r(21) containing subcentromeric euchromatin was shown. The application of new molecular cytogenetic approaches reveals the identification of origin, structure, DNA-content of sSMC, discover the mechanisms of formation and genotype-phenotype correlation.

FROM DNA CONTEXT-DEPENDENCE OF MUTATIONS TO MOLECULAR MECHANISMS OF MUTAGENESIS

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Mutation frequencies vary significantly along nucleotide sequences such that mutations often concentrate at certain positions called hotspots. Mutation hotspots in DNA reflect intrinsic properties of the mutation process, such as sequence specificity, that manifests itself at the level of interaction between mutagens, DNA, and the action of the repair and replication machineries. The nucleotide sequence context of mutational hotspots is a fingerprint of interactions between DNA and repair/replication/modification enzymes, and the analysis of hotspot context provides evidence of such interactions. The hotspots might also reflect structural and functional features of the respective DNA sequences and provide information about natural selection. I will discuss analysis of 8-oxoguanine-induced mutations in pro- and eukaryotic genes, polymorphic positions in the human mitochondrial DNA and mutations in the HIV-1 retrovirus. Comparative analysis of 8-oxoguanine-induced mutations and spontaneous mutation spectra suggested that a substantial fraction of spontaneous A•T > C•T mutations is caused by 8-oxoGTP in nucleotide pools. In the case of human mitochondrial DNA, significant differences between molecular mechanisms of mutations in hypervariable segments and coding part of DNA were detected. The observed differences suggested that a higher rate of mutations in HVS regions is caused by intrinsic properties of mutations. Analysis of mutations in the HIV-1 retrovirus suggested a complex interplay between molecular mechanisms of mutagenesis and natural selection. Mutation specificity for GG sequences, which is frequently a part of the TGG tryptophan codon, results in frequent generation of a TAG nonsense codon which leads to a premature termination of protein synthesis. This might be a genetic strategy to kill the virus, and the hotspot specificity is determined by its biological role.

MOSAICISM IN CONSTITUTIONAL KARYOTYPE-CYTOGENETICAL AND CLINICAL CHARACTERIZATIONS

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"Mosaicism" in constitutional karyotype is actual problem of clinical genetics and counseling. Affected persons demonstrate variability of phenotypic expression and need genetic prognoses. Patients with phenotype abnormalities were counseled and investigated using GTG-banding and FISH methods (lymphocytes). 175 carriers (130 female / 45 male) of sporadic mosaic aberrations were found among aberrant karyotypes during 1983-2003 years. We studied "mosaicism" phenomenon of the spectrum of mosaic aberrations and phenotype's expression. **Cytogenetical data** include mosaic numerical (88 cases), structural (18 cases, including 6 cases of marker chromosomes) and combinated rearrangements (69 cases). Sex chromosomes abnormalities comprise 74.3% of aberrant karyotypes; aberrations of autosomes - 18.9%; "gonosome-autosome" variants and markers - 6.8% of cases. Mosaic gonosomal abnormalities combined with balanced rearrangements (inv(9); inv(X) were identified in 2% of cases. Karyotypes with 2 (96% of cases); 3 (3,4% of cases) or 4 (0,5% of cases) aberrant cell lines in single karyotype including combinations of clones with normal and aberrant karyotype and clones displayed abnormal karyotype only were registered. **Phenotype:** *gonosomes' abnormalities* manifested by growth/sex development delay, hypogenitalism, amenorrhea, azoospermia, infertility, reproductive failure, miscarriges. Turner syndrome prevails among affected females (104 patients); 5 cases of polysomy X, including pentasomy X were found. 9 males presented Klainfelter syndrome and 2 – polysomy Y. Patients with structural gonosome's abnormalities manifested different sings of hypogonadism. *Autosomes' abnormalities:* 24 cases presented Down syndrome phenotype. 20 patients with unbalanced rearrangements of autosomes and markers showed multiple congenital malformations/mental retardation (MCA/MR) complex. *Combined "autosome-gonosome" mosaic aberrations* presented MCA/MR and hypogonadism. **Conclusion:** Carriers of mosaic abnormalities demonstrated wide spectrum of aberrations and considerable variability of clinical effects from normal or mild through severe affected persons (depending on type of aberrations, chromosome involved and rate of aberrant cell lines). Rare mosaic variants and "karyotype-phenotype" correlations will be presented in details.

STRUCTURAL FEATURES OF LIPID LIQUID CRYSTALLINE FORMATIONS OF BLOOD ERYTHROCYTES BY DIFFERENT STAGES OF PATHOLOGIC PROCESSES

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Mesomorphism of lipid liquid christalline formations in biological membranes leads to appearing of new characteristics of membranes and conditions of pathologic processes, as the liquid christals of lyotrop type in biosystems are functional environment, providing the proceed of life activity processes.

This report represents the results of investigations of structural characteristics of lipid bilayers, selected from blood erythrocytes of healthy people and also sick people in different stages of disease.

The research of submolecular formations of phospholipid erythrocite (PhlE) –water system by means of polarization macroscopy has shown independent of domenic system organizations on plenty of water. The dynamic of disclination development, the degree of christallisation, submolecular structure of liquid christalline mesophase in norm and in different stages are established.

The morphological submolecular investigations show, that in PhlE –water system there appears liquid christalline phase, where molecules of phospholipids form domens, which contain inclined structure of layers. During disease a change of structure of liquid christalline submolecular formations of phospholipid are observed. To find out the changes of molecular structures, the models were subjected to X-ray analysis.

The dependence of liquid christalline mesomorphic molecular structural transformations in a given system on amorph – christalline characteristics of total phospholipid membran of blood erythrocites are estimated.

MYOTROPIC MESOMORPHISM OF POLYCOMPONENT LIQUID CRYSTALS

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The qualitative and quantitative change of set of components of lyotropic liquid christals (LLC) has its influence on hydrophilic and hydrophobic balance of interactions. It causes good conditions for polymorphic transformations to be created, which on its turn determines possibilities to operate structures and properties of LLC. We represent the results of X-ray and morphology investigations of molecular and submolecular packings of polycomponent lipid formations of pentadecisulphonate natrium (PDSN)–water, ganglioside (G) -water, ganglioside – cerebroside (GC) - water, ganglioside – lecithin (GL) – water systems, which differ by their size, structure and molecular mass.

Characteristic parameters of structural formations and their dependence of density, the degree of compactness of hydrophobic nucleus, thightness of lamel are determined. The coexcistence of lamel structure of interchanging parallel layers of PDSN of mono- (L2 phase) and bimolecular (L1 phase) thickness and water – layers are estimated. It has been shown, that such kind of system is in gel condition. Under the influence of heat treatment the system turns into coagel condition, in which the hydrocarbon chains of PDSN molecules are in rectangular centered packing.

The investigations of G-water system made it possible to reveal mesomorphism in this system. It has been established, that C and L, being included in lamel of G-water system, decrease the degree of micelle swelling in relation to water and stabilize liquid christalline mesophase.

ALTERATIONS OF 5S rRNA GENES IN *TRITICUM-AEGILOPS* ALLOPOLYPLOIDS

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Synthetic polyploids represent a convenient model system to study early events in the formation of polyploid genomes, allowing to determine precisely the timing and processes of genome changes. To date the extensive genomic alterations occurred immediately after new polyploids formation were found. Some of the alterations, occuring early in allopolyploid formation, probably play an important role in successful allopolyploid genome stabilization, and its' investigations may be useful for understanding the plant genome functioning. It was found in a number of natural allopolyploids that the entire arrays containing 5S rRNA genes of one of the progenitors have been completely lost or replaced by the genes of another progenitor. We tried to determine whether this alterations resulted from allopolyploidy formation or they occur independently on the following stages of polyploid genome evolution. We analyzed the three newly synthesized allopolyploids of *Aegilops* and *Triticum* as a model system to study 5S rRNA genes organization in the early generations after polyploidization. Using PCR analysis, Southern-blot assay and comparing primary structures of fourteen 5S rDNA sequences cloned from allopolyploid *Triticum urartu* (TMU38) x *Aegilops tauschii* (TQ27) and corresponding parents we found the changes in 5S rRNA genes organization in the allopolyploid, namely, the increasing of relative amount of 5S rDNA sequences, specific for *T.urartu*. Analyzing different allopolyploid generations (S_2 - S_5) we showed that quantitative reorganization occured early in polyploid formation and held in the next generations. This work was supported by grants from INTAS (01-0537).

PARALOGS OF Rad51 PROTEIN FAMILY FROM *Chlamydomonas reinhardtii:* RECOMBINATIONAL CHARACTERISTICS

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Unicellular green microalga *Chlamydomonas reinhardtii* is a promissing model for basic and applied research. However, little is known about its system of homologous recombination underlying recombination repair of double-strand DNA break. Sequencing of *C. reinhardtii* nuclear genome has revealed many repeats which account for a low level of nuclear homologous recombination compared to that of nonhomologous recombination. Analyses of the *C. reinhardtii* EST (expressed sequence tag)- and genome libraries made it possible to reconstruct and clone cDNA of the *RAD51, RAD51B and RAD51C* genes. In this work, these cDNAs were expressed, their slightly modified products (CrRad51, CrRad51B and CrRad51C, respectively) with 6His-tag at their C-terminal ends were purified and the main biochemical activities were studied. The recombination strand exchange reaction measured in a real-time fluorescent format as exchange between a short oligonucleotide and a short double-stranded DNA molecule showed the following order in the DNA-transferase activity: CrRad51 >CrRad51B> CrRad51C. Interestingly that activity of the mixture of these three proteins was a two-fold higher than a total activity of all three proteins alone. The data suggest a possibility of complex formation between some proteins in this mixture that results in activation of the strand transfer reaction. The results show that Rad51 paralogs from lower eukaryote *C. reinhardtii* are identified as typical representatives of the Rad51-like proteins of higher eukaryotes.

The research was supported by Intas (grant 2001-0069) and Program of the Presidium of RAS "Dynamics of Plant, Animal, and Human Gene Pools"(2004-2005).

HSP70 GENES OF *D.MELANOGASTER* ARE "HOT SPOTS" FOR P ELEMENT INSERTIONS

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Drosophila hsp70 genes (especially promoter regions) were hypothesized to be "hot spots" for transposable element (TE) insertions. It was shown that several natural populations of *D. melanogaster* contain TE insertions in promoters of *hsp70Ba* and *Bb* (87C1 locus). We analyzed the frequency of P element-based constructs insertions in the genes *hsp70Aa* and *Ab* (87A locus) using *D. melanogaster* model system. Two laboratory strains which carried P element-mini-white construction at appropriate locations (5-8 kb far from *hsp70Ab*) were crossed *en masse* to a transposase source stock, and local transposition events were detected by eye color pigmentation and subsequent Southern-blot analysis and PCR screening. We screened 375 strains with first consruction (starting element located 8 kb from *hsp70Ab*) which contain additional insertion in the same chromosome as a starting element. 8,2% of these insertions were restricted to promoter regions of *hsp70Aa* and *Ab*. All insertions were localized by sequence analysis. 60% of these insertions are located upstream (-113bp) from transcriptional start, and the rest 40% were located in different positions of *hsp70* regulatory region within 44-256bp interval upstream from transcriptional start. Analysis of second construction (starting element located 5 kb from *hsp70Ab*) confirmed our results. The high frequency of P element insertions into *hsp70* promoters indicate that these regions are hot spots for P element insertions. Basing on thermotolerance experiments exploring the transgenic strains we speculate that such insertions may play an important role in the evolution of *hsp70* genes and in organism adaptation to thermal environment.

AGE-ASSOCIATED CHANGES IN MARKERS OF OXIDATIVE STRESS IN RATS WITH INHERITED OVERGENERATION OF FREE RADICALS

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An inbred rat strain (OXYS) was derived from Wistar rat (Albino, *Rattus norvegicus)* stock by selection for the susceptibility to a cataractogenic effect of a galactose-rich diet and siblings mating of highly susceptible animals. The main characteristic of the OXYS strain is the inherited overgeneration of free radicals, lipid peroxidation, protein oxidation, and pathological conditions paralleling several human degenerative diseases. We have determined age-dependent levels of activities removing 8-oxoguanine, hypoxanthine and uracil from DNA in liver cells from OXYS rats in comparison with those of control Wistar rats. Our results suggest an induction of 8-oxoG, uracil and hypoxanthine specific repair pathway with age in both types of rats. The levels of 8-oxoguanine DNA glycosylase/AP lyase activities in nuclear extracts of both strains of rats are comparable and approximately tenfold higher than in mitochondrial extracts. On the contrary, 8-oxoguanine DNA glycosylase/AP lyase activity in mitochondrial extracts of OXYS was remarkably higher than that of Wistar old rats, and a significant increase of this activity occurs earlier in OXYS than in Wistar rats. The activities of antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase were also estimated. A complicated enzyme-specific pattern of age-dependent changes in the activities of antioxidant enzymes was observed in cytosol and mitochondrial extracts. Dietary effects of some antioxidantes on levels of protein carbonyl groups and activities of an antioxidant enzyme, catalase, in cytosol and mitochondrial extracts of liver cells from Wistar and OXYS rats were studied. Long-term uptake of dietary supplements sharply decreased the level of protein oxidation in cytosol and mitochondrial extracts of hepatocytes of Wistar and of OXYS rats. Dietary supplements increased activity of catalase in liver mitochondria of OXYS rats.

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A NOVEL PATHWAY CONTROLLING 6-HYDROXYLAMINOPURINE INDUCED MUTAGENESIS IN *ESCHERICHIA COLI*

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High fidelity of DNA replication is necessary for the maintenance of genetic information. One important mechanism for ensuring optimal levels of mutagenesis is control of the qualitative and quantitative composition of the DNA precursors. A broad variety of mutagenic analogs of natural nucleotides have been identified at the present time. Some of them, for example 8-oxoguanine, are generated endogenously as products of oxidative stress. Others may be environmental pollutants. Here, we have studied the genetic control of mutagenesis by the base analog 6-hydroxylaminopurine (HAP) in *E. coli.* We have shown previously that in *E. coli* the main HAP detoxification system is a pathway dependent on the molybdenum cofactor. Several other mechanisms protecting the cell from mutagenic base analogs have been described in other organisms, but these are not well studied in bacteria. To obtain a better understanding of the different ways of HAP detoxification in bacterium *E. coli*, we have carried out a genome-wide screen for genes controlling HAP-induced mutagenesis using a library of about 30,000 independent insertion mutants in 3 different bacterial strains. Here, we describe one newly found gene conferring HAP resistance in *E. coli.* We show that mutations in the *fre* gene, encoding flavin reductase, increase HAP sensitivity by three-fold in complete medium and by greater than ten-fold in minimal medium. Genetic experiments suggest that *fre* may operate, at least in part, outside the molybdenum cofactor-dependent pathway. In addition, we were able to create a phenocopy of a *fre* mutant: in the presence of lumichrome (an inhibitor of Fre protein) a wild-type strain displays HAP sensitivity comparable to that of a *fre* mutant. The mechanism of *fre*-mediated sensitivity is likely related to certain biochemical functions of Fre flavin reductase. Fre may be involved in providing electrons for the reduction of HAP to adenine by a hypothesized HAP reductase. Alternatively, since *Fre* is known to be an activator of the enzyme ribonucleotide reductase, the HAP sensitivity of *fre* mutants may involve DNA precursor (dNTP) pool disturbances. The detailed mechanisms of the HAP sensitivity of *fre* mutants are currently being investigated.

PHENOGENETIC STUDY OF THE FERTILITY IN THE LINE OF TR'VC'ER'GL'AN ARABIDOPSIS THALIANA (L.) HEINH MULTIPLE LABELLED WITH DIFFERENT SIGNAL GENES

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Phenogenetic study of the fertility on the basis of the indices of seed productivity – weight of 1000 seeds, number of seeds in a pod and total number of seeds per plant was carried out on the experimental model containing 32 lines with different combinations of labeled genes (an – angulosa, narrow extended leaf – 1^{st} chromosomal coupling group; er – erecta – the plant of erectoid type, the 2^{nd} coupling group; gl – glabra, lack of fuzzy, III^{rd} coupling group; vc – viridicaulis - light-green stem, IV^{th} coupling group; tr – triplex – triple pods (fruits), V^{th} coupling group). The limit of changeability is determined according to the weight of 1000 of seeds i.e. min. – 1.07 gram, max. – 2.0 gram. The variation of the seeds' weight in the mutant lines in the comparison with normal (initial race Columbia) in the most cases is statistically reliable. It's worth to note that maximal index of the character under study (2 gram) id registered only for gene *an*. Whereas its combination with other signal genes resulted in the decrease of the weight in 1000 of seeds.

The results of our investigation carry evidence of the fact that in the case of introduction into genotype of *Arabidopsis thaliana* of one, two and even three genes, the mass of the seeds per one plant doesn't change in the comparison with standard. But this rule is broken in the case when genotype of *Arabidopsis thaliana* has more that three mutant genes. Maybe in the case of introduction of four, five or more mutant genes into *Arabidopsis* genotype they cause the changes in the plants' cell homeostasis which prevent to reveal the determined rules.

So we came to the conclusion that phenogenetic study of such complex character as seed fertility makes an important contribution into design of the effective approaches to improvement of the productivity of crop cultures based on the scientific achievements.

Mt-DNA POLYMORPHISM AND MACRO- AND MICROEVOLUTIONARY PROBLEMS OF *TESTUDO* TURTLES

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The terrestrial turstles of the genus *Testudo* are represented by many geographically and reproductively isolated groups what is reason to consider them as a good model system for the studying of population genetics and macrophylogenesis of vertebrate. Polymorphism of mt 12S rRNA gene has been studyed for 71 samples of *T. graeca*, n=35; *T. kleinmanni* (Egypt,n=4); *T. marginata* (Greece,n=2); *T. (Agrionemys) horsfieldii* (Uzbekistan,n=30) and five subspecies: *T.g. ibera* (Georgia,n=2; Azebaijan,n=3; Turkey,n=2), *T.g. armeniaca* (Armenia,n=3), *T.g. nikolskii* (Russia,n=6), *T.g. pallasi* (Dagestan,n=11) и *T.g. terrestris* (Israel,n=8). Based on our results and those received by Alvarez et al. (2000), Van der Kuyl et al. (2002) the phylogenetic relationships of different terrestrial turtles species and subspecies were reconstructed. All species formed 3 main clusters. One of them contains two groups with the first one grouping two *T. hermanni* subspecies and the second grouping two supposed subcpecies of *T. horsfieldii* which we discovered. The second cluster includes *T. kleinmanni* and *T. marginata* samples, while the third cluster gathering together all *Testudo* subspecies consists of 3 subclusters. One of them includes of *T.g. nikolskii*, *T.g. ibera* (Georgia, Turkey, Bulgaria) and *T.g. terrestris*, the other includes *T. g.graeca* (Spain, E. Morocco) with the third grouping *T.g. armeniaca*, *T.g. pallasi* и *T.g. ibera* (Azebaijan) and *T. g.graeca* (W. Morocco). Thus it is possible to suggest the presence of two caucasus evolutionary lines of *T. graeca*. The problems on phylogeny, expansion and time divergency estimation of the Mediterranean Sea, the Caucasus and the Central Asia groups of terrestrial turtles are discussed. The authors would like to thank R. Lapid, T.J. Papefyss, J.F. Perham for the blood samples. This work was supported by the Russian State Program "Leading Scientific Scools of Russia" (grant no. NSh-1995.2003.4), Russian Program "Dynamics of Gene Pools" (project no. 2417-01-2004).

LOCUS-SPECIFICITY OF PARTICIPATION IN GENETIC DIFFERENTIATION OF MOLECULAR-GENETIC MARKERS

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The importance of hexaploid wheat forced the search of markers, which could be suitable for genetic identification of varieties. However the inter-varieties differences on the numbers of genetic-biochemical systems emerged very rarely. Moreover, there are absent the differences on electrophoretic mobility between enzymes, genes of which are three-time repeated and localized in homeologous chromosomes. So, on our data, between 16 wheat enzymes one zone of activity was observed in electrophoretic gels for 7 enzymes; 2 zones – for 8 ones, and only 1 enzyme was presented by 3 zones of activity. The relation between polymorphism of structural genes and biochemical function of their products was observed. The new generations of molecular-genetic markers, such as microsatellite loci or DNA fragments flanked by them (ISSR-PCR markers) allowed receiving the polyloci, high polymorphous spectra. However their interpretation required the preliminary researches. In our researches the three wheat varieties (Kiev awned, Yatran' 60 and Mironovskaya 30) was investigated with the use of ISSR-PCR markers. The strong dependence of amplification product spectra from nucleotide sequences used as primer in PCR was observed. The use as primer microsatellite fragment (TG)9A did not lead to obtaining of any amplification product (amplicon), but similar to it sequence (AC)9T in PCR produced 7 amplicons, the similar ones in investigated varieties. The primers (AGC)6T and (GAG)6C allowed to receive 32 amplicons, 12 from them were polymorphous between varieties. All spectra included zones, which were less sensitive to different conditions of amplification ("major" zones) and those, which not always reproduced at the repeated analyses ("minor" zones). The genetic phenomena was described, when with the using of 7 primers in ISSR-PCR in self-pollinated wheat variety the 49 polymorphous from 131 loci in 20 individual plants were observed (Galaev et al., 2004). The obtained data testified the variability of evolution rate from one microsatellite locus to another ones and the absence of common regularities even for microsatellites with similar nucleotide sequences. Thus, the structural genes, microsatellite loci, their inverted repeats in hexaploid wheat essentially differed from each other on a genetic variability.

DYNAMICS AND FEATURES OF MUTATIONS IN NATURAL POPULATIONS OF *DROSOPHILA MELANOGASTER*

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Monitoring of mutational process in natural populations of *Drosophila melanogaster* enables to make a conclusion about regularity of mutability bursts. The phenomenology of this event is as follows: (1) the bursts of mutations occur only in separate genome loci or in the group of genes with the similar phenotypical manifestation; (2) the bursts of mutations may be local or global; (3) a definite burst continues for 7-11 years; (4) return of a fashion on mutations of a definite gene type is possible; (5) some mutation bursts are based on temporal activation or invasion of a Transposable Elements (TE). Mutability burst of *singed* and *yellow*-mutations in natural populations and their allelespecificity concerning mutability properties are related to activation of different types of mobile elements and *hobo*-element among others. The mechanism of insertional mutagenesis involves interaction between host genome and TE. We propose the model for the study of interactions between insertional mutations and the different components of reparation system. The model is based on generation of flies combining unstable mutations with different *mus*-genes in their genome, leading to the early embryonic deficiency in different reparation enzymes. Unstable alleles with the mutability ranging from 10^{-2} to 10^{-4}, namely, $w^{883\text{-}6}$, $sn^{m859\text{-}2}$, sn^{s99}, $y^{+743\text{-}66}$, $y^{1\text{-}85}$, $y^{2\text{-}717}$, $y^{2\text{-}771}$ and $y^{2\text{-}836}$, were selected for analysis. Our results show that *mus207*, *mus302*, *mus304*, *mus308*, and *mus312* system genes take part in reparation of insertional events. The unidirectional change of mutability of all studied unstable alleles by the particular reparation deficiencies (in different *mus*-genes homozygotes) testify to a non-casual effect of *mus*-genes on the reparation of insertional events. Thus, our data suggest that the processes of genomic reparation are involved in the locus-specific insertional mutagenesis. Obviously, the transposon-generated events induce the DNA reparation pathway controlled by *mus*-genes.

POSSIBLE GENETIC EFFECT OF IONIZING RADIATION AMONG THE OFFSPRING OF BELARUS POPULATION EXPOSED DUE TO THE CHERNOBYL ACCIDENT

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In Belarus, one of the mostly contaminated republics of Former Soviet Union due to the Chernobyl accident, a population based study of over 2 thousand Down's syndrome (DS) cases was performed in the areas contrasting by the level of radiation exposure for the period of 1981 to 2001. The data of Belarus national registry (BNR) of congenital malformations (CM), functioning since 1979 and covering the whole population of the republic (~100 thousand annual births), were used as a material for the investigation. All CM diagnosed within the neonatal period as well as prenatally found anomalies are routinely registered in BNR data base. All prenatally revealed DS cases and over 50% of newborns with DS have cytogenetic confirmation of their diagnosis.

No pronounced adverse effect of chronic low-dose radiation exposure due to permanent residence on the territories with high radionuclide contamination was observed. Significant access of annual DS prevalence at birth was registered only in 1995, when relative risk for defined contaminated area with mean estimated 5-year effective dose of ~12 mSv reached RR=2.26; 95%CI=[1.29-3.97]. Moreover a pronounced increase of monthly DS prevalence was found in Belarus in January of 1987, the highest one for the whole period under study. Thirty one cases vs. 14 expected were registered. The highest increase was found in the mostly contaminated Gomel region (8 cases v.s. 3 expected).

The causes of the increased DS prevalence in 1995 remain unclear. Postponed effect of intensive radiation exposure within the first post-accident period could not be completely ruled out; however, no clear radiobiological explanation could be reveled for the finding. The time of January 1987 DS cluster appearance and its spatial distribution confirm the hypothesis suspecting existence in human, similarly to other mammals, a highly radiosensitive phase of oogenesis, taking place around the time of conception. Thus, the accepted clinical experience to recommend women, panning a pregnancy, to avoid any additional radiation exposure during the period of suspected conception seems reasonable.

DYNAMICS OF THE PLANT POPULATION'S SEXUAL STRUCTURE

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Sexual differentiation is a typical, hereditary and stable feature of plant population. Sexual polymorphism is a one form of the adaptive polymorphism (Timofeev-Resovski, 1964), which is of great importance and is directed to increase a plasticity and stability of populations. The external environments exert great influence to a plant's sexual signs. Sexual polymorphism is well seen in the dioecious species. The monoecious species display it's less and the bisexual are least of all. We have investigated some species from Fabaceae, Lamiaceae, Caryophyllaceae in order to estimate the dynamics of sexual structure in populations of plants. There are three forms of sexual dynamics: a change of the proportion of individuals of different sex, a change of vitality and an increase of the reproductive organs sterilization. The dioecious plants have genetic determination of the sex structure of population and there is crossing between different genotype forms only (cross-pollination). For example, male/female individuals of Melandrium album are characterized with a statistically significant complex of morpho-physiologycal reproductive and vegetative features. The bisexual plants also have genetic determination of sexual structure of population and it's dynamics. For example, the sterilization of the reproductive organs take place in Fabaceae. The external conditions and lethal mutations are the causes of this process in perennial species. The opportunities for both cross-pollination and for spontaneous pollination exist in population of annual clover species. The spontaneous pollination is the second phenomenon, that compensate the absence of cross-pollination. The gynodioecious plants are effectivly adaptated for cross-pollination between the individuals of different sex and they have an opportunity for spontaneous pollination in bisexual plants. In normal conditions the plants of different sex plants vary in flower structures only. The strong vegetative and reproductive heterogeneity arises only in an unfavourable conditions. It caused by the decrease of the vitality of androsterile plants.Thus, the dynamics of the population's sex structure is a specific, stable and adaptive phenomenon. The important consequence is the change of the pollination system, that results in a change of heterozygosis level of population.

RADIOBIOLOGY

INFLUENCE OF MILLIMETER ELECTROMAGNETIC WAVES ON BLOOD SYSTEM

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In the recent years the increased interest of the researchers in electromagnetic waves Influence on organism has been observed, especially millimeter diapason waves. The changes of the blood system morphofunctional indicators under the long-lasting exposure of the millimeter electromagnetic waves was researched (30 days by 2 hours daily). On the 15^{th} day of the experiments the hypochromic shift of erythrocytes and hemoglobin quantity, moderate reticulocytosis, leucocytosis, neutrophilosis with nucleus left shift was observed. By the 30^{th} day normochromic decrease of erythrocytes and hemoglobin quantity, reticulocytopenia and also decrease reticulocytes maturation speed index. The increase of the quantity of basophilic and polychromatophilic erythronormoblasts and decrease of oxyphilic erythronormoblasts was observed in marrow. That brings to reduction of the erythronormoblasts maturation speed index from 0,7 to 0,5. The quantity of leucocytes in that period of time remains within norm. However neutrophilosis, monocytosis and leucopenia were observed in leucocytic formula. The number of mature neutrophiles have increased which proves the acceleration of the maturation process. The prove of this is the decrease of neutrophiles protoplasm maturation index. Hence, observ! ed in the dynamic of long-lasting exposure of millimetric electromagnetic waves on organism, neutrophilosis and monocytosis testifies to increase of organism nonspecific resistance. That fact is caused by the activation of the hypothalamic-pituitary- suprarenal system, which determines intensive release of glucocorticoid s and catecholamines. The mechanism and the ways of influence of glucocorticoid s and catecholamines to blood system are discussed.

THE ROLE OF BIOSYNTHESIS AND CATABOLISM OF PROLINE IN THE CELL METABOLISM IN COMPARATIVE ASPECT

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A through argumentation is given for the fact that the increasing demand for proline results in the induction of nonureothelic arginasa to ensure its biosynthesis from its predecessors - arginine and ornithine.

In this respect the correlation, established by us, between the arginasa activity and the enzymes of proline biosynthesis in the regeneration of the rain warm is unique in case the organism demands for proline, which in its turn is essential in ensuring the protein biosynthesis, mainly of the collagen, and when the regenerating and prolipheratic processes are much strengthened.

It has been established that the organisms in which a low activity of proline enzyme oxidation is observed, this process can be stimulated by citrate and adenylic nucleotides. In the meantime, the organisms that have a higher activity of the mentioned enzymes, on the contrary, inhibit these compounds the following way: citrate and adenylic nucleotides in all the studied organs of sezan significantly stimulate the oxidative process of proline. In the kidneys compound citrate + ATP stimulate the abovementioned process for more than 10 times.

Similar results were detected in pea shoot (Pisum sativum), where the proline oxidation enzymes have reduced activity. Under the influence of these compounds the activity of the enzymes of proline oxidation significantly decreases in beetles and larvae of haricot, where the proline oxidation is 215 times more active as compared with similar processes in rat brain.

Thus proline has a role of energy reserve for insects. It is established that the amount of alanine formed in proline oxidation in haricot equals the amount of proline used. When flying, proline can completely cover the energetic needs of the organism and in this respect it is likely to compete with glucose in insect hemolymphe.

MODELING OF GENOME INSTABILITY BY THE METHOD OF BIOLOGICAL DOSE ACCUMULATION UPON FRACTIONATED γ –IRRADIATION OF LYMPHOCYTES OF CHILDREN AND THEIR PARENTS

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After the accident the ChPP much importance has been attached to the phenomenon of genome instability in the organism of people exposed to radiation – liquidators, their children and residents of territories contaminated with radionuclides. For the purpose of further elucidation of this phenomenon in somatic cells experiments were started on modeling of genome instability by the method of dose accumulation upon fractionated testing in vitro γ - irradiation of peripheral blood lymphocytes of children and their parents examined in the Children's Center of Antiradiation Protection of the RF Ministry of Health. Chromosome aberration were analyzed both in intact lymphocytes and after testing γ –irradiation with Cs^{137} in vitro in G_0 phase .Single doses were 10, 20, 30cGy. Fractionated doses were 10cGy + 10cGy and 10cGy + 10cGy + 10cGy. The blood samples were irradiated at 24h interval. Before cultivation all blood samples were stored at 37°. 5-BDU was added to determine the ordinal number of mitosis. On single exposure to 10, 20 and 30cGy an increase in the frequencies of aberration of all noted types was observed with a predominance of aberration of the chromosomal type (increased frequencies of paired fragments and dicentrics + rings). The dose dependence in the range of 10-20cGy was increasing in character and upon irradiation with 30cGy a slight decrease in the yield of aberrations was observed. It is likely that the repair mechanisms come into action in this case. Upon fractionated γ –irradiation with cumulative doses of 20cGy and 30cGy the spectrum of chromosome aberration was extended and their frequency was increased. Aberration of the chromosomal type prevailed. However upon fractionated irradiation with a cumulative dose of 30cGy, as well as upon single exposure, the spectrum and frequency of both chromosome and chromatid aberrations were slightly reduced. The experiment has demonstrated that at the same irradiation doses (both single and fractionated) the yield of aberration is mitosis I is practically the same. However upon long-term exposure to low doses individual radiosensitivity is observed. Further investigation of dysgenomic effects upon fractionated irradiation in mitoses II and III is required. This experimental model can show that a long-term exposure of the human organism to low doses of radiation can cause a greater dysgenomic tffect than single exposure to high doses.

BACTERIAL EFFECTS OF MILLIMETER WAVES: ON THE ROLE OF WATER

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The study of bacterial effects of the weak extremely high frequency electromagnetic radiation, millimeter waves (MW), is of significance in revealing the mechanism to use these effects in recovery of bacterial diseases as well as in protection of cells from this radiation. The mechanism involves the resonance interaction of this radiation with bacterial cells and could be mediated by the membranotropic action [1].

With a non-coherent sound and weak radiation (53.5-68 gHz, 0.01 mW) different effects on the *Escherichia coli* growth rate those were depending on the growth phase, genetic characteristics of bacterial strains, growth conditions irradiation exposition and the other factors were found [1]. It was shown that separate irradiation (exposition of 10 to 60 min) of distilled water had bactericide effect whereas irradiation of the buffer containing inorganic ions, where bacteria were transferred after the growth in anaerobic conditions upon fermentation of glucose, caused opposite effects changing further growth of bacteria: water irradiation had bactericide effect whereas irradiation of buffer with ions stimulated bacterial growth. A bactericide action disappeared upon increasing irradiation exposition to 2 hours or repeated irradiation [2]. With coherent irradiation (49.0, 50.3 or 51.8 gHz. 0.06 mW) bactericide effect was further shown that was stronger with increasing the frequency (pH 7.5). Irradiation of water (pH 6.0) and of water with higher pH of 7.5 or 8.0 (adjusted with NaOH) led to decrease in pH with maximal effect of 51.8 gHz or 50.3 gHz correspondingly whereas the effect disappeared upon acidification of water (with HCl). Bacteria irradiated in appropriate water solutions covered the effects but not at higher pH.

These results point out the role of water in bactericide action of millimeter waves.

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ASSESSMENT OF INTERNAL DOSE DUE TO INCORPORATED PLUTONIUM-239 BY MEANS OF BIOLOGICAL DOSIMETRY

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Intra- and inter-chromosomal aberration frequency in Mayak PA nuclear workers has been studied by mFISH and mBAND techniques.

It was found that intra-chromosomal aberrations in lymphocytes from the peripheral blood are a biomarker of densely-ionizing radiation (alpha-particles) in workers exposed to radiation many years ago.

A dependence of the frequency of intra-chromosomal aberrations in plutonium workers on absorbed dose to the red bone marrow from internal exposure to incorporated plutonium-239 was found. Thus, the preliminary "biodosimetry system" was developed. Its capability to estimate internal doses from incorporated plutonium-239 in plutonium workers based on the frequency of intra-chromosomal aberrations detected in these workers was tested. Estimates of internal doses from incorporated plutonium-239 obtained using the preliminary "biodosimetry system" were compared with doses calculated by a model based on measurements of plutonium excretion in urine ("Mayak Doses-2000"). Estimates of internal doses from incorporated plutonium-239 obtained using both independent techniques demonstrated a highly significant correlation (correlation coefficient, $R^2 = 74\%$).

At the current research phase, the developed "biodosimetry system" is to be adjusted, which will allow to estimate ^{239}Pu body burden with an uncertainty less than 30%.

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INFLUENCE OF MILLIMETER ELECTROMAGNETIC WAVES OF NON-THERMAL INTENSITY ON THE STABILITY OF AT AND GC NUCLEOTIDE STEAMS OF DNA

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At present, the concept is more acceptable which states that non-thermal influence of millimeter electromagnetic (ME) waves on the biosystems is conditioned by the influence of ME waves on the water which cause the changes in characteristics of bound water.

Usually, during investigation of the influence of different factors on the nucleotide steams of DNA it is necessary to investigate the influence of the given external factor on DNA from different sources with different GC-content. In some cases these differences can be insignificant and lay within the limits of the experiment's mistake. In order to avoid this, it is possible to consider the influence of the investigating factor on the certain part of the same DNA (hence with different GS-content). For that the differential melting curve (DMC) of DNA decomposes into gauss components.Allgaussias on DMC of DNA is considered as certain part of DNA with some average GC-content. Therefore, studying the behavior of these parts under influence of the investigated factors, based on the received data, we can judge about selectivity of the influence on AT and GC nucleotide steams of DNA.

Generator G4-142 was used for irradiation. Irradiation of the samples was carried out at a room temperature. The power of irradiation in the location of sample at the frequency of 64,5gHz was 50mcW/cm^2. This frequency coincides with resonant frequency of fluctuations of hexagonal rings of water molecular structure.

Densitometric measurements showed that density of buffer and DNA solutions increases in consequence of the irradiation. At the same time the melting temperature of the investigated components of DMC DNA also increases during irradiation. The increase in the melting temperature becomes more apparent for AT-rich, more hydrated parts. Hence, ME waves changing the structure of the bound water have different influence on the thermostability of AT and GC nucleotide steams.

RADIATION CYTOGENETICS: THE COLOR REVOLUTION

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Analysis of radiation-induced chromosomal aberrations have long been a powerful tool to understand the mechanisms of radiation action in living cells. The concept of genetic target of radiation, introduced in 1935 by Timofeef-Ressovsky [1], was elegantly used by D. Lea to describe the effects of radiation on chromosomes [2]. Early cytogenetics was based on solid-stained chromosomes, although banding techniques were soon developed for karyotyping human cells. Banding is a complex, error-prone, and time-consuming methodology, especially when applied to radiation-induced aberrations that, unlike genetic syndromes, are randomly induced in the genome and in the cellular population. The "color revolution" occurred in the 80's with the introduction of fluorescence in situ hybridization (FISH) at the Lawrence Livermore National Laboratory [3], and was initially confined to painting of 1-3 chromosome pairs with the same color. More recently, combinatorial multi-fluor FISH (mFISH) [4], high-resolution multi-color banding (mBAND) [5], and rainbow cross-species FISH (RxFISH) [6], have greatly expanded the number of colors, providing an amazing detail in the analysis of inter- and intra-chromosomal rearrangements. The first great improvement provided by FISH-painting was the opportunity to analyze symmetrical, transmissible aberrations (such as translocations and inversions) simply and rapidly, whereas dicentrics had long been the main endpoint analyzed by solid staining. Multi-color painting demonstrated that radiation-induced rearrangements are much more complex than previously thought [7], and even low doses of densely ionizing radiation produce mostly complex-type exchanges. The impact of multi-color painting on the understanding of radiation-induced genetic effects will be discussed here.

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LOW LEVEL RADIATION: CYTOGENETIC AND CANCEROGENIC EFFECTS

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Group of liquidators is the main object in the steadfast studies of the postradiation consequences of the Chernobyl NPP accident as the morbidity parameters in it are 2-5 times higher if compared with the rest population of Ukraine (Serdyuk A., 1998). It is known that the low levels of radiation induce mutations, chromosomal aberrations, which can be the reason for the malignant transformation of cells (Hagmar L. et al, 1994). Parallelism in the frequency of oncologic diseases and radiation-induced chromosomal aberrations in exposured persons was observed earlier (Knight et al, 1993). In this connection cytogenetic study in human peripheral blood lymphocytes (HPBL) as the model somatic cells during solid tumors development is also actual today (Monakhov A.S., 2001). The purpose of the presented study was to estimate the influence of the absorbed dose values on the cytogenetic effects and risk of malignant formation (MF) in group of liquidators. Material and methods. The method of "internal comparison" made it possible to study epidemiological parameters in dependence with the documented exposure doses for 17 thousand liquidators. The cytogenetic analyses for 500 liquidators were carried out on the base of test- system of HPBL. The dose dependence of MF frequency was studied with the application of piecewise-linear splines and hypothesis about the equality of two probabilities on the basis of the statistical criterion *2S*. Results. Tendency toward reduction in the frequency of MF with increase of dose in the interval of 1-85 cGy for both age groups (younger and older than 40 years) was observed. Probability of random event when the liquidators with MF were exposed to dose in the ranges from 1 to 3 cGy or from 3 to 5 sGr statistically significantly exceeds the appropriate probability for the cohort as a whole. Significant differences in the probabilities for other classes of diseases within any dose ranges were not observed. In spite of the remote terms of cytogenetic analysis and partial elimination of dicentric chromosomes from blood only in the group of liquidators with MF dependence for the aberrations "dose- effect" remains. This fact can be explained by insufficient intensity of the anticancer protection of organism against radiation effects in the dose range which includes the elimination of the cells with chromosomal aberrations by immune mechanisms of supervision of the antigenic constancy of organism, reparation etc. Complex of the parameters of chromosomal injuries in HPBL which allows determining individual relative cancerogenic risk in liquidators was developed.

ECOLOGICAL STANDARDIZATION OF ELECTROMAGNETIC CONTAMINATION OF BIOSPHERE

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It is equally important with chemical and radioactive pollution to take into consideration electromagnetic contamination of biosphere. The effects of electromagnetic radiation (EMR) ranging from 8-10 GGc and the energy-flux density (EFD) at 1 mkVt/cm^2 on prokaryote and eukaryote cells have been studied. Cell survivability of wild-type strain *E.coli* WP_2, mutant strain *E.coli hcr⁻ exr⁻* and spontaneous motional activity dynamics (SMA) of unicellular paramecium *Spirostomum ambiguum* have been chosen for observation.

The biological effects revealed in this experiment are probably caused by cooperative processes, based on resonant interactions of biological macromolecules. They are considered to be protein molecules which are part of the membrane structure. According to Frelich's theory explaining non-thermal effects, EMR may cause polar restructure of biomolecules capable of providing high amplitude fluctuations at the resonant frequency due to energy swapping.

It is known that living organism susceptibility to ionizing radiation grows with increase of morphological and physiological complexity. Apparently, protozoa are more susceptible to EMR at SHF-rangers. The established conformity to natural laws has probably been coursed by another circumstance connected with characteristics of environmental properties during biosphere evolution. In particular, it is known that every living thing has evolved shielded by atmospheric ozone from short wave ultraviolet which perniciously affects the majority of living organisms, among which microorganisms demonstrate high susceptibility. EMR with parameters essentially different from EMR of natural origin has found wider application at the present stage of civilization development. These radiations may conceivably affect some organisms and we can attribute these effects to the impact of more habitual and investigated factors. Evidently, studying living organisms susceptibility lines to physical and chemical factors must be the task of ecological standardization.

POSITRON LIFETIME SPECTROSCOPY AND MONTE CARLO METHOD FOR WATER-PHOSPHOLIPID AGGREGATES RESEARCH

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The method of lifetime spectroscopy positron annihilation is an efficient research instrument of the physical and chemical properties of lyotropic liquid crystalline biological structures, as the primary units of the matter organization. The method is based on distinction of lifetimes of a singet state of positronii (*p*-Ps), free positron and triplet Ps state (*o*-Ps) in solution with lyotropic aggregates[1,2]. Positron-lifetime measurements have been made on phospholipid bilayer aggregates of 40% water–DPPC (DL--dipalmitoylphosphatidylcholine) systems in the temperature range 23 to 63 °C [3]. The increase of *o*-Ps lifetime is associated with the pretransition as well as with the main transition of the lipid aggregates. The phase temperature transition may be define in terms of the onset temperature and the differential lifetime methods [3].

Yet both these critical temperatures may be defined by the Monte Carlo technique [4] in the framework of spin models. We have carried out the microcanonical simulations with Metropolis algorithm for the main transition temperature and confirmed the advantages of the positronii annihilation method.

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RELATIVE BIOLOGICAL EFFICIENCY OF ACCELERATED CHARGE PARTICLES

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The work is devoted to the comparative analysis of relative biological efficiency (RBE) of accelerated charged particles for a wide range of energy and linear energy transfer (LET) in dependence on the dose level. The investigation was conducted on the organism's level both in acute and in late period after irradiation. The animal mortality, the cytobiological and cytogenetic indexes in cell systems of mammalian organism *in vivo* and in the culture of donor blood *in vitro*, the frequency of cataractogenic and carcinogenic effects as well as other indexes were considered. Biological objects were irradiated by heavy charged particles with doses from 0.03 to 4.0 Gy. As the standard radiation, gamma-rays ^{60}Co and ^{137}Cs and 180 kV X-rays were used.

The studies show that the RBE coefficients of accelerated charged particles are determined by many factors both of physical and biological nature, the most important among them being LET, radiation dose, reparation capability of cells and their radiosensitivity. It is revealed that in the energy range from 50 MeV to 645 MeV the protons are, practically, not distinguishable, in biological efficiency, from standard radiation. In turn, 9 GeV protons, 4 GeV/nucleon helium ions are characterized by higher RBE coefficients that may be related with the effects of secondary irradiations. It is found that RBE coefficients for radiations with various LET are increased when the radiation dose decreases. The most large values of RBE coefficients are obtained for 300 MeV/nucleon carbon ions at low doses (0.03 – 0.5 Gy). These RBE coefficients, which were determined by analysing the frequency of cataracts in mice in late period after exposure, reach the values of 30.0 – 66.0. The results obtained are relevant in solving the problem of ensuring the radiation safety of long term space missions.

The work is supported in part by RFBR (Grant # 05-04-49573-a).

CONDITIONAL DOMINANT LETHALS IN DROSOPHILA AND THE REMOTE CONSEQUENCES OF RADIATION IN HUMAN

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According to current concepts, the causes of the remote consequences of radiation (malformations, cancer and leukaemias) in humans are gene damage. However, once arisen and inherited, these pathological conditions, do not obey the rules of the inheritance of the dominant and recessive mutations of the classical (Mendelian) genes. The genetic nature of the instability arisen in the irradiated genome is also unclear. There is no doubt that the genetic material is damaged by radiation and that the radiation consequences are of genetic nature. However, it should be suggested that the nature of the above pathology is damage of genetic structures that have not so far studied.

Based on performed studies, it is assumed that the cause of the bulk of genetic pathology resulting from radiation are mutations in the regulatory genes that control ontogenesis (mutations of *ontogenes*).

Radiation-induced mutations of ontogenes (over 100 mutations in the X, autosomes 2 and 3) were discovered in *Drosophila melanogaster*, using the developed methods. On the bases of formal genetics, these are facultative dominant lethals that start to express in the first generation of irradiated individuals. Somatic expression of the mutation is clonal defects of development (*morphosis*). The penetrance of the mutations is incomplete, and their expression is dependent of sex, direction of cross, chromosomal rearrangements in the genome, and others. Mutation formation leads to genomic instability expressed as a number of phenomena: secondary mutagenesis (new gene mutations and chromosomal aberrations), disturbance of chromosome distribution in meiosis (chromosome loss and nondisjunction) and mitosis (mosaics and gynandromorphs), formation of modifications, phenocopies etc.

The detected features of the mutations comply with the appearance of a genetic abnormality pattern of malformations, cancer, and leukaemias and also with genomic instability phenomenon after irradiation.

FUNCTIONAL ACTIVITY OF Ca^{2+}-SENSITIVE K^+-CHANNELS IN ERYTHROCYTES IN DIFFERENT PERIODS POST MULTIPLE EXPOSURE OF THE ORGANISM TO LOW INTENSIVE EMW OF MM-RANGE

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Numerous experimental and epidemiological data testify that the ever-growing exposure of living organisms to non-ionizing electromagnetic waves (EMW) of super-low intensity promote the occurrence of different disturbances in functional activity of cells and organisms, which stimulate development of several sicknesses in humans. Biological membranes present themselves structures, which reveal the expressed sensitivity to both external and internal factors. It is well established that Ca^{2+}-activated K^+ -channels in biological membranes play a critical role in many cell functions, such as regulation of cell volume, cell differentiation, proliferation and apoptose, modulating calcium signaling of cells. Ca^{2+}-activated K^+-channels manifest high sensitivity to the changes of cell environment. In this light the study of functional activity of Ca^{2+}-depended K^+-channels of erythrocytes in animals, which underwent multiple exposure to low intensive EMW of millimetre (mm) range, would provide information concerning the mechanisms of action exerted by non-ionising mm-range EMW to the living organisms. With this aim adult white inbred male rats were exposed to low intensity mm-waves of coherent and incoherent (noise) origin during 5 days for 30 minuts daily. The exposure to coherent radiation was performed with the help of "Yav-1" apparatus with the power of irradiation 10 mWt/cm^2 and emitted EMW with the wavelenght = 5.6 mm. Generator "Artsakh-2" with output power at 0.5 mWt was used as a source of incoherent EMW with 4 – 6 mm wavelenghts. The activity of erythrocyte membranes Ca^{2+}-activated K^+ channels was studied on days 1, 15, 30 and 40 after the treatment course.

It was revealed that multiple exposure of rats to EMW of mm-range resulted in significant changes of the activity of erythrocyte membranes Ca^{2+}-activated K^+-channels as compared with Norm. In case of animal exposure to incoherent mm-waves the dynamics of observed changes had undulating character with the maximal splashes of activity on days 15 and 40. In case of coherent mm-waves, there was revealed gradual attenuation of Ca^{2+}-dependent K^+-channels activity. It is supposed that these changes are revealed due to the long-lasting shifts in Ca^{2+} homeostasis in blood plasma and erythrocytes under the influence of mm-range EMW.

DOSE RATES AND EFFECTS OF CHRONIC ENVIRONMENTAL RADIATION ON HYDROBIONTS WITHIN THE CHERNOBYL EXCLUSION ZONE

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We studied the rate of chromosome aberrations in cells of freshwater snail (*Lymnaea stagnalis* L.) embryos and in the apical meristem of roots of the higher aquatic plant common reed (*Phragmites australis* (Cav.) Trin. ex. Steud.) and arrowhead (*Sagittaria saggitifolia* L.). The samples has taken in different seasons of 1999–2004 in reservoirs within the Chernobyl exclusion zone – Azbuchin Lake, Dalekoye-1 Lake, Glubokoye Lake, cooling pond, Yanovsky Creek, Uzh River and Pripyat River. The chromosome aberration rate was registered by anaphase method. The results of the analyses compared to the data received for hydrobionts from Goloseevo lakes located within Kiev City territory. The absorbed dose rate for hydrobionts, living within littoral zone of the researched water objects, due to external irradiation and radionuclides incorporated in tissue was in a range from 2.5E-04 to 3.4 Gy $year^{-1}$. The highest value was found for hydrobionts from lakes within the embankment territory on the left-bank flood plain of the Pripyat River (Dalekoye-1 Lake and Glubokoye Lake), the lowest – for specimens from the running water objects (Uzh River and Pripyat River). The molluscs from Dalekoye-1 Lake and Glubokoye Lake were characterised by the maximal rate of chromosome aberration – about 20–25 %, that in 10 times exceeds a level spontaneous mutagenesis for hydrobionts. A little bit less rate is registered for snails from Azbuchin Lake and Yanovsky Creek. The chromosome aberration rate of hydrobionts from Goloseevo lakes on average was about 1.5 %, and the maximal rate did not exceed 2.5 %. The maximal aberration rate in roots of higher aquatic plants (7.8 %) has registered in Glubokoye Lake; in plants of Goloseevo lakes this value was about 1.8 %. The cytogenetic research of aquatic biota within the exclusion zone convinces that the organisation of regular genetic monitoring of the contaminated territories is the important measure, extremely necessary for understanding and forecasting of negative remote consequences of long-term irradiation.

ON THE ROLE OF SPONTANEOUS ADDITIONAL SYNTHESIS IN FORMATION OF RADIATION INDUCED ABERRATIONS OF CHROMOSOMES

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The sudden discrepancy between the amount of primary damages in DNA and a final number of chromosomal aberrations is one of a phenomena which was found out during studying of features of the process of chromosomal mutagenesis in eukaryotic cells and cannot be explained from the point of view of classical concepts. Biologically significant dozes of ionizing radiation produce primary damages in DNA, only very insignificant part of which (about 1-2 %) constitutes a non-repaired component (Rydberg, 1985).

One more fact demanding special attention and studying is differential sensitivity of euchromatin and heterochromatin chromosome parts to ionizing radiation action (Slijepcevic P., Natarajan A., 1994). Moreover, distribution of targets for formation of exchange chromosome aberrations (ECA) on the length of a chromosome is also not casual (Thucker J., Senft J., 1994). Besides a number of researchers note a special role of minor fraction DNA, associated with a nuclear matrix, in formation of EAC (Yasuyi, et al, 1996, Ganassy, et al, 1986).

In our own researches which were carried out earlier on animal and plant cells, a specific character of radiosensitizing effect of thymidine analogue 5-bromo-2'-deoxyuridine (BrdU) in various phases of a cellular cycle was found out. It proved to be, that incorporation of BrdU into the minor fraction of DNA (a component, which is no more than 1-2 % genome DNA) in GO, G1 and G2 - phases results to cytogenetic effect (on a criterion of chromosome aberrations), which is comparable with the same in S-phase. Analysis of our own data and other research's outcomes allows to make the assumption that this effect is caused by incorporation of BrdU into DNA within the framework of spontaneous additional synthesis (SAS) DNA, which is topologically connected with nuclear matrix.

In the present work, incubation of PHA–stimulated human lymphocytes with Ethoposyde - an inhibitor of Topo II led to specific character of modifying of ionizing radiation effect by BrdU. Studying the spectrum of aberrations of the chromosomes formed as a result of combined action of BrdU and Ethoposyde in GO, G1 and G2-phases of a cellular cycle, indicate a specific role of spontaneous additional synthesis DNA in forming EAC.

USING TOOTH ENAMEL MINISAMPLES FOR INDIVIDUAL DOSE DETERMINATION BY EPR SPECTROSCOPY

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The problem of determination of the individual absorbed dose is one of the key issue of radiation medicine. Conventional dosimetry methods have several disadvantages which is especially important at individual accidental exposure.

The method of tooth enamel electron paramagnetic resonance (EPR) dosimetry is one of the most sensitive and specific among all main methods of biological indication of ionizing radiation dose. It allows to determine radiation dose to organism after accidental exposure. The most essential limitation of this method is the need for tooth extraction in order to get the dental enamel. This cannot be admitted as expedient action solely for irradiation dose estimation. Use of the part of the enamel taken during dental treatment, and the following restoration of anatomic shape and functions would allow wider application of EPR method in medical practice. Method of taking enamel mini-samples from a person followed by restoration of anatomic shape and functions has been developed. Optimal enamel mini-sample mass is 50,0 ± 10 mg. This value allows not only the determination of irradiation dose with rather high accuracy but also complete restoration of tooth functions by dental products.

We recommend to take enamel samples from lingual and palatine sides of permanent teeth according to their eruption in oral cavity: **6,4,5,7,8** to determine the most accurate value of the dose accumulated during the lifetime. Light-curable fine-dispersed hybrid composites are proposed for the restoration of hard tissues defects. Materials of this group are the most similar to the hard tooth tissues by their physical parameters. Distinctive feature of these materials is the possibility of universal using for restoration of both frontal and masticatory teeth. Three-years follow-up of patients shows that taking mini-samples of enamel during dental treatment by the developed method does not cause pathologic changes of hard tooth tissues and fully ensures its functional recovery.

EVALUATION OF THE DNA STABILITY AND GROWTH PARAMETERS OF BEAN SEEDS OF ARMENIAN NPP LOCATION REGIONS

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The goal of this work is the radiation monitoring of the beans selected from six areas of Armenian NPP location regions was conducted. The bean is known as the accumulator of radionuclides. The choice of regions was determined by direction of the wind rose in this region.

We separated the analyzed territory on three zones: zone I- territory in the radius of 1-3 km from NPP, zone II – territory with radius of 3-5 km, zone III- located on 30 km radius around NPP. For the control we used the bean from region 60 km away from NPP. The specific content of the radionuclides was determined in this bean samples. We investigated the grow physiology of the bean seeds (growth linear-weight parameters analysis). We studied parameters of seeds quality, such as germination energy, germination, yield of empty seeds; this let us to testify the viability of grains.

Our results have shown, that

- zone I - the main growth parameters were decreased, and the yield of empty seeds was increased, compared with control.
- zone II – (at the leeward side of NPP) was detected a low suppression of the grow and slight increase in the yield of empty seeds.
- zone III - the beans sprouts were the same sizes and viability index, as the control.

DNA has been isolated from the dry sprouts. After that, we investigated DNA melting characteristics. It is well known, that this method allows to establish defects of structure (single and double stranded breaks, inter- and intramolecular crosslink's) of irradiated DNA molecules.

The results have shown that the parameters of DNA melting of beans from various zones of NPP practically are not changed in comparison with control.

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THE INFLUENCE OF X-RAYS ON PROLINE HEAP AND PROLINE METABOLISM

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It has been determined, that the quantity of proline in haricot beetles increase during the its ripen. In young beetles, which were exposed to the influense of X-rays, contents of liberty proline is increased by 3 times, at comparison by non- radiated beetles, and in radiated matur (10 dayly) beetles, the quantity of liberty proline is increased only a few. The contents of liberty proline also increase in beetles, whiche are ripen from radiated larvaes.

It has been determined too, that the activity of the enzymes of proline biosynthesis is rised by influence of X- rays, and on the contrary, the activity of the enzymes of proline catabolism is falled.

In a result of X-rays influense during germination of been seeds in buds increases the content of free aminoasids in comparison with non-radiated seeds buds. During germination of bean seeds in different environments, it is established, that there is an opposite correlative connection between free prolin quantity and intensity of buds growth.

Vitamin E fully overwhelms the growth of buds in radiatied seeds, but vitamin C, the opposite, promotes the intensive growth ob buds.

The extracts of some drug-plants (milfoil, lionstail, wormwood) stimulate the activation of the enzymes of prolin biosynthesis as in radiated so in not radiated been buds. But that stimulaythion in radiated been seeds in a presence milfoil and lionstail extracts is noticeably high in comparison whith non-radiated seeds buds, but in a presence of wormwood that index is high in non-radiatied seeds buds.

In all mentioned extracts and in a presence of proline activation of the enzymes of proline katabolism stimulates approximately twios and fully overwhelms in a presence of KNO_2 , Vitamin E and C.

METHOD OF BIOCHEMICAL -BIOPHYSICAL INDICATION AND ORGANISMS' "LIFE'S QUALITY" FORECAST IN CONDITIONS OF RADIATION POLLUTION

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The purpose of work - development of the biochemical-biophysical integrated approach of an estimation of organisms' "life's quality" in polluted environments which includes cytological, biochemical and biophysical methods of an estimation of stress factors action on a homeostasis of growing cells of various organisms. Simultaneous definition of inclusion ^{3}H-thymidine in DNA, ^{14}C-leucine in synthesized fibers and mitotic index of growing cells for an estimation of differential processes activity of replicative and reparative DNA synthesis, and also translations of fibers with $\tau_{1/2}$> 24 hours is carried out. All characteristics are estimated in relative units in relation to the control. The biophysical component consists in registration of secondary radiation parameters (S) of cellular culture in an aquatic environment, describing a degree of structurization and conformational alteration in system water klasters of mediums - permolecular biopolymeric structures of cells.

The method is approved on barmy cells *Cuf liver* culture at action on them of radiating or chemical stress factors of the various nature, for example, at reception of a total absorbed dose by them γ-radiation 100÷350 microRoentgen.

It is shown, that a total absorbed dose 100 and 350 microRoentgen have not change cells' "life's quality" due to the balanced activation of a reparation and translation, and also minimization of conformational reorganizations of klaster structures of aquatic environment. At absorption 250 microRoentgen there was a decrease in cells' "life's quality" because of activity reduction of reparation, especially translations and by essential reorganizations of aquatic klasters structure environment.

Thus, the suggested and approved method allows to estimate the contribution of various biochemical and biophysical processes to cells on their change of "qualities of a life" at action of radiating or others stress factors of environment.

CYTOGENETIC ANOMALY DYNAMICS IN BLOOD CELLS OF HOLSTEIN CATTLE IN CONDITIONS OF THE RADIONUCLIDE CONTAMINATION

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The comparative analysis of cytogenetic anomaly frequencies in peripheral blood cells of Holstein cattle breeding in territories with high level of radio pollution after Chernobyl's accident was carried out (experimental farm "Novoshepelichi": contamination by Cs-137 ≈ ~200 Ci/km^2; "Vladimirovka" - ~100 Ci/km^2). The frequencies of the number of erythrocytes (EMN) and one-nuclear lymphocytes with the micronuclei (LMN), two-nuclear lymphocytes (TNL) and apoptotic cells (A) in ‰ were evaluated. The archive collection of blood smears of animals from farm "Vladimirovka" (1987 – 1990 yy), and from ones "Novoshepelichi" were investigated. In cattle of farm "Vladimirovka" at 1988y the number of the cytogenetic anomalies was authentic above, than in animals at 1987 y: EMN - 5,1±0,3 against 3,4±0,2 ($P < 0,01$); TNL - 2,4±0,3 against 1,4±0,1 ($P < 0,01$); and LMN - 5,8±0,1 against 3,0±0,3 ($P < 0,001$). The frequencies of all characters in cattle at 1989y were authentic below, than in animals at 1988y: EMN - 3,7±0,3 ($P < 0,01$); TNL - 1,7±0,1 ($P < 0,05$); LMN - 4,6±0,4 ($P < 0,05$). In 1990y the authentic increase of these characters in comparison with ones at 1989 y was again observed. Thus, the frequency changes of EMN, TNL and LMN in animals from farm "Vladimirovka" in different years after Chernobyl's accident had non-linear character: it increased in 1988y in a comparison with 1987y, then decreased in 1989y and again increased in 1990y. The analysis of the same cytogenetic anomalies of cattle from farm "Novoshepelichi" was carried out also. The herd was subdivided to parent generation (which was born before Chernobyl's accident) and three generations, which were born in conditions of increased ionizing irradiation. The frequency LMN in parent generation (F0) was 4,5±0,4 ‰, that was authentically above, than in first (FI) 2,4±0,4 ($P < 0,01$), second (FII) 3,0±0,3 ($P < 0,01$) and three (FIII) 1,5±0,4 ($P < 0,001$) animal's generations. It is interest to note that in different generations the frequency of all cytogenetic anomalies had non-linear dynamic also: it decreased in FI in comparison with F0 generation, then increased in FII and decreased again in FIII generation.

THE ROLE OF UNTARGETED MECHANISMS IN BIOLOGICAL EFFECTS OF LOW DOSES OF IONIZING RADIATION

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The findings in rapidly developing researches of radiation-induced low-dose effects show them to be considerably more complex than predicted within the framework of the linear no-threshold (LNT) theory. The regions of hypersensitivity and induced radioresistance were observed on survival curves of mammalian cells irradiated with low-doses of ionizing radiation. Our study of chromosome damage induced by 0.01 Gy to 1 Gy of γ-irradiation from ^{60}Co and carbon ions ^{12}C in peripheral blood lymphocytes has exhibited similar phenomena. At 0.01 Gy to 0.,05 – 0.,07 Gy the cells show the highest radiosensitivity mainly due to chromatide-type aberration induction which are usually considered to be a feature of spontaneously generated ones. With increasing dose the frequency of the aberrant cells and aberrations decreased significantly (in some cases to the control level). At doses higher than 0.5 – 0.7 Gy the dose-effect curves become linear with a smaller slope compared to the initial one.

The idea of what process significantly affects cell survival and chromosome rearrangement in the low-dose region has not been identified yet. Recently discovered amplification of reactive oxygen species (ROS) executed by mitochondria in consequence of radiation-induced mitochondrial permeability transition is quite a probable candidate for such a process. As a result of extensive generation of ROS, cells experience oxidative stress which also may produce deleterious effects, including generation of chromatide aberration. In this report, current knowledge about the process is reviewed. The molecular-signalling pathways associated with radiation exposure are also discussed to explain how cells are able to induce resistance to further radiation exposure.

CONTRIBUTION OF BYSTANDER EFFECT IN VARIABILITY AND VIABILITY OF SEED PLANT POPULATIONS IN THEIR HABITATS AROUND THE RADIATION SOURCES

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Here we evaluate the contribution of bystander effect in variability and viability of plantain seeds *Plantago major* (indigenous populations) collected in their habitats (a 20-km radius around a nuclear power plant (NPP); a low-polluted Chernobyl trace territory; a Joint Institute for Nuclear Research (JINR) territory) in 1998-1999. Both radiation exposure and Cs^{137} soil concentration (C_{Cs}) did not exceed natural ground excluding the Chernobyl trace territory (C_{Cs} ~30 Bq/kg) and the JINR territory (the radiation level over all the particles exceeded the background ~ twofold for dose and ~eightfold for dose rate for two months, 1998). In populations near the NPP, the seed survival (S) can decrease by up to 20%, whereas the frequency of rootlet meristem cells with abnormalities (CAs) increases (or not) depending on the seeds' antioxidant status (AOS), and the mitotic index (MI) increases up to threefold. In JINR plant, the MI exceeded the same value averaged over the other populations approximately twofold in 1998. We suggest a "relay-race" scheme of non-targeted mechanisms that acted under radiation stress. Statistical simulations showed that CAs can appear both independently and Poisson-distributed (P) and correlative enhanced and geometrical-distributed (G). P-subpopulation did not change significantly under stress irradiation, whereas value of G-fraction decreased and its sample mean increased with dose/dose rates. This correlative enhancing contributed to variability (up to 60% of CAs, JINR) and non-viability (up to 65%, around the NPP). We conclude that P-mechanisms provide population stability, and the correlative enhanced G-machinery plays adaptive role.

POPULATION LEVELS OF ADAPTIVE RESPONSES OF THE CHILDREN'S POPULATION TO THE RADIATIONAL STRAIN OF A MEDIUM

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The notion of populational adaptive conflict was introduced by V.P.Mikhalev in 2001. It consists of the responses of not registered by receptors and low intensive, but sharp modifications of structure of a medium and in a consequence of discordance to phenotype. This conflict flows as an adaptive syndrome, but, owing to the absence of starting classical mechanism of a link of responses, it is exhibited with reliability (as well as other low intensive evolutionary modifications of a medium) only at a level of populations (the investigator must have sufficient time for observations).

The primary processes of such responses (as against to emergent externally generated hypophise-adrenaline replies), flow according to primary gradual accumulation of metabolic breakages at a cell level, as a corollary of a discordance of a spectrum of the newest metabolites, which have joined in exchange between the medium and the organism, to the spectrum of phenotypic pherment-hormonal regulators of exchange.

The first system of adaptation here, that suffers from massive overloading, (as against of emergency responses of an organism), is the immune system, more precisely its T-lymphocitic part (we registered sharply increased frequency of a strain of fiber).

Further transport of an information (epophisar blood flow and the hypothalamus) conducts to consequent secondary splash of similar overloading of nervous and endocrine systems. For an evaluation of a degree of populational strength of adaptive processes we investigated the responses of immune systems (on diagnosed diseases), nervous systems (finger-nose test, test of Romberg, seizing reflex, chronoreflexomethria), endocrine system (constant of proportionality, indexes of Erisman and Quetelet, frequency of arythmies and vegetho-vascular responses), and vegetho-vascular responses themselves (dynamic tests of pulse and blood pressure, dermographism, microvascular test, the velocity and character of restoring of vascular responses after loads).

Analysis of responses shows the expressed functional strength of adaptive systems of children in radioactively infected areas with powerful industrial saturation of a medium.

The corresponding responses of children that live in radioactively infected territories – equal by radiational circumstances, but more ecologically pure – are opposite directed. Pathological processes are negatively connected with stored dozes of an internal exposure.

RADIOSENSITIVITY OF CHROMOSOME APPARATUS OF VOLES FROM ALIENATION ZONE OF CHERNOBYL'S ACCIDENT

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Traditionally small-sized rodents were used for bioindication of territory pollution by different gene toxic agents. However, so far the spontaneous mutation spectra in bone marrow cells of different rodent species and species-specific traits of them in conditions of the chronic low-dose ionizing irradiation were studied insufficiently.

The goal of the work was the study of the 9 cytogenetic characters in bone marrow cells of various vole's species, trapping in Chernobyl's zone with various levels of radio nuclide pollution (20 – 100 - 1000 Ci/km^2). Species differed by number of acrocentric and metacentric chromosomes in the karyotypes. It was studied the bone marrow cells the following species of voles: *Microtus arvalis* (2n=46, Fna=84), *Microtus subarvalis* (2n=54, Fna=54), *Clethrionomus glarealus* (2n=56, Fna=56), *Microtus oeconomus* (2n=30, Fna=56), *Microtus agrestis* (2n=50, Fna=54).

In the zones with high-level radio pollution in all species of voles the increase of the mitotic activity and related with it the little decrease of metaphase frequency with asynchronous fission of centromeres were observed. Also the increases of frequencies of those cytogenetic anomalies which were unstable in the zones with low-level radio pollution (*Microtus arvalis* – aneuploidy, the voles with acrocentric autosomes – centric fusion of the chromosomes) were revealed. The high frequency of chromosome inversions in group of little-sized chromosomes in *Microtus agrestis* was marked.

Thus, the chronic ionizing irradiation lead to increase the frequency of those cytogenetic anomalies, which were species-specific characteristics of spontaneous mutagenesis in bone marrow cells in investigated vole's species.

SEQUENCING ANALYSIS OF MUTANT ALLELE CDC28-SRM OF PROTEIN KINASE CDC28 AND MOLECULAR DYNAMICS STUDY OF GLYCINE-RICH LOOP IN WILD TYPE AND MUTANT ALLELE G16S OF CDK2 AS MODEL

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The central role that cyclin-dependent kinases play in the timing of cell division and the high incidence of genetic alteration of CDKs or deregulation of CDK inhibitors in a number of cancers make CDC28 of yeast *Saccharomyces cerevisiae* very attractive model for studies of mechanisms of CDK regulation. We have found that certain gene mutations including *cdc28-srm* affect cell cycle progression, maintenance of different genetic structures and increased cell sensitivity to ionizing radiation. A *cdc28-srm* mutation is not temperature-sensitive mutation and differs from known *cdc28-ts* mutations because it has the evident phenotypic manifestations at 30°C. Sequencing analysis of *cdc28-srm* revealed a single nucleotide substitution G20S. This is a third glycine in a conserved sequence GxGxxG in the G-rich loop positioned opposite the activation T-loop. Despite its demonstrated importance, the role of the G-loop has remained unclear. The crystal structure of the human CDK2 has served as a model for the catalytic core of other CDKs, including CDC28. Nanoseconds long molecular dynamics (MD) trajectories of the CDK2/ATP complex were analyzed. The MD simulations of substitution CDK2-G16S (CDC28-G20S) shows a conformational change of CDK2 structure resulting in the moving of the G-loop away from ATP and a new rearrangement of amino acids in the T-loop.

THE PHENOMENON OF GENOMIC INSTABILITY IN THE CHILD'S BODY EXPOSED TO PROLONGED RADIATION AT SMALL DOSES AND HEALTH STATE

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Genomic instability was investigated in 90 children living on territories contaminated with radionuclides (Novozybkov district, Bryansk region, 16-18 Ci/km^2, ^{137}Cs) as a result of the accident at the Chernobyl NPP. The children were delivered for examination to the Federal Children's Scientific-Practical Center of Antiradiation Protection. (prof. L.S. Baleva). Significantly increased frequencies of certain radiation-induced chromosomal aberrations (CA) were observed in all examined groups of children exposed to radiation at different stages of ontogenesis: postnatal-irradiated in 1986, uterine-irradiated in 1986, born after the accident in 1987-1992 and in 1994-2000 from irradiated parents. No dependence of the expression of cytogenetic disturbances on the accumulated dose was established. Testing of genomic instability was carried out in 15 children with the use of ^{137}Cs γ-irradiation of lymphocytes in vitro. An elevated radiosensitivity of lymphocyte genomes to testing irradiation in vitro at doses of 10 and 100 cGy and peculiarities of the dynamics of CA frequencies in 3 successive mitoses of intact and in vitro irradiated lymphocytes were discovered both in uterine-irradiated in 1986 children and in children born after the accident from irradiated parents. The level of reciprocal sister chromatid exchanges in lymphocytes of the 3rd mitosis turned out to be significantly higher in the children from the contaminated territories as compared to the children of the control group. A wide range of interindividual variability of the frequencies of dysgenomic effects (DE) was observed. In most of irradiated children with pronounced DE combined abnormalities in the immune system were observed. No significant differences were revealed for CA frequencies depending on the structure of morbidity. In children having disturbances of the central nervous system only the frequency of chromatid fragments is higher than in the group of children having infective-allergic diseases. The highest sensitivity to testing irradiation and expression of DE in lymphocytes of the 3rd mitosis were observed in 5 children having the following pathologies: disturbances of the central nervous system, chronic gastritis, chronic pneumonia, extremity malformation The data obtained suggest the reality of induction of genomic instability in a growing organism exposed to prolonged low-intensity radiation and the necessity of further studies of the relationships between individual peculiarities of the expression of genomic instability and the health of children.

THE CHARACTER OF ADAPTATION REACTIONS ON RADIOGENIC DEFORMATIONS OF THE ENVIRONMENT

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A sharp momentary switch in the power and structure of sociogenic and radiation forces which took place in the conditions of unevenly distributed technogenic background of the environment was one of the causes of the growing health deficiency of the children's population of the country. The reasons for such reactions, quite different from those of the children in the analogical but stable ecosystems (Western Europe, Northern America and others), are not deciphered. The attempts made to reveal the leading, depending on factors, specific reactions in the structure of the competing forces are still unsuccessful. And it excludes the possibility to work out strategic medico-social bases for correction and prevention of the growing rise in children's pathologies.

The advanced conception of population adaptation conflict suggests as the main cause of reactions not factor as such, but a sharp change in the structure of the environment with the following formation of massive non-specific reactions of reorganization of the population phenotype – a spectrum of genetically fixed, stored populational nuclear memory; ferments, hormonic, neuroreflexive metabolism regulation, which is adequate to the adapted structure of the environment.

The character of adaptations in such, equal in the degree of new deformation of the environment, ecosystem situations will depend on the background state of the phenotype evolution. In case of the completed adaptation of ecosystems (third – fifth generation) and corresponding freedom of reserved population reactions of adaptation, the newest deformation of the environment can serve a strong stimulus of the functional state of populations. But in case of background massive technogenic many spectral pollution of the environment, lasting (in contrast to the Western environment) for several decades and, accordingly, massive background tension of adaptation systems and reactions, despite the possible limit of forces accepted in the sanitary practice, this deformation will involve marked functional tension of the classical triad of adaptation systems with the following rise in frequency of their intrapopulation breakages.

STUDY OF THE PHOSPHOLIPID COMPOSITION OF WHEAT SEEDLINGS NUCLEAR SUBFRACTION UNDER INFLUENCE OF ELECTROMAGNETIC RADIATION

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The nuclear membrane structure plays an important role in determination of the dynamic balance between nuclei and cytoplasm. The direct relationship between the internal structures of nuclear membrane and chromatin predetermines their possible participation in the change of gene functional activity. At present there is an evidence that nuclear structures of phospholipids have a definite contribution in the regulation of the genetic activity.

Depending on the functional state of a cell, the phospholipids content of nuclear membrane and soluble nuclear fraction (including chromatin) may vary, and this may alter the activity of a genome as a whole. Phospholipids being a significant component of nuclear membrane can sensitive respond to change in growth conditions, having placed in surrounding environment.

In present study we carry out a study influence of coherent non thermal electromagnetic irradiation on wheat seeds during germination and influence on phospholipid content in composition of nuclear subfraction. The EHF generator with the range of working frequencies 37,5-53,5 GHz was used as a source of monochromatic radiation of mm-waves. The irradiation was carried out in a distant zone of radiation of the generator.

The results of our investigation have revealed the reliable influence of the irradiation on phospholipid content in composition of nuclear subfraction. These resonant frequencies are close to those, found out in several works for aqua solutions and biological liquids that allows assuming the important role of water in biological effect of influence of low intensive electromagnetic waves (EMW). Our experimental data allows us to expect that phospholipid composition of nuclear subfraction is an sensitive parameter, hanging from external factor, acting upon plant.

THE INFLUENCE OF ELECTROMAGNETIC IRRADIATION OF MILLIMETER WAVES ON BACKGROUND IMPULSE ACTIVITY OF SUPRAOPTIC NUCLEUS' NEURONS OF RATS' HYPOTHALAMUS

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Low intensity millimeter-wave electromagnetic radiation has nonthermal effect on the organism and it is widely used in biology and medical practice for treatment of various diseases. The aim of this was to investigate the effects of millimeter-wave irradiation on the background impulse activity on the supraoptic nucleus neurons of rats' hypothalamus, which has of great importance in mechanisms of adaptation and maintance of homeostasis of organism in conditions of various external and internal factors' influence. In acute experiments on white rats anaesthetized by Nembutal the registration and analysis of background impulse activity of supraoptic nucleus' neurons of rats' hypothalamus in norm and after local single exposure of electromagnetic irradiation The millimeter-wave exposure characteristics were: frequency - 50,3GHz, power density – 0,48 mW/cm^2, duration – 40 min. It was estimated the distributions of neurons by the degree of rhythm regularity, the character of types of dynamics of the following of impulse flows, the average frequency of impulsation, the modality of histograms and the coefficient of variation of interspike intervals. It was revealed changes of the background impulse activity, which were concerned mainly the changes of the inner structure of registered impulse flows. It was shown that the statistically significant shifts after exposure are observed in the distribution in the degree of regularity of impulse activity ($p<0,01$) and in the character of dynamics of neuronal current flows ($p<0,05$). The changes of the average frequency of impulsation and the coefficient of variation of interspike intervals were no essential. Statistically significant changes ($p<0,05$) of the average frequency of discharges of different frequency range neurons' populations were revealed. The reorganization of the background rhythmical pattern, which has detected after local single exposure of low intensity millimeter waves to healthy rats testifies about nonspecific initial adaptive reaction of brain.

SOMATIC GENE MUTATIONS IN PATIENTS WITH BENIGN TUMORS LIVING IN RADIATION CONTAMINATED REGIONS

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The aim of this study was to research level of somatic mutagenesis in women with benign tumors of reproductive system living in radiation contaminated regions. 139 persons were investigated and divided in two groups. The first group consisted of 97 patients with myoma who had been living in Novozibkovskiy region of Bryansk oblast during 18 years since the moment of the Chernobyl accident. Mean ^{137}Cs density in this region was 799kBq/m^2. The second (control) group included 42 age-matched unexposed healthy individuals. Flow cytometry was used to evaluate the frequency of peripheral blood lymphocytes bearing mutations at T-cell receptor (TCR) locus. The mean frequency of the TCR-mutant cells (±SE) in patients with myoma was significantly higher than in control group: $(5,3\pm0,5)\cdot10^{-4}$ vs $(4,0\pm0,2)\cdot10^{-4}$ ($p<0,05$). 18 patients (18,6%) had the TCR–mutant cell frequencies exceeding the 95% confidence interval in control group ($>7,0\cdot10^{-4}$). The frequencies of mutant cells in other patients corresponded to those in control group. Our results confirm, that the significant elevation of the TCR variant frequency was observed in the certain proportion of persons with benign tumors of reproductive system living in radiation contaminated regions. Individuals with elevated TCR-mutant cell scores might belong to high-risk group potentially prone to the development of neoplasm and need more thorough medical observation then the rest of population.

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INFLUENCE OF IRRADIATION ON DROSOPHILA LIFE SPAN IN CORRESPONDENCE TO GENOTYPE

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It is shown, that not irradiated Drosophila lines with defects of DNA repair, antioxidant protection and apoptosis have higher speed of ageing, than a wild type line. At the same time the irradiation results in change of life span depending on the line genotype. The longevity and physical activity of organism (reflecting life quality level), change in unidirectional way. It is offered the mechanism of the remote action of low dozes of ionizing radiation on the life span. As the cells with the weakened protection will accumulate damages and to be exposed to ageing with the greater speed, than steady cells, they radio-induced elimination at early development stages will be resulted in delay of age - dependent changes and will lower speed of ageing. In the subsequent irradiated generations the given somatic answer to stress (hormesis), on the population level will be replaced by negative genetic effects therefore life span will be reduced.

PROLONGED STRESS INDUCES MUTATIONS AND PROVIDES UNSPECIFIC ADAPTATION OF DROSOPHILA POPULATIONS

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Earlier we have shown adaptation of natural drosophila populations from radiocontaminated regions of Belarus to mutagenic effect of radiation. It was interesting to study the possibility of remaining this adaptation in a number of generations after radiation exposure termination. This question was investigated in natural drosophila populations caught in 2000-2001 in the settlement Vetka of Gomel region (24 Ci/km^2) and in Berezinsky reserve (the control) with the test of dominant lethal mutations. For genetic resistance evaluating males from the populations studied were exposed to acute γ-radiation 30 Gy.

The population from the settlement Vetka proved to be more adapted to radiation exposure than the population from Berezinsky reserve. This adaptation was shown to be unspecific – irradiated population became more resistant to chemical mutagen ethylmethansulfonate as well as to radiation.

Then the population samples were kept under laboratory condition without irradiation. for 8 generations. It should be noted that the mutation level in both populations increased at keeping under such conditions. Acute irradiation 30 Gy was used after 8 generations. Adaptation of Vetka population to irradiation remained.. Besides the control population became also more resistant to ionizing radiation but its adaptation was not so expressed than that of Vetka population. It means that keeping of natural drosophila populations under laboratory conditions is a strong stress (limited space, overpopulation, other temperature and light conditions), which increases mutation process and induces unspecific adaptation.

These facts should be considered in studying dynamics of the mutation level during radionuclide removal in animals caught in radiocontaminated regions and placed in vivarium conditions.

INDUCTION OF DNA POLYMORPHISM IN OFFSPRING OF MICE, γ-IRRADIATED IN DOSE RANGE FROM 1 Gy TO 3 Gy

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It is known, that cells at various stages of spermatogenesis have various radiosensitivity. The aim of the present work is study effects of a chronic and acute irradiation in various doses and also comparison genetic sensitivity of spermatogonia and spermatids to an irradiation in a range of doses 1-3 Gy. The analysis carried out with the use of the RAPD-test (random amplified polymorphic DNA assay) which is based on amplification total genomic DNA with a series of random primers and enables multi-locus scanning of hypervariable regions. Mice BALB/c were irradiated in doses 1-3 Gy on gamma-unit GYPOS (dose rate- 4,5 Gy/min, source- Cs-137). For comparison of sensitivity of different stages of spermatogenesis males were crossed with females the same strain in two weeks and in three months after an irradiation. The offspring in both cases contained in standard conditions and killed in the age of 3-4 weeks. Purification and amplification of DNA were performed, by using commercial kits of Izogen. PCR condition were Td-95 ^{0}C, 25 s, Tm - 42 (cycles # 1-4), then 58 ^{0}C (cycles # 5-35), 5 s, Ts- 74 ^{0}C, 80 s. For PCR amplificator PT-48 (TDL Company) were used. Products of RAPD were separated into 1,5 % agaroze gel. Analysis of offspring patterns carried out on the basis of comparison with parental patterns, with the purpose of registration of new, "not parental" bands. Results of the analysis processed statistically. In case of an acute irradiation we received increasing of a level of the polymorphism expressed as frequency of occurrence of new strips on one descendant in family, which significantly distinguished from the control. Comparison of results of the analysis of sensitivity of spermatogonia and spermatids at all used doses, has shown, that in pre-meiosis cells genetic effects of an irradiation are expressed in the greater degree, than in post-meiosis cells. Result of our work is is revealing dependence of levels of polymorphism in hypervariable sites of DNA in the field of doses up to 3 Gy, and also detection of genetic effect at influence of low-levels chronic radiation.

CHANGES OF SOME MOLECULAR BIOLOGICAL PARAMETERS OF WHEAT GERMINATING SEEDLINGS UNDER INFLUENCE OF NON-THERMAL ELECTROMAGNETIC RADIATION

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The electromagnetic radiation (EMR) is an environmental factor, which influence is very important for growth and development of living organisms as well as for human. However recently with increasing of number of artificial source having different frequency of irradiation, may led to the unpredicted consequences. The influence of low intensive non thermal coherent extremely high frequency electromagnetic radiation (EMR) of mm-diapason on intensivity of wheat seedlings growth, general activity of lactate dehydrogenase (LDH) and peroxidase (PO) as well as on the biochemical composition of plant cell nuclear sub-fractions structures have been investigated.

It is shown, that the preliminary irradiation of wheat seeds accelerates growth of seedlings. General activity of LDH and PO of germinating seedlings determined for 3-6 days after an irradiation. The changes of LDH activity are different directed and depend on frequency of radiation and period of growth. The activity of peroxidase rises in all cases. The rising of nucleic acids content in composition of nuclear membrane and soluble nuclear fraction had been obtained as well. Have been shown that content of DNA increase two times and five times of RNA.

The significant effect was observed at expositions 20-30 min. The biologically effective frequencies in a narrow range 49-53 GHz with the expressed resonant frequencies close to 50 GHz and 51,8 GHz are revealed. On the basis of our study we suggested that EMW influence direct on the genome and lead to increase of preservation system of living organisms. Biological replay directs to the surviving of organisms and is an adaptive reaction of organism against to the stress of physical factors of environment.

TOTAL ACTIVITY AND ISOENZYME COMPOSITION OF LACTATE DEHYDROGENASE OF WHEAT SEEDLINGS UNDER THE INFLUENCE OF NON-THERMAL ELECTROMAGNETIC IRRADIATION

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The total activity and isoenzymatic composition of lactate dehydrogenase (LDH) were investigated after irradiation of germinating wheat seedlings by low intensive non-thermal coherent electromagnetic radiance (EMR) with frequency of 45-53GHz and density of a flow of capacity 0,6mW/cm^2. As the source of monochromatic radiance the generator Г4-141 with area of working frequencies 37,5-53,5GHz was used. The irradiation was carried out in a distant zone of radiation of the generator during 30 mines.

It is revealed, that the irradiation of germinating seeds leads to changes of total activity and ratio of the molecular forms of LDH. The value of deviations depends on frequency of EMR. During growth the change of an orientation and increase of a degree of the biological reply was observed, the resonant character of changes which is known for other biological systems was kept. The maximal deviations from a control level of total activity of LDH are found out in seedlings, irradiated with frequency in a range of 49-53GHz. In 3-6 day-old seedlings, irradiated with frequency of 50,3GHz and 51,8GHz the increase of the contents of fast migrating fractions of LDH characteristic for embryonal cells (LDH_1, LDH_2) was observed. In seedlings irradiated with frequency of 49GHz and 50GHz – middle fractions of pattern (LDH_3, LDH_4), and with frequency of 51GHz and 53GHz - slow migrating fractions (LDH_4, LDH_5) is revealed. The change of a ratio of LDH isoenzyme content may be result of destabilization of enzyme molecules due to increasing of amplitude of auto fluctuations under action of external EM field, which may cause the dissociation and reassociation of subunits of enzyme, different sensitivity of subunits of LDH to this external factor. The increase of general activity of LDH allows us to assume, that the change of LDH isoenzymes ratio may be caused by change in activity of genes regulating the synthesis of LDH subunits.

STUDY OF DNA STRAND BREAKS IN SPLEENOCYTES OF MICE EXPOSED TO VERY LOW DOSE-RATE γ-RADIATION USING THE COMET ASSAY

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The aim of the present work was to study of DNA strand breaks induction in spleenocytes of mice continuously exposed to very low dose-rate γ-radiation (0.07 mGy/h) within a dose range of 7 to 61 cGy using the single cell gel electrophoresis (comet) assay. It was shown that very low dose-rate irradiation resulted in statistically significant increase in DNA strand breaks level, starting from a dose of 20 cGy. Further prolongation of exposure time and, hence, increase of a total dose did not, however, lead to further increase in the extent of DNA breaks. To take into account the contribution of an apoptotic cell subpopulation to a final readout of DNA breaks in our experiments, we measured the percentage of apoptotic spleenocytes from irradiated and untreated animals using the "DNA diffusion" assay. At the days 120, 270, and 365 of the chronic irradiation (20, 45, and 61 cGy, respectively), approximately two-fold increase over a control level in the apoptotic cell fraction was observed. These observations prompted us to recalculate overall DNA damage levels in irradiated vs. untreated groups. When performed without counting highly damaged cells, the comet assay yielded in less, but still statistically significant, difference in DNA damage levels between irradiated (20-61 cGy) and untreated mice. The appearance of apoptotic cells with highly fragmented DNA in irradiated animals cannot, therefore, have direct impact on overall DNA strand breaks level. To summarize, chronic irradiation at a dose-rate 61 cGy/year lead to statistically significant increase in a number of DNA strand breaks in spleenocytes within a 20-61 cGy dose range. Overall increase in the level of DNA strand breaks as a result of low dose-rate γ-radiation exposure can be associated with the chromatin rearrangement accompanied by gene overexpression, increase in ROS production rate, and DNA repair activation, processes known to be triggered after low doses of ionizing radiation. Although insignificant, a contribution of apoptotic cells to an overall level of DNA strand breaks was also recorded.

MODELLING OF CHROMOSOME ABERRATION YIELD IN WIDE DOSE RANGE

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Mathematical description of dependence of frequency of chomosome aberrations in wide dose range is important task in both theoretical and applied aspects.

It should be noted that in assessing of genetic hazards of low doses of ionizing radiation is complicated with non-linearity of dose-effect relationship and existing of a plateau in low-dose region (Sevankaev A.V., Luchnik N.V., 1977, Geraskin S.A., Sevankaev A.V., 1996., Zainullin V.G., 1997). From the other hand in case of high doses of ionizing radiation, which take place in case of accidents for example, the dose-effect ralationship could be described by linear-quadratic function.

We present in our current publication the generalized analytical model for quantitative assessment of frequency of chromosome aberrations. We formulate source, multi-parametric Koshi task as a differential equation with initial condition and then as a solution of this task we define analytical form of generalized dependence; accounting for initial condition allows include in mathematical model a level of a threshold as an additional parameter.

It is shown that in absence of reparation and recovery processes in irradiated biological system the current model in particular case provides regular linear-quadratic non-threshold model.

Thus, developed model generalizes in natural way theoretical and experimental data on frequency of the chromosome aberrations after radiation exposure.

EXPERIMENTAL SUBSTANTIATION AND ESTIMATION OF HUMAN INDIVIDUAL RADIOSENSITIVITY

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Test- system of human peripheral blood lymphocytes (HPBL) with the subsequent analysis of level and spectrum of chromosomal aberrations makes it possible to obtain objective information about the instability of cell genome. Chromosomal aberrations have been observed in many types of tumor and it was shown, that the increased level of structural rearrangements in chromosomes positively correlates with the increased risk of tumor development. From the other side, HPBL are the most sensitive indicators of radiation effects, proposed by WHO and IAEA for conducting the biodosimetry of radiation injuries. Radiation causes different types of DNA damage, among which double- strand breaks (DSB) are of special importance being one of the most dangerous in their consequences for cell and can result in chromosomal aberrations. An increase in chromosomal aberrations is associated with radiation-induced genome instability due to inappropriate repair of DBS or recombination. Recent reports have suggested that observed elevated chromosomal aberration yields following G_2 phase irradiation of somatic cells from cancer patients are consequences of impaired DNA repair and hereditary predisposition to cancer.

The analysis of literature data carried out by us showed significant variability in the parameters of radiosensitivity among the individuals in group of healthy control. Our studies of chromosomal radiosensitivity of healthy individuals after G_2 phase irradiation confirmed our assumption. Application of the modified G_2- assay made it possible to determine, that the variations in the parameters of individual radiosensitivity of 25 healthy donors were formed due to the chromatid aberrations, up to 90% of which were chromatid breaks. The developed statistical approach made it possible to reveal that 12% of the examined individuals have showed an increased chromosomal radiosensitivity. The role of DNA repair processes in the formation of variations in individual radiosensitivity was examined.

DISTRIBUTION OF TCR-MUTANT LYMPHOCYTES ON NAÏVE AND MEMORY CELL SUBSETS IN INDIVIDUALS EXPOSED TO LOW DOSES OF IONIZING RADIATION

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It was found earlier that frequency of lymphocytes with mutant (variant) phenotype at T-cell receptor (TCR) locus increased in a some individuals exposed to low doses of ionizing radiation (Saenko et al., 1998). To understand the reason (s) for this effect it is worth to study nature of the mutant cells and, first of all, to elucidate their distribution on memory and naïve cell subsets. In this work frequency of the all TCR-mutant cells, percentage of $CD45RO^+$ memory cells among TCR-mutants and frequency of $CD45RO^-$ naïve TCR-mutant cells were assessed by flow cytometry in 46 workers of atomic industry and 31 inhabitants of Novozybkov district of Bryansk region. Mean cumulative doses (±SE) in these two groups were 120,8±21,0 мSv and 29,0мGy (according to data of Russian National Medical and Dosimetric Registry) correspondingly. The percentage of $CD45RO^+$ memory cells among TCR-mutant lymphocytes was in average 67±3 in inhabitants of Novozybkov district and 72±3 in workers of atomic industry. Thus, the most of the TCR-mutant lymphocytes are memory cells, those are known to be able to recirculate in organism and proliferate for a long time even in lack of the TCR unlike naïve cells. Some proportion of individuals (12/77) had the TCR–mutant cell frequencies exceeding the 95% confidence interval in unexposed age-matched control group. It was found that the mean frequency of $CD45RO^-$ naïve TCR- mutants was significantly higher in individuals with the elevated TCR-mutant frequencies than in others: $(2,4\pm1,3)\cdot10^{-4}$ vs $(0,9\pm0,1)\cdot10^{-4}$ correspondingly ($p=0,003$). This fact implies that the elevation of mutant frequency occurs among not only memory but naive cells that, in turn, indicates high intensity of mutational process for a relatively short period before analysis.

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THE BREACHES IN PHOSPHOLIPID AND GLYCEROLIPID METABOLISM DURING IONIZING IRRADIATION IN HEPATOCYTES.

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After ionizing irradiation there are detected changes in the rate of into total hepatocyte membrane phospholipids. Liver phosphatydilinositol and phosphadtydilethanolamine synthesis is pressed while the level of phosphatidic acid and lysophosphatydilcholine is increased.

After ionizing irradiation in hepatocytes the involvement of 14C –glycerol in vivo into mono-, di- and triglycerides is decreased whereas the content of nonestherified fatty acids is elevated. It was established that there is an infringement of glycosphingolipid metabolism, which is expressed by an increase of N-acetyl neuraminic acid amount in experimental animal's liver. Unlike to them the level of hexoses from acidic glycosphingolipid constituents was decreased. It was shown a decrease in the rates of glycerokinase and glycolitic pathways in glycerolipid biosynthesis, which is expressed by the inhibition of catalytic activity of glycerokinase and glycerophosphate dehydrogenase. Both mitochondrial and cytoplasmic glycerophosphatedehydrogenase activities are diminished which is accompanied with lowered glycerophosphate shuttle mechanism.

Thus it can be stated that after ionizing irradiation there are significant changes in hepatocyte lipid metabolic pathways leading to adverse changes in other metabolic processes.

INFLUENCE OF INTERLEUKIN-1 AND ITS RECEPTOR ANTAGONIST ON FREQUENCY OF RECIPROCAL TRANSLOCATIONS FORMATION IN THE SPERMATOGONIES OF MICE IRRADIATED IN SUBLETHAL DOZES

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The purpose of the present research was studying harmful action of sublethal dozes of X-ray radiation on sexual cells and an estimation of an opportunity of pharmacological protection of the revealed changes by recombinant interleukin-1β or its receptor antagonist.

EXPERIMENTAL RESEARCHES HAVE BEEN EXECUTED ON 100 MALE OF BALB/C MICE SUBJECTED TO A SHARP X-RAY IRRADIATION IN DOZES 1, 2, 3 AND 4 GY. THE CYTOLOGICAL ANALYSIS OF FREQUENCY OF THE RADIATION-INDUCED RECIPROCAL TRANSLOCATIONS FORMATION IN SPERMATOGONIES CARRIED OUT THROUGH 45-60 DAY AFTER AN IRRADIATION AT A STAGE OF DIAKINESE-METAFASE OF THE FIRST MEYOSIS DIVISIONS INTO THE CONSTANT AIR - DRY PREPARATIONS BY METHOD E.P. EVANS ET AL. (1964).

It has been shown, that the quantity of cells with reciprocal translocations after an irradiation in a doze 1 Gy increases in 10 times, 2 Gy – in 17 times, 3 Gy – in 21 times and 4 Gy – in 24 times in comparison with not irradiated control. Preventive application of interleukin-1β or its receptor antagonist in terms when these preparations show radioprotective effect by criteria of survival rate of the irradiated animals, did not cancel mutagenious action of radiation. It is necessary to note, that introduction of interleukin-1β, its receptor antagonist, as well as joint application of both preparations did not increase frequency of reciprocal translocations formation both at irradiated, and at not irradiated mice. The received results allow to make the conclusion that these radioprotective preparations do not have own mutagenious action.

RADIATION-INDUCED TERATOGENIC EFFECTS IN HUMANS: CONCLUSIONS FROM FINDINGS AFTER THE CHERNOBYL ACCIDENT

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The evaluation of radiation risks by international radiation protection committees is based on the findings in the A-bomb survivors of Hiroshima and Nagasaki. The effects after prenatal exposure observed in this collective were mental retardation and reduced head sizes while no other significant detriment was detected. Only the time from the 8th to the 15th week of gestation is assumed to be the period of risk. A threshold dose of 100 mSv is postulated for teratogenic effects in publication No. 90 of the ICRP (2003). A variety of observations about congenital malformations, fetal loss, stillbirths, and infant deaths, as well as Down´s syndrome after the Chernobyl release demand to point out the incompleteness of the Japanese data. The main problem is that the investigations of the Radiation Effects Research Foundation did not begin before 1950, 5 years after the explosion. The review of observed developmental disturbances after exposure in pregnancy of experimental and human collectives shows many analogous effects to findings after Chernobyl. The latter were originated by a chronic low dose exposure. Effects by Chernobyl contaminations, especially in great distances, are usually denied because of the low values of the estimated human exposures. The reliability of the dose factors of the ICRP which are used for dose calculation is, however, highly questionable. Several investigations by biological dosimetry in contaminated regions show that physical dose estimations lead to considerable underestimations of the true exposure. The threshold hypothesis of the ICRP can therefore not be accepted. The findings after Chernobyl should be considered as the main reference for low dose risks after prenatal irradiation.

THE POPULATION CYTOGENETIC STUDY OF CHILDREN LIVING ON THE AREAS CONTAMINATED AFTER CHERNOBYL ACCIDENT

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The Chernobyl accident, which was the most large-scale nuclear accident in a mankind history during peace-time, took place about 20 years ago. It was appeared the unprecedented case when hundred thousands children were exposed both at the moment of accident including internal irradiation with incorporated radioiodine and protract due to living on the contaminated areas. It is well known that child nature is the most sensitive target for ionizing radiation especially in an antenatal period. That is why the exposed children appear to be the critical population group injured as a result of Chernobyl accident. The study groups were comprised of children constantly living on the territories of Zhizdra and Ulianovo districts of Kaluga Region. It was examined 740 subjects, among them were 333 from Ulianovo and 407 from Zhizdra districts respectively. It was carried out repeat cytogenetic examinations - 5 times for Ulianovo district and 7 times for Zhizdra district in the period from 1989 to 2003 years. The significant part of children was inspected repeatedly from 2 to 6 times. The control group was comprised of 192 children and teenagers living on un-contaminated areas and examined in 1991, 1993 and 2004 years. The average ^{137}Cs contamination of two inspected districts are 111 and 200 kBq/m^2, the mean whole body absorbed doses over the period 1986-2001 years are 6.7 and 11.4 mGy for Zhizdra and Ulianovo districts respectively. It was found the enhanced level of chromosomal aberrations in the lymphocytes of peripheral blood in the part of examined subjects ranged from 30% to 60% by way of cytogenetic inspection over 18 years after accident. The mean frequency of all unstable aberrations (acentric fragments, dicentrics and centric rings) was about 0.4 per 100 cells over the observation period (control level was 0.22 per 100 cells). The mean frequency of radiation markers (dicentrics and centric rings) ranged 0.04-0.19 (0.03 as a control) per 100 cells kept enhanced over the observation period. The observed enhanced level of an aberrations – radiation markers in a human groups living on the contaminated areas after Chernobyl accident which has no tendency to decline with time over 18 years is a clear indication of constant radiation mutagen environment stress to the population.

UNTARGETED MUTATIONS IN THE MATERNALLY DERIVED X-CHROMOSOME IN F1 *DROSOPHILA MELANOGASTER* PROGENY OF IRRADIATED MALES

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The untargeted mutations in the X-chromosome of unirradiated oocytes after fertilization by irradiated spermatozoa were firstly observed in *Drosophila* by Abeleva et al. in 1961. The similar effects were found by several authors in *Drosophila* and mice in recent studies, but the molecular mechanisms are still unknown.

We assume that the main role in this effect may play three mechanisms: Activation of error prone repair in response to preliminary DNA lesions in irradiated chromosomes; DNA damage induced transpositions of transposable elements; DNA damage induction by long-lived free radicals. Also we suggest that the same mechanisms may be responsible for the non-linear effects of low-dose irradiation.

In this study we analyzed the frequencies of the recessive lethal mutations in the maternally derived X-chromosome in F1 progeny of irradiated males. We used two irradiation conditions: chronic low-dose irradiation during one generation with a dose rate of 60 cGy and acute irradiation with a dose rate of 10 Gy. For testing our hypothesis we used females from the repair deficient mutant strains (*mus209* and *mei-41*), females with lack of radicals detoxification mechanism (*sod*), and with active transposable *P*-elements in genome (*Harwich*).

We failed to found untargeted mutations after chronic low-dose irradiation. After acute irradiation we observed decreasing of mutation rate in *mei-41* and increasing in *sod* strain. The obtained results suggest involvement of DNA repair as well as radicals in mutation induction in unirradiated X-chromosome.

INFLUENCE OF THE INCREASED NATURAL RADIATION BACKGROUND OF GROWTH ON PHOTOSYNTHESIS OF AN *DUSCHECIA FRUTICOSA* AND ON FERMENTATION ACTIVITY OF SOILS

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Research of fermentation activity of soils on refuse dumps urine-thorium deposits is carried out. On refuse dumps of a deposit it has been incorporated 5 sample plots having a γ-background 20, 60, 100, 750 and 1000 microRontgen/h, with the maintenance of uranium in them - from 6,3 up to 564 mg / kg. In this background range the *Duschecia fruticosa* is prevailing. On sample plots in leaves of an *Duschecia fruticosa* (in vivo) determine photosynthetic activity under the account of carbonic gas (marked ^{14}C) in a current of air past through the specific area of a sheet. Sample drawing melkozem from upper root-inhabited an edaphic layer (0-10 sm) has simultaneously been carried out.

The enzymes investigated by us invertase, urease, catalase and polyphenol oxidase play the important role in material and energy exchange of soils. As the received data testify, nuclear pollution lowers a level of fermentation activity of soils. It concerns some all enzymes investigated by us, both hydrolytic, and redox.

1. It is fixed, that in process of increase in the maintenance of uranium in soil from 6,3 up to 564 mg / kg, its amount in leaves of plants from 0,01 up to 15,6 mg / kg increases.
2. It is shown, that nuclear pollution by natural radioactive nuclides in the investigated range of concentration (the increase γ-background makes from 20 up to 1000 microRontgen/h) reduces in 2,0-3,5 times a level of fermentation activity of the investigated soils, as hydrolytic enzymes, and oxydoreductases (invertase, urease, catalase and polyphenol oxidase). Decrease in fermentation activity of soils, apparently, is consequence of edaphic microflora oppression as a result of increase of radioactive nuclides maintenance.

It fixed, that with increase γ-background of growth from 20 up to 1000 microRontgen/h the maintenance of a chlorophyll in leaves of an alder statistically authentically does not change.

INVERSE DOSE-RATE EFFECT ON MICRONUCLEUS FORMATION IN BONE-MARROW ERYTHROCYTES OF BANK VOLES CHRONICALLY EXPOSED TO RADIOACTIVE CHERNOBYL FALLOUT

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Genetic effects after low dose-rate chronic irradiation, which we had observed in bank voles (somatic and germ cells, embryos), pond carp (fertilized eggs, embryos, fry) and laboratory mice (somatic and germ cells) in the dose range from near normal background up to 10 cGy per animal generation, have been significantly higher than expected from the known dose-effect relations of high dose-rate experiments. For further analysis of this inverse dose-rate effect, direct comparison between the genetic efficiencies of low dose-rate chronic irradiation and higher dose-rate acute irradiation was carried out in natural populations of bank vole which inhabited two sites in Belarus differing in radionuclide ground deposition. Dose-effect relations for the formation of bone-marrow erythrocytes containing micronuclei were obtained for chronically irradiated bank vole populations with absorbed dose rates in the range of 2 to 40 μGy/d and for acute irradiation by ^{137}Cs γ rays with 5 cGy/min.

In the case of chronic exposure, the doubling dose for the fraction of bone-marrow erythrocytes containing micronuclei observed in low dose-rate experiments was 2,61 cGy, whereas the doubling dose determined in high-dose rate experiments was 31,4 cGy. We conclude that this inverse dose-rate effect on micronucleus formation in bone marrow erythrocytes is due to the fact that the chronic radiation exposure was extended over all precedent differentiation stages of these bone-marrow cells including the stem cells, whereas in the case of acute irradiation merely the cell generation immediately preceding the examined cells had been irradiated. This interpretation was confirmed by a similar chronic exposure of laboratory mice over a single animal generation.

THE PROBLEM OF TRANSGENERATION GENOME INSTABILITY IN CHILDREN BORN FROM FATHERS-LIQUIDATORS AND UNIRRADIATED MOTHERS

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The action of radiation in the liquidators can be classified as fractionated. In connection with this, of basic importance is complex genetic monitoring of their families. The following parameters was studied: the spectrum and frequency of chromosome aberration, activity of unscheduled DNA synthesis in lymphocytes in 14 full families living on territories without radioactive contamination. The irradiation doses accumulated by the liquidators did not exceed 25 Ber. The average age of the parents before the moment of conception was 34 years for men and 26 years for women. In most fathers liquidators an increased level of complex radiation-induced chromosome aberrations after almost 20 year following the accident were revealed: dicentrics, rings, paired fragments, deletions, centromeric breaks, translocations and inversions. In some, rare multiaberrant cells with two and more aberrations were discovered. The levels of chromatid fragments were increased. The repair activity of genomic DNA was reduced on the average both after UV- and after γ-irradiation. An increased level of chromosome and chromatid aberrations was observed in several mothers, wives of the liquidators. In some cases the DNA repair activity was reduced after UV- irradiation. The children of the above-mentioned parents were subdivided into two groups: group-I – children born in 1986-1991, group-II - children born in 1993-1998. An increased level of chromosome aberrations was recorded in both groups. In children born 7-10 years after the Chernobyl accident an increased level of dicentrics + rings without paired fragments is observed. In several children born soon after the accident a high level of stable aberrations (inversions, translocations) was noted. Sporadic aneuploid, polyploidy cells were revaled. The number of chromatid aberration is higher in the children of group-I. No dependence of the spectrum and frequency of chromosome aberrations on the sex of the children were revealed. In half of the children after UV- irradiation and after γ-irradiation the repair activity of genomic DNA is reduced. In was noted that the children showed individual expression of dicgenomic effects. The examined children were found to have pathologies: of the central nervous system, digestive, urogenital, osteomuscular and other system. The obtained data point to individual peculiars of the induction of transgeneration genome instability in the children born from irradiated fathers - liquidators of the Chernobyl accident.

INCREASE OF REGIONAL TOTAL CANCER INCIDENCE IN NORTH SWEDEN DUE TO THE CHERNOBYL ACCIDENT?

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Study objective. Is there any epidemiologically visible influence on the cancer incidence after the Chernobyl fallout in Sweden?

Design. A cohort study was focused on the fallout of caesium-137 in relation to cancer incidence 1988-1996.

Setting. In Northern Sweden, affected by the Chernobyl accident in 1986, we categorised 450 parishes by caesium-137 deposition: <3 (reference), 3-29, 30-39, 40-59, 60-79 and 80-120 kiloBecquerel/m^2.

Participants. All individuals 0-60 years living in these parishes in 1986 through 1987 were identified and enrolled in a cohort of 1 143 182 persons. In the follow-up 22 409 incident cancer cases were retrieved in 1988-1996.

Main results. Taking age and population density as confounding factors, and lung cancer incidence in 1988-1996 and total cancer incidence in 1986-1987 by municipality as proxy confounders for smoking and time trends, respectively, the adjusted relative risks for the deposition categories were 1.00 (reference <3 kiloBecquerel/m^2), 1.05, 1.03, 1.08, 1.10 and 1.21. The excess relative risk was 0.11 per 100 kiloBecquerel/m^2 (95% CL 0.03;0.20). Considering the secular trend, directly age standardised cancer incidence rate differences per 100 000 person-years between 1988-1996 and the reference period 1986-1987, were 30.3 (indicating a time trend in the reference category), 36.8, 42.0, 45.8, 50.1 and 56.4. No clear excess occurred for leukaemia or thyroid cancer.

Conclusions. Unless due to chance or remaining uncontrolled confounding, a slight exposure-related increase in total cancer incidence has occurred in Northern Sweden after the Chernobyl accident. Published in *J Epidemiol Community Health 2004;58:1011-6*

8-OHDG IN BELARUSSIAN CHILDREN RELATES TO URBAN LIVING RATHER THAN CHERNOBYL RADIATION: A PILOT STUDY

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Background: After the Chernobyl accident in 1986 Belarus got considerable fallout of caesium-137 (Cs-137) and other isotopes. The exposure from these radioactive isotopes is still a concern in the contaminated regions.

Objectives: Ionizing radiation generates free radicals *in vivo* that can cause oxidative damage to DNA and contributes to carcinogenesis. Increased levels of 8-hydroxydeoxyguanosine (8-OHdG), a marker of such oxidative stress, have been demonstrated in subjects under radiation treatment, but also at much lower dose rates. We therefore tested the hypothesis that long-term radiation exposure from the Chernobyl accident might increase the urinary excretion of the 8-OHdG in Belarussian children.

Methods: Urinary 8-OHdG was determined in 31 and 46 children living in contaminated and uncontaminated areas of Belarus, respectively (the majority of the unexposed children lived in the capital Minsk). The annual summary effective dose was calculated from whole-body counting of Cs-137 and measurements of the terrestrial gamma radiation.

Results: The children from the contaminated areas had a significantly higher annual summary effective dose, but a significantly lower urinary 8-OHdG levels than the children from the uncontaminated areas. Unexpectedly, children living in uncontaminated urban areas had significantly higher urinary 8-OHdG levels than children living in uncontaminated rural areas. There was no statistically significant effect of sex or body mass index (BMI) on urinary 8-OHdG, but a weak significant inverse correlation to age as well as to the annual summary effective dose.

Conclusion: These findings suggest that radiation from the Chernobyl accident is now a less important contributor to oxidative stress in Belarussian children than is urban living. One explanation could be that the present radiation exposure is below the threshold of 8-OHdG excretion as the effective dose is about 1/1000 of the therapeutic doses after an increased excretion have been seen. The intriguing urban factor needs to be explored.

INVESTIGATION OF THE PARTICULARITIES OF THE LOW-DOSE ACUTE AND CHRONIC GAMMA-IRRADIATION EFFECT IN MOUSE BONE MARROW CELLS *IN VIVO* USING THE MICRONUCLEUS TEST

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In our previous studies the induction of cytogenetic damage (percent of cells with chromosome aberrations or micronuclei) by low doses of acute (dose rate of 47 cGy/min) and chronic (dose rate of 0.01 cGy/min) gamma-radiation was studied in the culture of Chinese hamster fibroblasts, human lymphocytes, *Vicia faba* seeds and seedlings. The sensitivity of these objects to low doses was higher than it was calculated by extrapolation from higher to lower doses. There is no statistically significant difference between the yields of cells with micronuclei induced by low doses of acute vs. chronic irradiation in the examined dose range. The aim of this study was to investigate the dose-effect, adaptive response and genomic instability induced by low-dose gamma-irradiation in mouse bone marrow cells *in vivo*.

SHK and BALB/C male mice were used in our experiments. The freguency of micronuclei was estimate in bone marrow cells of mice at doses of 5-50 cGy. At the moment of low-dose adapting gamma-irradiation (10 or 20 cGy with a dose rate of 1.25 cGy/min) animals were 2 months old. In the experiments using standard adaptive response protocol the challenge dose was 1.5 Gy (1 Gy/min). Bone marrow specimens for calculating micronuclei in polychromatic erythrocytes were prepared by a conventional method with minor modifications.

The results of our study demonstrate that: 1) the dose-response curve can be divided into three parts: a plateau and two linear segments. At very low doses the dependence of cytogenetic damage on dose can be fitted by linear regression with supersensitivity, which then turns to a plateau and at some higher doses the curve becomes linear again; 2) a single low-dose of gamma-radiation induces the cytogenetic adaptive response in mouse bone marrow cells which persists for up to 12 months after irradiation; 3) by using the test "adaptive response", it is possible to detect the transition of γ-radiation-induced genomic instability in sex cells of male parent into somatic cells of mice (F_1) either from changes in radiosensitivity or by the absence of the adaptive response induced by a standard scheme.

THE PHOTOCHEMILUMINESCENCE OF RAT'S ORGANS AT THE INFLUENCE OF GALARMIN IN ALUMINUM NEUROTOXICOSIS

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It is of great interest to evaluate the influence of recently discovered by Galoyan prolin-rich polypeptide (PRP or galarmin) that possesses also the immunotropic and neuroprotective properties, on intensity of photochemiluminescence (PCL) of homogenate of rats different organes and blood serum in aluminum toxicosis as experimental model of Alzheimer's disease (AD). It is well known that lipid peroxidation plays a significant role in the development of quite a number of human diseases included AD (Vladimirov Yu.A.,1999).

Experimental model of AD was created in rats by double subcutaneous injection of 0,2 ml of 10 solution of $AlCl_3$. For the experiments 72 white rats were used. Control and experimental animals were killed at 12th day after injection of $AlCl_3$. Serum and homogenates of organs (hypothalamus, hypophysis, thymus, adrenal, testicles, ovary, uteurs) underwent to action of ultraviolet (UV) radiation by UV-lamp (Medicor, Q-439, Budapest) ,exposition 1 min, distance to quivette 5 cm. PCL intensity was measured on a quantometric divise, equipped with FEU-140 photomultiplier (Zakaryan A.E.,1990).

The PCL intensity accompanying the oxidation reflects the concentration of peroxyl radicals of the lipid at each moment of time was always higher than the level of spontaneous CL on the aluminum neurotoxicosis. The highest degree of PCL intensity was recognized in thymus, hypothalamus and testicles of male rats. and besides that in the ovary and uteurs of female animales. The administration of galarmin normalized the level of PCL in famale rats hypophysis, thymus and hypothalamus as well as in male hypothalamus, and difinitely lowered it in genital organs.

Thus, we can cunclude, that galarmin plays an essential protective role for neurocells by lowering of PCL intensity in all investigated organs in course of UV-radiation changes.

CORRELATION BETWEEN INTRACELLULAR LEVEL OF NITRIC OXIDE AND FREQUENCY OF GENE SOMATIC MUTATIONS AFTER LOW DOSE RADIATION EXPOSURE

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As it was shown earlier, some proportion of individuals exposed to low doses of ionizing radiation was characterized by genomic instability of lymphocytes that manifested, in particular, in elevating frequency of cells with variant/mutant phenotype at T-cell receptor (TCR) locus (Saenko et al., 1998). There is a growing consensus that disturbance of cellular redox homeostasis plays an essential role in induction of genomic instability after irradiation. Some of the redox system components are nitric oxide (NO) and products of its oxidation, that are able to exert direct genotoxic action, on the one hand, and serve signal-regulatory functions providing active cell response to damage, on the other hand. The purpose of this work was to study possible relationship between intracellular NO level and genomic instability of lymphocytes after irradiation with low doses. The level of NO in peripheral blood lymphocytes and frequency of the TCR-mutant cells were assessed by flow cytometry in 112 workers of atomic industry with mean dose (±SE) 114,9±10,8 мSv, accumulated within 21,4±1,1 years. 25 individuals (22%) had the TCR–mutant cell frequencies exceeding the 95% confidence interval in unexposed age-matched control group ($>7.1 \cdot 10^{-4}$). The frequencies of mutant cells in other workers corresponded to those in control group. It was found positive correlation between the intracellular NO level and the TCR-mutant frequency (R=0,37, p=0,01). The mean level of NO in individuals with the elevated TCR-mutant frequency was significantly higher than in others: 1650±66 vs 1281±48 relative units (p=0,04). The results suggest that reactive nitrogen species may, in certain degree, influence on manifestation of genotoxic effects of irradiation with low doses.

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MECHANISMS OF HUMAN CELL DEFENCE AGAINST RADIATION

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Defence system determines cell homeostasis in human cells. This system includes genes controlling antioxidant properties (superoxid dismutase, catalase et.al.), biotransformation of xenobiotics (CYPIAI, CYP 2 E1, glutation-S-transferases et.al.), repair activity (BER, NER, MMR) et.al. Besides genome defence can be achieved by radioadaptive response (RAR), when pre-treatment by small doses of radiation or chemical can form the cell stability, to damaging doses.

The discovery of the new systems, affecting cell homeostasis generates some riddles. Among those could be the following:

1. How many systems are there in the cell not allowing the damaging influence of DNA;
2. Who is the first taking part in DNA repair;
3. What acting sequence is in different defence systems and there is any exchange in their order;
4. What is the way to explain RAR-paradox: relatively high RBE at small doses of radiation and simultaneous forming of resistance to damaging influence;
5. Is there any dependence and of that kind between RAR and DNA repair;
6. Are the mechanisms providing RAR at the differing adapting factors similar;
7. Why does not the people majority living in higher radiation regions form RAR but does form adaptive response to non-radiational factors as, for example, bleomycin.

This presentation is to be followed by the discussion on some items, mentioned above.

RADIATION-INDUCED BYSTANDER EFFECT – MODERN EFFECT OF LOW DOSE RADIATION. CRITICAL REVIEW ON BASIS OF LITERATURE AND OWN DATA

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Radiation-induced *Bystander effect* (BE) – phenomena of irradiated cell(s) affect to un-irradiated neighbouring ones. This phenomenon was observed by radiobiologists in 60^{th}. It became widely popular after a paper in 1992, demonstrated increased level of sister chromatid exchanges after alpha-particle irradiation of small part of Chinese hamster cell population. There are some specific features of BE known from the literature. It is shown that irradiation of small part of cell population (or even one cell through nuclei or cytoplasm) could lead to enhanced level of chromosome, chromatide aberrations, apoptosis, micronuclei frequency, mutations, transformation, cell death, and also to adaptive response, genetic instability and changing of genes expression. BE can be induced by high and low LET radiations, at low (1 cGy and lower) and at high doses. BE was demonstrated *in vitro* and *in vivo*. There is not known a lot about mechanism of BE despite of huge amount of experimental data: a) BE is induced not in all cell lines and not by all cells; b) laboratory investigation are sometime controversial and not always agreed with epidemiological findings; c) the mechanism is still unclear. There could be many different mechanisms related to effect which is called "Bystander Effect" now.

In our study evidences about absence of bystander effect were presented on primary human fibroblasts and on glioma cells. Three different approaches to induce bystander signal by high and by low LET radiation and three endpoints were used. The results are conflicting with other studies utilized similar protocols and cell lines. The possible explanations of nonappearance of the effect in the study and controversies found in literature related to BE were discussed.

RADIOECOLOGY

THE STOCHASTIC DESCRIPTION OF DISTRIBUTION OF RADIONUCLIDES IN SYSTEM "NPP-ENVIRONMENT"

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For a subsystem «Radioactive fallouts - ground - drain» on the basis of the law of mass action the stochastic differential equation for conditional probability of radionuclides distribution in the allocated subsystem is deduced. At the equations compiling the fallouts from the nuclear power plant (NPP) and global fallouts are taken into account. The conditional probability $P(x,t/x',t')$ is determined as probability that at the t time moment the concentration of radionuclides in soil is equal x, under condition that during the previous moment of time t' concentration of radionuclides in soil was x'. As parameters in the equation are inserted the constants of speed of radioactive fallouts on a soil surface from the nuclear power plant and atmosphere and a constant of speed of transition of radionuclides from soil into a drain. From the stochastic differential equation the equations for average concentration of radionuclides in soil ground and its dispersion are received. It is shown, that expression for average concentration of radionuclides in soil in accuracy coincides with the decision of a deterministic equations. It is shown also, that the dispersion of concentration of radionuclides in soil is equal to average concentration of radionuclides in soil, that specifies that casual process of distribution of nuclides in a subsystem «radioactive fallouts (from the nuclear power plant and from atmosphere)-soil - drain» is Poisson process. Correlation functions and spectral density of the radionuclides concentration in soil have been received. It is shown, that with increasing of constant of speed of radionuclides transition in a drain the time of correlation decreases. It is shown also, that the spectral density has Lorentzian type and frequency of "cut" increases with growth of a constant of speed of radionuclides transition in a drain.

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TRITIUM IN THE WATER ECOSYSTEMS OF THE URAL REGION

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Tritium (a radioactive isotope of hydrogen) is a widely spread pollutor of natural waters. Its natural background level is averagely 1 Bq/l, the technogenic background level is 5 Bq/l. Under operation of nuclear fuel enterprises additional quantities of tritium enter the environment and cause the local contamination of water ecosystems around such enterprises. The Institute of Plant & Animal Ecology conducts a systematic research of the effect of the Beloyarskaya atomic power station (Sverdlovsk region) and the "Mayak" enterprise (Chelyabinsk region) on the pollution of water ecosystems with tritium in the Ural region. Since 1980 tritium content has been estimated in the area adjacent to the Beloyarskaya power station: the cooling reservoir (Beloyarskoye reservoir), the Olkhovskoye bog-river ecosystem, snow and rain precipitation, drinking water, some soil types. Monitoring of the radionuclide in the cooling reservoir revealed pollution levels above the technogenic background in all observation points, including the upper reaches of the reservoir, where tritium level ranged from the technogenic background level up to 60-70 Bg/l. Near the dam it was up to 93 Bg/l, in the heating zone – up to 105 Bg/l, in the area of the Biophysical station – up to 1000 Bg/l. Tritium entered the Beloyarskoye reservoir mainly from the discharge canal and the upland ditch. During the first 35 years after starting the power station tritium content in the Olkhovskoye bog-river ecosystem was relatively high and varied in the range of 500-1000 Bg/l. After the discharge reconstruction and tritium content in the water of the bog ecosystem reduced sharply. It was experimentally estimated that besides open water ecosystems in the area of the power station tritium contaminated the air environment, as a result snow and rain precipitation contained the radionuclide in concentrations significantly exceeding the technogenic level. In the area of the "Mayak" enterprise tritium concentration decreased with the distance from the enterprise. This evidenced that the "Mayak" is a pollution source. In the 20-25 km zone around the enterprise tritium concentrations in ponds is averagely 4-10 times over the technogenic background level, at a greater distance – 2-3 times. Almost all tritium in Ural region, including the control plots, was of anthropogenic origin.

A SIMPLE METHOD FOR THE EVALUATION OF SIDE DOSES IN RADIOTHERAPY

Arrigo A. Cigna

Radiation protection is taken into account in radiotherapy practice from the point of view of the medical aspect in order to achieve the best ratio between the tumour control probability (TCP) and the normal tissue complication probability (NTCP). But any radiotherapy treatment implies a dose delivered to the patient's body also outside the beams. Therefore, there is a certain interest important to quantify that which can be considered a negative impact of radiotherapy also if it cannot absolutely be avoided and, in any case, the benefit of radiotherapy should prevail.

The dose delivered to the patient by the radiation scattered outside the direct beams has been measured in general in phantoms. This procedure has the advantage of avoiding the direct involvement of the patient but, on the other hand, a phantom implies a certain degree of approximation in the reproduction of the real characteristics of a human body.

In particular rather inaccurate phantoms are normally used. This simple solution supplies reliable results to some specific questions as, e.g., the measurement of the collimator scatter or the dose distribution in the penumbral region. But when the dose to the whole body or to a single organ has to be evaluated, such phantoms are totally unreliable. In fact the dose distribution within the body varies notably with the distance from the target volume and the average value can be identified with difficulty if only one value is available.

The scope of this study is the evaluation of the dose, outside the beams, delivered to the body of a patient submitted to external radiotherapy by means of direct measurements. The radiation scattered by the target was monitored by means of pen dosimeters distributed along the body. A virtual model was obtained by dividing the body into about 800 small volumes where the doses could be assumed uniform. The power functions obtained by the best fit of the experimental values were used to calculate the effective dose delivered to the body and single organs due to scattering only.

EVOLUTION OF INFUSORIA CULTURE GROWTH UNDER MN-PORPHYRIN INFLUENCE

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Porphyrins are fascinating compounds with biological significance. The sensitivity of Infusoria (*Paramecium caudatum*) in vitro to the Mn-porphyrin was studied. The dose-dependent effect was demonstrated: low (from 0,25 to 0,75 mg/ml per 2 ml of culture solution) concentrations stimulated the infusoria culture growth and high concentrations (1,5-2,0 mg/ml) were toxic.

These studies offer strong evidence regarding the influence of Mn-porphyrin to culture of Infusoria in vitro.

The main objectives of the study are:

1) to test the relative cytotoxicity and toxicity in order of newly synthesized porphyrin compounds, especially Mn-porphyrin influence to Infusoria cells;
2) to reveal the influence of low porhyrin's concentration to Infusoria culture .

Milestones

1.Optimisation of culture conditions to obtain the maximum infusoria cells.

2.Determination of susceptibility of infusoria to Mn-porphyrin influence.

3.Analyses of the cell growth in variable concentration of porphyrin compounds

Infusoria cells will be cultured by routine techniques in standard 20-squares' culture plastic flasks. Infusoria have been cultivated at 28-30^{0}C on banana substratum. The Mn-porphyrin different concentranion have been used to the culture (100 µl agent solution to the 2 ml infusoria culture).During few experiments Mn-porphyrin has been applied by concentrations 2.0-0,02 mg/ml, 1-0,25 mg/ml, and 2-1,0 mg/ml.

Experimental trials of Mn-porphyrin with infusoria culture indicate that infusoria density reduction is dependent of agent concentration level. The culture growth rate and cell death was determined. Experimental setting include identifying lowering of infusoria since 1.5 mg/ml, but the middle and low concentrations of Mn-porphyrin have been stimulated the growth of cells.

FRUIT TREES AND VINES MALE GAMETOPHYTE GENERATION IN SURROUNDINGS OF ARMENIAN NUCLEAR POWER PLANT

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Plant pollen grains fertility and size parameters, including fruit trees and vines, allow one to estimate gametocide effects of environmental mutagens. To this end it is reasonable to use plants that proceed in the investigated environment both sporophyte and gametophyte cycles. Reproductive parameters of male gametophyte of some species of fruit trees and some sorts of vine were analyzed in plants growing on distances of 3-5 km from the Armenian Nuclear Power Plant (ANPP), near to settlement Metsamor, in comparison with the control point on the distance more than 30 km from ANPP. Biometric parameters of pollen are investigated on the preparations stained by acetokarmin.

Table. Properties of pollen size of fruit trees and vines in different points of growth

Investigated taxon	Metsamor		Control point	
	Diameter, mcm	Volume, mcm^3	Diameter, mcm	Volume, mcm^3
Fruit trees				
Cherry wild	45.45	49151.1	45.47	48247.3
Cherry, sort Shpanka	49.79	43944.3	42.98	41550.63
Quince	47.40	53703.31	41.81	38248.90
Sorts of vines				
SHAHUMYANI	28.75	12671.37	27.97	11451.33
KISHMISH BLACK	28.92	8292.16	28.08	11488.19
KISHMISH WHITE	27.83	11391.32	26.91	106031.71
ARARATI	26.68	9871.92	26.32	9871.02
ARAKSENI	26.38	9871.42	26.10	9326.03
HADISI	26.68	9871.96	26.68	9871.72

The obtained results show that the size of pollen of investigated sorts of fruit trees and vines growing around the area of ANPP do not significantly change. Together with our previous data on the absence of difference on their fertility the influence of the ANPP on the male generative system of the investigated fruit trees and vines is not revealed.

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ALTERNATIVE APPROACH IN ARMENIA: ITS INTRODUCTION IN PRACTICE OF RESEARCH AND APPLICATION IN RADIOBIOLOGY

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Our Department is an only institution in Armenia using cells *in vitro* (cultures of normal and transformed human and animal cells) as alternatives to animal experiments. This approach meets international principles and ideas of 3 Rs (Refinement-Reduction-Replacement). Experimental cell systems *in vitro* are known to be pragmatic solution for the early screening of potential ecotoxicity of various physical and chemical agents, safety testing and risk assessment, determination of acute and chronic toxicity (cyto- and genotoxicity) of chemical compounds, including environmental pollutants and potential mammalian cell-based biopharmaceuticals. These systems are also applicable in toxicogenomics and for development of environmental standards. The main direction of our work is testing of new biologically active chemicals as potential pharmaceuticals. The techniques used at our Department are: cultured cell survival determination by vital dye exclusion and cell clonogenic activity, genotoxicity evaluation by induction of chromosome aberrations and micronuclei, Comet-assay and Comet-assay in combination with FISH (Fluorescent *In Situ* Hybridization). The cell system *in vitro* developed is employed also to assess radioprotective/radiomimetic and oxidative/antioxidant properties of chemicals. Earlier we have identified two new Mn-chelates possessing radioprotective and antioxidant activities [Bajinyan *et al.*, 2004]. This work is supported by ISTC Foundation (Projects A-361 and A-301.2).

EFFECTS OF CONTAMINANT EXPOSURE ON PLANTS. IMPLICATIONS FOR RADIOLOGICAL PROTECTION OF THE ENVIRONMENT

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Low-level chronicle exposures to a number of anthropogenic agents of various natures simultaneously are peculiar to a nowadays ecological situation. These exposures, however, are over background levels that biota has evolutionary adapted at. These influence systems of cellular level of biological organization, first of all. An assessment of consequences from increasing of man-made influence on biota should be based on the clear understanding of regularities of cell response to weak external exposures. In this report, results of laboratory, "green-house" and field experiments carried out on different species of wild and agricultural plants (barley, Scots pine, bulb onion and others) to study toxic and genotoxic effects of such common pollutants as γ-radiation, artificial and heavy natural radionuclides, heavy metals and pesticides are presented. The results of long-term field experiments in the 30-km Chernobyl NPP zone and at territories of radioactive waste storage facilities (Sosnovy Bor, Leningrad region; Vodny, Komi Republic; Obninsk) are discussed in detail. Consequences from data obtained are discussed for a network organization for biological monitoring of anthropogenic pollution and a development of scientific basis for radiation protection of the environment.

THE RADIATION PROTECTION STRATEGY ON THE TERRITORIES CONTAMINATED BY RADIONUCLIDES

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The biological radiation protection of the human being by means of chemical agents it is impossible to limit only using by radioprotectors. Now when million contingents of the population have find itself within the territories contaminated by long-lived man-made radionuclides and up to 80–95 % of a dose catch due to internal irradiation, it is necessary to consider as the basic way of radiation protection a minimization of radionuclide passing into the system with food stuff and water. On the one hand it is achieved due to production of plant-growing and cattle-breeding produce and, accordingly, food stuffs by means of special receptions and technologies which reduce a radionuclide accumulation, and on the other hand – due to use of chemical agents (radioblockators) which limit of radionuclide transfer into the human organism. Such blocking can effectively act not only at a stage of food stuffs – human being, but also on much earlier parts of a trophic chain: "soil–plant", "plant (forage)–animal", "plant-growing and cattle-breeding produce–food stuff". Among substances, which can block of radionuclide transfer in organisms, three basic groups are considered: antagonists of radionuclides, complexions and adsorbents. At the stage "plant–animal" the special value has got the preparations, which bind radionuclides in a gastrointestinal path of animals. First of all it is necessary to consider among them a ferrocin. Only its addition to forages allows to decrease a ^{137}Cs transition in meat and milk in 6–8 times. There are additives to forage like the natural adsorbents of above mentioned zeolites type. At last at stage "food stuff–human being" it is necessary to carry to radioblockators the provisions containing increased quantities of calcium and potassium, phosphorus, pectin substances, alginates etc., which binding radionuclides in a gastrointestinal path of the human. The important role should be assigned to the substances accelerating removing of radionuclides from the organism – radiodecorporants. Thus the strategy of radiation protection of the human being should represent multi-stage complex of methods and actions which includes a blocking transport of radionuclides at all stages of trophic chain, protection form ionizing radiation, both external, and internal, and an acceleration of radionuclide removing.

CASUALTIES AND RADIATION DOSIMETRY OF THE ATOMIC BOMBINGS ON HIROSHIMA AND NAGASAKI

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On August 6, 1945, the first atomic bomb *LITTLE BOY* exploded above Hiroshima, and three days later the second bomb *FATMAN* was dropped on Nagasaki. The liberated energy by nuclear fission (16 kton TNT in Hiroshima and 22 in Nagasaki) instantly ruined both cities. The numbers of inhabitants were 340,000 and 220,000, respectively, in Hiroshima and Nagasaki. The death rate of the people who were within 1 km from the hypocenter in Hiroshima was about 70 % in the first day and reached more than 90 % three months later. According to the reports from both city governments, 140,000 and 70,000 deaths occurred in Hiroshima and Nagasaki by the end of 1945.

In order to investigate late effects of atomic bomb radiation on humans, the US government founded ABCC (now RERF) in Hiroshima in 1948. Through the special inquiries attached to the national census in 1950, 284,000 people were confirmed to be atomic bomb survivors. Based on this information, ABCC selected 94,000 survivors as the subjects to be used in their epidemiological study *LSS: Life Span Study*. At the same time, comprehensive efforts were started to estimate individual radiation dose both by interviewing every survivor about the location and situation at the time of bombing and by developing methodology to calculate radiation field in both cities. The author has been involved in development of the latest version of dosimetry system *DS02: Dosimetry System 2002* as a member of Japan-US Joint Working Group. At 1 km from the hypocenter, radiation dose at 1 m above the ground in open field was estimated 4.5 and 8.7 Gy, respectively, in Hiroshima and Nagasaki, while at 1.5 km they were 0.54 and 0.99 Gy.

According to the latest LSS report (2003) for the period of 1950-1997, among 86,572 survivors to whom individual dose was estimated, there have been 44,771 deaths (52 %), including 9,335 from solid cancer and 31,881 from non-cancer diseases. The dose-response analysis of LSS data indicated excess relative risks of 0.47 Sv^{-1} and 0.14 Sv^{-1}, respectively, for solid cancer death and non-cancer disease death.

OPPORTUNITIES FOR USE OF *LECCINUM* AS BIOMONITORS OF RADIOACTIVE AND CHEMICAL POLLUTION IN WOOD COMMUNITIES

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Now use of fungi for bioindication is limited by the chemical analysis of heavy metals and radioactive elements in fruit bodies. The essential restrictions are caused as sporadic of fruit bodies production and small conformity between amount of polluting substances in sporocarps to quantity them in the soil.

The problem on search of species-biomonitors, capable to change morphological attributes under influence of polluting substances, will open new opportunities on use fungi for estimation of environmental condition. Potential biomonitors fungi should satisfy to following conditions: to have wide geographical distribution, to possess high variability depending on ecological conditions and forming ectomycorrhizas.

The genus *Leccinum*_S.F. Gray (*Boletales*, *Boletaceae*) corresponds to these requirements. The main reason influencing on choice Leccinum has become detection of abnormal peculiarities of a structure of rDNA site, playing a key role in albumen biosynthesis.

The *Leccinum versipelle* (AY237178) identified from ectomycorrhizal rootlets of Picea abies by PCR/ITS/RFLP method with further sequencing and comparison with INSD has shown abnormal length of ITS1-5,8S-ITS2 by the size 1600bp. The reason of this phenomenon that ITS1 is organized by repeating minisatellites sequences named also VNTR (Variable Number of Tandem Repeats). Sequence GAAAAGTA meet 12 time and CTAATAGA – 11. VNTR are usual at plants, animals and human DNA. They are characterized by high variability and are used for population analysis. Probably this is the first discovery of minisatellite-like repetitive sequences in ITS1 among basidiomycetes whose rDNA were investigated.

May be the *Leccinum* species are useful as biomonitors of radioactive and chemical pollution in wood communities? For answer this question is necessary to investigate species genetic structure (for example by RAPD) and understand what this species are: the organisms with high variability depending on ecological conditions or species complexes in the state of a divergence or independent species.

USING GIS MAPPING FOR AN ESTIMATION OF SURFACE CONTAMINATION OF BELARUS TERRITORY BY RADIONUCLIDES OF IODINE AT AN ACTIVE STAGE OF THE ACCIDENT

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Immediately after the accident at the Chernobyl nuclear power plant the short-lived isotopes of iodine represented the greatest radioactive danger. In April 1986, after the accident, the direct measurements of radioactive contamination by ^{131}I of the soil-vegetative cover of the country territory were not carried out. The maps of ^{131}I soil contamination, performed with the help of GIS, ver. 3.1 ARC/VIEW, let us to get the most visual estimation of radioactive situation at this stage.

At the creation of contamination maps of Belarus territory the following experimental data and aspects were accepted: as a basic unit of time scale the day was accepted; the experimental data of plane-table measurings of ^{131}I diurnal fallouts were utillized; for points, where ^{131}I was not measured, the data are acquired according to the accumulation of ^{137}Cs on the ground and reconstruction on ^{129}I; the additional formation of ^{131}I due to decay of ^{131m}Te is taken into account; the information for GIS was obtained from a central database (designed by the authors) in the shape of points. Each point contains the information on a level of radionuclides contamination of a subjacent surface, air bottom layer, population etc.

Thus, on the basis of the maps obtained on contamination of territory of Belarus by ^{131}I, radioactive conditions at active stage of the accident in dynamic from April 26 till May 5, 1986 were reconstructed. Approximately 35 % from all ^{131}I to be found in the active zone of reactor just before the accident has fallen out on the territory of Belarus. The basic contamination of the whole area is considered to be result of single-pass fallouts. The maximal levels of contamination were observed in Radin - Kruki spots of a near-field northeast track - $4*10^8$ Bk/m^2, in spots of far-field northern track Vetka -Dobrush – $5*10^5$ Bk/m^2 and Chudyany - $1,5*10^5$ Bk/m^2. The received cartographical information can be considered as a basis at a selection of regions, where it is necessary to carry out long-term medical screening of the population with age up to 18 years at the moment of the accident.

INVESTIGATION OF SOIL RADIOACTIVITY IN ECHMIADZIN

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Radioactivity level due to global radionuclides (^{90}Sr, ^{137}Cs and other short-lived isotopes) fall-out decreases . As an example, β-radioactivity level of atmospheric precipitations in Yerevan in 1989-1998 compared with 1969-1983 decreased for 52%.

We conducted study of soil radioactivity in Echmiadzin and its neighborhood in 2003 and 2004. β-radioactivity measurements were performed on radiometer ÐÏÃ-1åÌ, ^{226}Ra and ^{137}Cs were defined on γ-spectrometric facility CANBERRA.

In soil samples taken in 2003 ^{137}Cs concentration was in interval of 24,8-118,7 Bq/kg, average 47 Bq/kg. ^{226}Ra concentration was in interval of 29,0-41,1 Bq/kg and β-radioactivity - 708-758 Bq/kg, which exceeds natural level of β-radioactivity of 600 Bq/kg.

In samples taken in 2004 existence of ^{137}Cs was determined in seven probes. ^{137}Cs concentration was in interval of 13,4-93,0 Bq/kg, average 40,2 Bq/kg. Such a large spread of values is probably related to vegetation differences at the points of sampling. ^{226}Ra concentration was in interval of 24,9-39,7 Bq/kg and β-radioactivity - 657-770 Bq/kg,

Also study of radioactivity of field soil samples was conducted in the directions of Echmiadzin-Shaumyan and Echmiadzin-Armavir main-roads. The distance between sampling points was 1-2 km, field soil samples were taken at the distance of 15-20 m from the road. Field soil β-radioactivity level was in interval of 666-715 Bq/kg. ^{226}Ra concentration was in interval of 18,5-51,5 Bq/kg and ^{137}Cs – 18,9-46,5 Bq/kg.

β-radioactivity level and radionuclides concentrations variations can be explained by differences in microrelief, agrotechnical conditions and other. Not significant increase of soil radioactivity both in Echmiadzin and along main-roads is probably due to motor transport exhausts and continual fall-out of small amounts of ^{137}Cs.

^{137}Cs AND ^{90}Sr IN THE AQUATIC SYSTEM: THE KAKHOVKA RESERVOIR –THE NORTHERN-CRIMEAN CANAL-THE KARKINITSKY BAY OF THE BLACK SEA

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The problems of drinking and irrigative water supply for the South of Ukraine are solved significantly due to the Dnieper River water. The Northern-Crimean Canal (NCC) is the main source of fresh water for the Kherson Province and the Crimea. The Dnieper River water arrives to these Regions from the Kakhovka Reservoir via the NCC. The biggest part of this water is used for irrigation of agricultural crops and first of all of the rice. During the irrigation the Dnieper water goes through NCC main canal, agricultural fields, drainage systems, including discharged canals, and arrives to the Karkinitsky Bay of the Black Sea. So, radioactive pollutions in the Dnieper water is transfered not only to the Kherson Province and the Crimea but also to the Black Sea. We studied the distribution of ^{90}Sr and ^{137}Cs concentrations in bottom sediments of the NCC as the main accumulative object of the aquatic system. It was observed that they were decreased with distances from the NCC start point to the Karkinitsky Bay. After irrigation ^{90}Sr and ^{137}Cs concentrations in bottom sediments were 2.5-3 times lower than at the beginning of NCC (Fig.1). Hence, the role of bottom sediments in the extraction of these radionuclides from the NCC aquatic system is very significant. The values of ^{90}Sr and ^{137}Cs concentrations in water and sediments were used for the determination of their fluxes to the Black Sea with the Dnieper water via the aquatic system of the Kakhovka Reservoir-NCC-the Karkinitsky Bay.

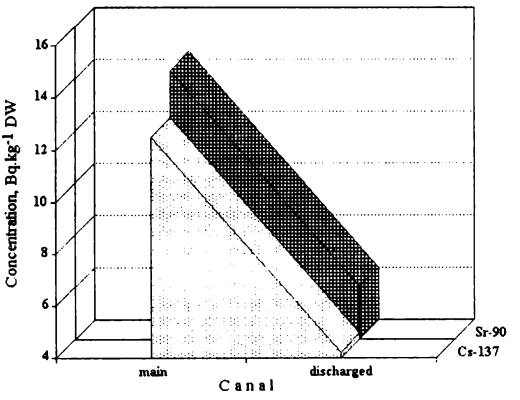

Fig.1. Concentration radionuclides strontium and cesium in the NCC bottom sediments

DOSES TO THE BLACK SEA HYDROBIONTS FROM THE NATURALLY OCCURRING RADIONUCLIDE ^{210}Po

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The investigation of the Black Sea hydrobionts ability to accumulate the natural radionuclide ^{210}Po was started in Ukraine in 1998. It covered net zooplankton, 4 species of algae, 17 species of fishes and 5 species of mollusks. The sampling places were located in different bays of Sevastopol and along the coast of the Crimea from the Cape Lukul to the Cape Sarych. ^{210}Po concentrations were increased from zooplankton and algae to fishes and mollusks. The ability of the Black Sea fishes to accumulate ^{210}Po depended on their belonging to different ecological groups, increasing from benthic and demersal to pelagic species. The highest ^{210}Po concentrations were determined in the Black Sea anchovy *Engraulis encrasicholus ponticus* (40 Bq·kg^{-1} wet weight), sprat *Sprattus sprattus phalericus* (28 Bq·kg^{-1} wet weight) and mussel *Mytilus galloprovincialis* Lam. (60 Bq·kg^{-1} wet weight) which are the main commercial species of fishes and mollusks in the investigated area. Hence, the absorbed doses delivered by this radionuclide to anchovy, sprat and mussels are equal to 1.1, 0.9 and 1.64 mGy·a^{-1}. ^{210}Po is accumulated by marine organisms through food intake. The concentrations of ^{210}Po in the viscera of the Black Sea sprat were 2.5-4 times higher than those determined in this fish whole body. The calculated rates of the absorbed doses in its viscera ranged from 2.2 to 3.5 mGy·a^{-1}. A radiation weighing factor equal to 20 was applied for the calculation of equivalent doses delivered by ^{210}Po to the investigated species of the Black Sea biota. The highest values were estimated in the Black Sea anchovy (22 mGy·a^{-1}), sprat (17.5 mGy·a^{-1}) and mussels (33 mGy·a^{-1}). The data presented above show that the highest doses, calculated in the Black Sea mussel *M. galloprovincialis* and pelagic fishes anchovy and sprat, are about two orders lower than the dose limit (4 Gy·a^{-1}) proposed by IAEA for the protection of aquatic organisms from ionizing irradiation.

CHERNOBYL URANIUM ON TERRITORY OF BELARUS

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Among all radionuclides, thrown out in biosphere in the result of the accident at the Chernobyl nuclear power plant (NPP), the biggest part in terms of mass is surely uranium. The estimation of contamination levels of territory of Belarus by Chernobyl-originated uranium was not carried out. In this report the calculation results of Chernobyl uranium total mass for Belarus territory counted for uranium dioxide are given. In the first case the tracer of the spent reactor uranium ^{236}U isotope was used to determine the total mass of fallouts. That fact was in case of the latter taken into account, that U and Pu have dropped out together in a composition of so called fuel particle.

Methodological basis for an estimation of uranium fuel amount with the help of ^{236}U in soil fallouts after the accident at Chernobyl NPP is formed first of all by calculated correlations between isotopes of uranium in an active zone of the reactor depending on the burn-up of the fuel, and also by experimental data providing isotopic ratios of naturally occurring isotopes and accident originated ^{236}U, ^{235}U, ^{238}U. The measurements of uranium isotopic ratios to ^{236}U are performed with the help of the mass spectrometer.

In case of the latter, knowing an integral reserve $^{239.240}Pu$ in soil and $^{238}U/^{239}Pu$ and $^{238}U/^{240}Pu$ ratios, it is possible to determine the amount of the Chernobyl originated ^{238}U, dropped out on the ground surface of Belarus as a result of the accident. The calculations by both methods were carried out for 30 km zone, for area, close to 30 km zone, so called near-field area, and far-field areas of contamination. The estimation of spent reactor uranium total mass for territory of Belarus counted for uranium dioxide by the first method is equal to 3,2 t; by the second method - 3,9 t (1) and 8,9 t (2) (the following data on an integrated reserve $^{239.240}Pu$ in soil were utilized: 1 - Institute of the radiobiology, Minsk, Belarus, 2 – Institute of the agroradiology, Kiev, Ukraine).

Just before the accident there were 214,6 tonnes of fuel ($^{238+235}UO_2$) in the building of 4th unit of the NPP; 190,2 tonnes were in the active zone and 24,4 tonnes were located in three technological rooms. Thus, the contamination of territory of Belarus by the irradiated uranium is in limits 1,5-4,1 % from the content in fuel of an active zone on the moment of the accident. Further clarifications are required.

N.W. TIMOFEEFF-RESSOVSKY'S VIEWS AND IDEAS AS THE BASIS OF THE RADIOECOLOCIGAL INVESTIGATIONS

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Theoretical fundamental and methodological approach to the investigations of the radionuclide behaviour in biogeocenosis was made by the outstanding researcher N.W. Timofeeff-Ressovsky. He used fundamental natural-historical doctrine for the soils, landscapes and biosphere elaborated by the Great Russian scientists: V.V. Dokuchayev, V.I. Vernadsky, B.B. Polynov, V.N. Sukachev. Considering the experience acquired during centuries of observing harmful environmental impacts of industrial waste, Timofeeff-Ressovsky aimed at a complete study of all possible effects of the development of the nuclear industry on the biosphere. He performed and supervised the first investigations of the radionuclide behaviour into the simple links as soil-solution, soil-plant, water-hydrobionts. He has emphasized the main role of the processes soil formations and runoff in the radionuclide distribution into terrestrial biogeocenosis, of the processes sediment formations and input of the radionuclides into water biogeocenosis. Later his students and adherents started a new trend in radioecology, i.e. a continental radioecology, studying the radionuclide migration and biological effects on natural ecosystems of land and internal reservoirs. The natural ecosystems, owing to their structural and functional properties involve radioactive substances into biogeochemical migration cycles and, as consequence, the accumulation of rather high concentrations of the radionuclides in some links of an ecosystem is being observed. Based on this statement one of the main paradigms of radioecology was formulated: a natural environment can not be considered as a passive diluents of radioactive contaminants. An extensive release of radionuclides of various origin leads to the formation of both "hot" spots (impact zones) and buffer territories. Owing to separating of these territories the peculiarities of the radionuclide migration and their biological effects into terrestrial and water ecosystems on the concentrations gradient are investigated. N.W. Timofeeff-Ressovsky's views and ideas are corresponded with the theory of the sick plates of the planet which is being elaborated as a part and a parcel of the contemporary ecological doctrine of Russia.

COMPLEX ASSESSMENT OF ENVIRONMENTAL RADIOACTIVITY IN YEREVAN

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Today, the issue of radioactive pollution of biosphere is acquiring a special topicality worldwide due to rapid development of nuclear technologies, nuclear energy, nuclear weapon testing, accidents in nuclear power plants. This dictates a necessity of radioecological monitoring of urban environment in particular.

The paper highlights the results of first ever complex radioecological research in system atmospheric precipitation-soil-plant performed in Yerevan - the capital of Armenia.

Collating all the results obtained in study period 1969-2003, compiling relevant database and producing the radioactivity distribution maps are important constituents of this research aimed to getting an exhaustive picture of radioactive pollution of Yerevan environment.

The study materials were atmospheric precipitation, soil, plant and moss samples taken in the limits of Yerevan. In the research β- and γ-radioactivity measurement methods were applied. Based on the results, the Yerevan soil β-radioactivity distribution maps (1:10000) were produced using GIS Arc View 3.2 and Surfer 6.04 Programs.

The basic outcomes of complex radioecological monitoring for the entire study period 1969-2003 in Yerevan allow establishing 1) the trend for the decrease of radioactivity level in all the study objects in period 1989-2003 vs. 1969-1988; 2) radionuclide composition of atmospheric precipitation is mainly represented by ^{40}K, ^{238}U, ^{232}Th, ^{137}Cs, ^{90}Sr as of 2000; 3) considerable decrease of soil gross β-radioactivity in Yerevan in 2002 vs. 1990 and the extension of areas with natural background (500-600Bq/kg); 4) the decrease of global radionuclides ^{137}Cs and ^{90}Sr concentrations in all the study objects; 5) that high level of gross β-radioactivity in plants results from man-made pollution (as of 2000); 6) mosses may serve indicators in radioecological monitoring due to their ability of accumulating radionuclides and ^{137}Cs in particular. The map of Yerevan soil gross β-radioactivity distribution is included into General Urban Development Plan for Yerevan (2003).

ROLE OF MOSSES AND LICHENS IN RADIOECOLOGICAL MONITORING OF ENVIRONMENT

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Development of nuclear technologies, functioning of centers for treatment and embedding of radioactive materials and waste, make it necessary to examine the behavior of technogenic radionuclides in biogeocoenoses components. Evaluation and forecast of radio-contamination levels in natural ecosystems, as well as monitoring of the process, call for the usage of bioindicators. In this regard, lichens and mosses are of special interest. Both lichens and mosses play significant role in formation of terrestrial vegetation covering vast areas in tundra, forest-tundra, and forest complexes. Wide distribution of mosses and lichens is favored by their tolerance for many extreme factors of environment and durable life-cycle span. Highly expressed accumulation function of these organisms is determined by special anatomical and morphological features, their physiology and water regime. Complex approach combined with the results of experimental studies of radionuclides' accumulation mechanisms in mosses and lichens allowed to receive a significant data array in order to reveal regularities of accumulation and storage of different technogenic radionuclides. For a sequence of years (over 30), we examined spatial dynamics of radionuclides contents from global fall-out in moss and lichen cover of the tundra, forest-tundra, taiga and pre-taiga regions. Modern (background) levels of 90-Sr and 137-Cs have been determined for the whole moss-and-lichen terrestrial cover and separate taxa of mosses and lichens, in different regions of the Urals and Siberia. General amounts of radionuclides stored in lichens and the whole moss-and-lichen cover were estimated for the South and North Subarctic tundras and the Polar Ural mountain tundras. All the data received indicate to the lichens and mosses making important part in soil vegetation cover as useful indicators for monitoring and forecasts of the environment radioactive pollution values from both global and accidental fall-out of technogenic radio-nuclides. As the role of global fall-out has been registered to decrease and stabilize, significance of mosses and lichens in the process of radionuclides' migration and redistribution within the soil and vegetation cover is marked to grow.

CARCINOGENIC RISK ASSESSMENT AS THE PART OF INTERDISCIPLINARY STUDYING OF ECOLOGICAL RISK

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Radioecological situation has been studied in details in six areas of Gomel region and four areas of Mogilev region. Levels of radiation risk have been determined for these areas. Quantitative modeling of probable number of additional oncologic diseases ascertained by the values of radiation contamination has been carried out. Radiation contribution to the total oncologic diseases of the population of Belarus has been estimated. It has been shown on these grounds that real dynamics of oncologic diseases does not keep within the forecast based on conservative notions about linear dependence «dose-effect».

In connection with this the necessity has been established in order to clear up the whole complex of reasons that generate an absolute value and the growth of oncologic diseases, among them chemical and other ecological aspects should occupy a considerable place.

Risk levels for pathology of breathing organs have been estimated from the data on contamination of air basin with effluents out from stationary sources in concrete populated points of Gomel and Mogilev regions. Ecotoxicological and epidemiological analysis of a complex assessment of the effect of atmospheric pollutants on induction of tumors of breathing organs has been carried out. It has been determined that comparison of risk for health of the population from radiation with the risk from other sources allows to make the conclusion that the rate of ecological danger is determined by chemical contaminants in the Republic in the first instance. It is also true even for those regions which have suffered mostly from radiation contamination as the result of the Chernobyl NPP accident.

It is shown that objective assessment of ecological danger can be developed only by the methods of a comprehensive multifactor theoretical and experimental modeling.

The mathematical model of the combined effect of factors of radiation and chemical nature has been developed for the purpose of detection and analysis of synergism effects in biological experiment with animals.

At the doses of 0.35 Gr a good agreement of experimental dependencies with theoretical points of view on kinetics of reparation processes in cells has been demonstrated.

EFFECTS OF CHRONIC LOW-LEVEL IRRADIATION ON RADIOSENSITIVITY OF MAMMALS: MODELING AND EXPERIMENTAL STUDIES

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Effects of low-level chronic irradiation on radiosensitivity of mammals (mice) are studied experimentally and by making use of the methods of mathematical modeling. Own and reference experiments show that chronic low-level short-term and long-term exposures induce, respectively, elevated radiosensitivity and lowered radiosensitivity (radioresistance) in mice. The manifestations of these radiosensitization and radioprotection effects are, respectively increased and decreased mortality of preirradiated specimens after challenge acute irradiation in comparison with those for previously unexposed ones. The reason of the animal death in the experiments was the hematopoietic syndrome of acute radiation syndrome. Therefore the theoretical investigation of the influence of preirradiation on radiosensitivity of mice is conducted by making use of the biologically motivated mathematical models which describe the dynamics of hematopoietic system in mice exposed to challenge acute exposure following the chronic one. Modeling results indicate that the radiosensitization effect of chronic low-level short-term (less than 1 month) preirradiation on mice is due to increased radiosensitivity of lymphopoietic, granulocytopoietic, and erythropoietic systems accompanied by increased or close to the normal level radiosensitivity of thrombocytopoietic system. In turn, radioprotection effect of chronic low-level long-term (more than 1 month) preirradiation on mice is caused by decreased radiosensitivity (radioresistance) of the granulocytopoietic system appeared at the level of one functional cell pool, blood granulocytes. It is important that modeling estimations of the duration of low-level chronic preexposures, which result in radioprotection and radiosensitization effects on mice, are in agreement with the relevant experimental data. The developed models can be useful in planning of prospective experiments in this field and in estimating the risks of low-level chronic irradiation.

UTILIZATION OF CONTINUAL CYTOGENETIC MONITORING OF COSMONAUTS IN THE PROBLEM OF RADIATION SAFETY DURING SPACE MISSIONS

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The problem of radiation safety of space crews becomes very urgent in conjunction with long-term space flights, such as a mission to Mars. This is connected both with the peculiarity of radiation environment in space and with the duration of a flight to Mars. This problem can be solved by means of creating an effective radiation protection system, which structure must include radiation environment monitoring subsystem. Since biological effectiveness of space radiation is substantially higher than that of standard radiation, such levels of radiation exposure can be very hazardous to cosmonauts. To assess space radiation hazard it is necessary to have reliable information on radiation doses absorbed by crewmembers. This problem can be solved with the use of cytogenetic examination of cosmonauts. Analysis of chromosomal aberrations in human blood lymphocytes is widely used both for indication and either for quantitative assessment of radiation exposures.

This work presents results of cytogenetic examination of cosmonauts, participated in long- and short-term space flights on MIR station and International Space Station.

The results of cytogenetic examination of cosmonauts showed that:

- long-term space flights result in an increase in the frequency of chromosomal aberrations;
- the frequency of dicentrics depends on the flight duration, dose and dose rate;
- the frequency of dicentrics decreases with time after flights, remaining elevated even in several years after the completion of flights;

chromosome aberration analysis will be widely applied to estimate radiation risk in cosmonauts of long-term space missions.

ECOLOGICAL GENE ASSESSMENT

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Ecological genes are considered as ones controlling the interaction between a cell or an organism and external environment and providing for their stability. At present the strategies to reveal cDNA and such gene functions are realized for studying the mechanisms of aluminium plant tolerance. Aluminium is actually considered to be the primary phytotoxic factor in plant occurrence or cultivation on acid soils and amounts to 40% of the total world tilled lands. Over the evolutionaty history of development, however, plants have developed the aluminium tolerance mechanisms. One of these mechanisms is controlled by the wheat Alt gene (aluminium tolerance) [1] and manifests itself by releasing the organic acids from root cells coming into contact with aluminium. Apple, citric and other acids bind aluminium in the near-root zone and make it intoxic for plants [2]. Ryan et al. [3] have supposed that the Alt gene product is a transporter (shaperon) which removes the organic acid from the root tip cells into the near-root area. It is found that various wheat and barley cultivars [2] are aluminium tolerant differently, however, the root ability to release the organic acids does not always correlate with aluminium tolerance. The Alt gene activity in aluminium tolerant cultivars of cereals is assumed to be broken or modulated by other genes. To establish the pleiotropy of ecological gene functions, the barley sprouts were simultaneously treated with aluminium and iron ions in concentrations comparable with those in the acid soil solution. Three types of chromosome aberrations(genomic, chromosomal and chromatid) appear under the action of aluminium tolerant cultivars of barley. Iron ions do not cause aberrations in the root merisystem of bearley sprouts. A prounced decrease in chromosome aberrations is observed for tolerant barley cultivars under a combined action of aluminium and iron. Tolerant cultivars are assumed to induce a transduction signal, as a result of which activated are the genes similar to transferrin or lactoferrin genes of animals [4]. These gene products are capable for inactivating aluminium genotoxicant. A strategy to identify a family of ecological genes is discussed.

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MICROEVOLUTION PROCESSES IN POPULATIONS OF AQUATIC ORGANISMS AFFECTED BY RADIOACTIVE AND CHEMICAL POLLUTION

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Among radiochemoecological problems the study of radiation and chemical effects on aquatic organisms as well as the investigation of microevolution processes in affected populations are of great interest. For several years before and after the ChNPP accident we have studied chromosome mutagenesis in populations of aquatic organisms (worms, mollusks, crustaceans and fishes) that inhabited the Black and the Aegean Seas, the Danube and the Dnieper Rivers, the 30-km ChNPP zone and the region polluted with sewage. The highest level of chromosome mutagenesis was observed in populations of aquatic organisms of the 10-km zone of ChNPP. In this area radiation dose rates are in the range of the 'Ecological Masking Zone' where radiation effects on populations/communities level may be hidden (masked) and/or modified by the influence of other ecological factors, including chemical contamination. In order to identify the deleterious factors we used the methodology proposed by us earlier. This methodology includes a comparison of cytogenetic effects in mutagen equivalent doses, an analysis of chromosome aberrations distribution in cells and a mean number of aberrations per an aberrant cell. The results obtained show that the obvious damaged effects of ionising radiation were observed only in populations in the 10-km ChNPP zone. In other regions chromosome aberrations in cells of aquatic organisms were induced mainly by chemical pollution. In connection with the problem of ecological risk assessment and of "critical" species identification it is important to investigate adaptive possibilities of aquatic organisms populations. It is obviously that reproductive strategy of populations plays a great role in their adaptation. A comparison is made in relation to adaptation rates of aquatic crustaceans and worms populations with different reproduction modes in the region polluted with sewage and in the Chernobyl zone. It is found that the studied species with sexual reproduction have higher rate of adaptation to anthropogenic pollution in comparison with species with the prevalent asexual reproduction. Hypothetic mechanisms of population adaptation are discussed. Criteria of "criticial" species identification are proposed.

ECOTOXIC EFFECTS IN *PINUS SYLVESTRIS* L.POPULATIONS GROWING IN THE VICINITY OF A RADIOACTIVE WASTE STORAGE FACILITY AND IN THE 30-KM ZONE OF THE CHERNOBYL NPP

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The study into cytotoxic effects in the populations of Scotch pine *Pinus sylvestris* L. growing in the vicinity of a plant for storage and reprocessing of radioactive wastes "Radon" and in the town of Sosnovy Bor in the Leningrad region was carried out for 6 years (1997-2002). Previously a similar study was performed in the 30-km ChNPP zone where pine populations were investigated in the region of an asphalt-concrete plant (ACP) and Cherevach (relatively clean area in the 30-km zone).

The levels of cytogenetic damage to the intercalar meristem of needles, as well as the root meristem of germinated seeds of pine populations from the "Radon" and Sosnovy Bor territories were found to significantly exceed the relevant control levels, though being markedly lower than similar values for pine populations from the zone of sub-lethal tree damage (ACP, 30-km ChNPP zone). While for the populations from the 30-km zone typical is a regular growth of the level of cytogenetic disturbances with increase in the exposure dose rate, an increased rate of cell aberrations in both vegetative and reproductive organs of trees from the Leningrad region cannot be explained by radiation effects alone.

A more detailed information on acting factors can be derived from an analysis of structural mutations, which has revealed that in the "Radon" region the main contributors to the pollution of the environment are chemical agents.

A regression analysis of variations (1997-2002) in the rate of aberrant cells in the root meristem of seeds has indicated that pine populations growing in the vicinity of "Radon" and Sosnovy Bor have been experiencing over the whole study period constantly increasing mutagenic effect.

Acute gamma-irradiation at a dose of 15 Gy of part of the seeds collected at the study sites of the Leningrad region has revealed their increased radioresistance, which together with a significant difference in the variance of the study parameters ariance of the study parameters anthropogenic effects populations, is indicative of the ongoing processes of cytogenetic adaptation.

RADIOSENSITIVITY *ATRIPLEX PATULA L.* ECOFORMS DEPENDING ON VARIABILITY OF ITS PHYSIOLOGY-BIOCHEMICAL CHARACTERISTICS

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The purpose of the given research - to reveal influence of variability of physiological and cytology-biochemical characteristics on radiosensitivity goose-foot sprawling, growing in different climatic parameters (South Yakutia, the Central Yakutia, Average Ural).

For comparison of radiostability, both at individual plants, and at ecoforms, the normalized estimation natural – logarithmic doze curves anamorphous of survival rate of plantlets has been applied, which has enabled to establish values quasi-limit dozes (Dq, gray) and a corner of an inclination curve "doze - effect" (tgα).

Cleared up, that high values of factors of a variation of survival rate of plantlets and domed forms of variational curves southern and central yakutian ecoforms of goose-foot sprawling (as against Ural) allow to assume: a) these ecoforms have high adaptive potential; b) in one year of gathering of seeds they grew in approximately in an identical weather environment. High variability of factor antioxidant protection of cells yakutian ecoforms of goose-foot determines its high radiostability (on Dq). And at the Ural population it has been revealed reduced dispersion of chromatin conformation, that has led to increasing of irradiation stability in a range of high dozes (above 250 gray).

The additional acute exposure has led to two-peak occurrence of variational curves of plantlets survival rate distribution. Similar forms of curves have appeared at an irradiation dozes 100 gray (southern and central Yakutian) and 250 gray (Ural). It is assumed, that unitary radiating influence has caused reorganization genomes individuals which was showed in decrease and (or) increase of survival rate of the plantlets, distinct from normal distribution. It allows to speak about occurrence of radio-induced instability genomes cells investigated goose-foot ecoforms, capable to arise in reply to action of any specific force factors (in this case - sharp radiating influence).

INFLUENCE OF ADAPTIVE POTENTIAL OF WILD PLANTS OF YAKUTIA ON THEIR RADIOSTABILITY

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As material for researches seeds and plantlets of 50 kinds of wild plants of Yakutia served. Due to the cytology-biochemical methodological approach dependence between physiological, cytology-biochemical characteristics and radiostability of plants has been shown. It is fixed that result of adaptation to extreme conditions of the North at plant ecoforms of Yakutia is the increased contents of antioxidants (k_{aop}) and increase in functional genome activity (k_{gga}) which makes gene more vulnerable at an acute exposure. Simultaneously it facilitates inclusion enzyme DNA-reparation systems of cells (k_{rep}), chromosomes restoring integrity.

As a result of the carried out researches by us division into 4 groups of radiosensitivity of plants of Yakutia was offered, in connection with their cytology-biochemical characteristics (tab. 1).

Table 1.

Classification of wild plants of Yakutia on a degree of their radiostability (Dq and tgα) depending on the generated adaptive potential

group of radiostability	conditions	Dq, Gy	tgα	k_{aop}	k_{gga}	k_{rep}	k_{gt}
I	Dq>20, tgα<1	69,5	0,8	2,6	1,9	0,2	2,8
II	Dq>20, tgα>1	79,0	2,1	2,1	3,1	0,7	2,1
III	Dq<20, tgα<1	8,1	0,7	0,6	2,1	1,2	2,2
IV	Dq <20, tgα>1	10,9	1,7	0,5	4,8	2,2	1,7

Cleared up, that in Yakutia plants (60 %), radiostability concerning to II group on the classification suggested by us predominate. At them, owing to centuries-old adaptations to sharply continental climate of the Central Yakutia, high levels of the genera genome activity and antioxidant protection were generated at rather low activity of systems of DNA reparation.

BIOSPHEROLOGY

SOME OUTLINES TO THE SPACE MICROBIOLOGY

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Microbiological issues are of primary importance to ensure the reliable and safe exploitation of inhabited spacecrafts. They include large scopes of problems in which microbial deterioration and degradation of polymeric materials and equipment are of the most important ones. The development of integrated methodology for evaluation of the stability of polymeric materials to microbial deterioration should be the background to solve the problem. The presence of extremophilic, opportunistic and toxinogenic forms in microbiota of spacecrafts must attract the priority interest and to be a substantial task in forthcoming microbiological researches.

The paper summarizes the data of long-term researches in study of microbial deterioration of synthetic polymeric materials used in space technique for the creation of the Culture Collection of fungal degradants with DBs and development of integrated evaluation of biostability. It includes generalization of data of previous long-term R&D and methodological approaches for prediction of microbial deterioration and biostability of polymers for space technique.

The data obtained within the ISTC Project A-092.2 carried out in cooperation with Russian and Ukrainian institutions will be presented and discussed.

BIOSPHERE AND HUMANITY: FROM IDEAS OF N.W.TIMOFEEFF-RESSOVSKY UP TO PROBLEMS OF MODERNITY

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Creative environment of Obninsk was favourable to crystallization of N.W.Timofeeff-Ressovsky's ideas in inaxhaustible area of research on Biosphere and Humanity (1968). For today the ideas have not been realized to the full yet. Their essence is formulated as « ... in terms of physics biosphere of the Earth is open thermodynamic system. ... It is an essential component of general life of the Earth as planet and power screen between the Earth and Space and that film, which transforms the certain part of space energy, mainly solar one in valuable high-molecular organic substance, ... it forms also balanced atmosphere composition, and solutions composition in natural waters, and it creates energy of our planet through atmosphere ... Careless attitude to biosphere results in disturbance of proper athmosphere functioning, it means, that people in improper biosphere cannot live on the Earth ". He has united diverse processes into unified system of knowledge: - "energetic entrance into biosphere in the form of a solar energy - biological circulation of biosphere - leaving the biosphere for geology". The main sense of the offered triad is in understanding of mechanisms which increase efficiency of biosphere.

The strategic importance of the problem and its complicated analysis leads to the necessity of creation of uniform and adequate language for the formal description of observable processes. We could create the first version AIE on a complex problem "Biosphere and Humanity" (N.G.Gorbushin, 2003) on the basis of concepts and senses in area biospherology. It becomes clear, that AIE, generated on the basis of conceptual - semantic images, promote perfection of the mechanism of thinking of natural intelligence in understanding and decision of problems coevolution of biosphere and humanity. The property of biosphere, shown as the power screen - converter in system Space - the Earth, causes biosphere-power strategy of thinking and development of Humanity. This idea forms global complete system as a triad "society-political - socio-economic - information-power processes". Understanding of mechanisms and the realization of technology of interaction of elements of this triad will supply development N.W.Timofeeff-Ressovsky's ideas in the field of biosphere and humanity XXI of century.

STRESS RESPONSE TO HARD AND ULTRA-HARD RADIATION OF FREE LIVING BLACK YEAST - A MODEL OF SURVIVAL UNDER SPACE AND OTHER SEVERE RADIATION CONDITIONS

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Dematiaceous fungi have been found recently as survivors in all radiation stressed environments of planet Earth. Fungi in the fossil record were also detected as main inhabitants of highly radiation exposed environments of uranium ore deposits such as the ore deposit of Menzenschwand (Black Forest, Germany) or in the natural reactor systems of Africa. Our experiments with hard UV and other radiation stress demonstrate, that black yeast-like free living fungi are excellent candidates to survive high doses of hard radiation including cosmic radiation and radioactivity. Despite the general view, that only prokaryotes as e.g. *Deinococcus radiodurans* may survive hard radiation doses, these eukaryotic micro-organisms seem to be as capable to cope with and survive high irradiation of the shortest wave lengthes. A description is given of the metabolic products and pathways protecting this interesting organism group from environmental stress. Experiments under radiation stress and response mechanisms such as melanin and mycosporine formation are described in this contribution. Some of the fungi under investigation have been isolated from the monuments on the Crimea peninsula, others were isolated from desert environments in the Negev, the Namibia, Gobi and Mojave deserts, where intensive and lasting irradiation stress rules the environmental conditions and eliminates most other organisms from rock surfaces. Presently comparative experiments are underway to test survival under space conditions for prokaryotes (*Deinococcus radiodurans*) and eukaryotic black fungal isolates (*Coniosporium sp.*) from Chersonessus, Crimea.

ROLE OF SOIL MICRO FLORA IN THE ACCUMULATION AND REDISTRIBUTION OF RADIO NUCLIDES

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Among diverse factors of radionuclide migration in nature the least studied is the chapter which concerns the research of the role of micro organisms in concentration, dispersion and alteration of the migration rates of these radio nuclides in the soil. The main purpose and task of the experiments was the determination of the accumulation co-efficients of radionuclides by micro organisms and clarification of the role of the soil micro flora in radioisotope migration in a relatively simple system "meat peptone agar-soil microorganism colony".

The experiments were done in Petri cups. There were 21 isotopes chosen for the experiment: 32P, 35S, 45Ca, 54Mn, 59Fe, 65Zn, 73-74As, 86Rb, 90Sr, 91Y, 95Zr+ 95Nb, 106Ru, 110Ag, 115Cd, 131J, 137Cs, 140Ba, 144Ce, 185W, 203Hg. These elements can be divided in three groups by the level of concentration.

1. The most intensively concentrating elements (concentration coefficient varies from 10^2 to 10^3) ^{32}P, ^{54}Mn, ^{115}Cd.
2. Elements with medium intensity of concentration ($10 - 10^2$) ^{35}S, ^{45}Ca, ^{59}Fe, ^{65}Zn, $^{73\text{-}74}As$, ^{86}Rb, ^{90}Sr, ^{137}Cs, ^{140}Ba.
3. Elements with low intensity of concentration (less then 10) ^{91}Y, $^{95}Zr+^{95}Nb$, ^{106}Ru, ^{110}Ag, ^{131}J, ^{144}Ce, ^{185}W.

It was determined in the study of the soil micro flora influence on radio nuclide migration that the migration of elements where the experimental system were Petri cups with agar medium and micro organisms' colonies consists mainly of two processes: diffusion of elements in the environment and their redistribution in the colonies of micro organisms.

Radioautographics obtained from sterile cups and cups with colonies helped to compare the migration and the diffusion processes and gave the possibility to classify these elements by their diffusion and migration ability.

DIRECTED EVOLUTION OF MANKIND AND BIOSPHERE

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Directed evolution means the following: living organisms have a predisposition to vary in the certain directions, and this very predisposition defines trends of evolution first of all, adaptation causes only small part of evolutionary events, the evolution reacts on the change of environment, but it could take place even without the changes of external conditions. The concepts of directed evolution are numerous. During last 150 years they appeared more then 20 times. These concepts were always criticised. However now they partly penetrate in modern biology through back entrance (e.g. in the critics of adaptationist programme, in the studies on evolutionary constraints, in the searches for specific mechanisms of macroevolution, etc.).

The essence of the idea of directed evolution means the belief in the unity of all processes of development observed in nature. Some of its adherents tried to reveal the similarities among the processes of evolution of the inorganic world, the life cycle of cell, ontogenesis, evolution of species. They formulated some generalisations in biology by analogy of the generalisation made in chemistry and physics (the law of inertia, periodic law, the regularities of the growth of crystals).

The attempts to include man and biosphere in such analysis are also known. However in the most cases they seem to be either not consecutive, or not finished. Even the adherents of the idea of directed evolution at the most cases were inclined to make an exception for man concerning the laws of nature. They considered man as a special phenomenon, which could control its own evolution and the evolution of biosphere in accordance with his intellect. However if to develop the idea of directed evolution consistently in respect to man and biosphere, the opposite conclusion should arise: the mankind and its impact on biosphere undergoes spontaneous transformation like other species irrespectively of the desire of individuals. The facts on ecological history and the development of technology are compatible with this viewpoint.

ECOLOGY OF ECTOMYCORRHIZAL ASSOCIATIONS – RESEARCH IN NATURAL CONDITIONS

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Obligate mycotrophysm (fungi-depended nutrition) of the tree-plants from temperate zone is important characteristic its biology and ecology. Actually, in nature, it is not exists really and adapts for environment single plant, but symbiotic systems "plant – ectomycorrhizal fungi". Accedence in a symbiosis amplifies adaptive ability both partners and permits them to play dominate role in forest ecosystems.

Mechanism of the interrelation of trees with ectomycorrhizal fungi and different effects this interrelation is intensive studied, but now it is not enough information about way for achievement of symbiotic effect in natural condition. We have studied reaction ectomycorrhizas on environmental changes with several ecological gradients – range adjoin ecotope and use for it complex anatomic featured of ectomycorrhizal tips which estimates fettle of each symbiont.

Object of studies is ectomycorrhizas of the boreal coniferous trees (*Pinus, Picea, Abies*). Determinate that construction of ectomycorrhizal tips is specific for different species of trees and characterized considerable variability. "Fine tuning" of tree absorbing apparat on actual ecological condition is executed by two modus. First modus: changes in ratio of ectomycorrhizal tips with fungal mantles of different structure (analogy – changes of species list in biological community). Second modus: changes of symbiont dimension and vitality. It, generally, brings to changes in ratio them volume (analogy – changes in a level of development and vitality of separate species individuals in ecological gradients). According our data, increasing of fungal component significance of the ectomycorrhizas is observed under deterioration of edaphic condition or linked with increase of press on absorbing organs of plants.

The above allow to impeach a fact about appropriateness of absolute contraposition of the ecological functions controlled autotrophic (plants) and heterotrophic (symbiotic fungi) organisms.

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EVOLUTION

ONTOGENES IN *DROSOPHILA MELANOGASTER*: GENETIC FEATURES AND ROLE IN ONTO- AND PHYLOGENESIS

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A new class of mutations, *conditional dominant lethals*, were identified in Drosophila. The total number of these mutations is about 100 in the X chromosome, autosomes 2 and 3. The mutations are manifest as dominant lethals in particular genotypes only. The manifestation of lethality is dependent on the sex of the mutant, cross direction, presence of chromosomal rearrangement in the genome, etc. A part of the conditional dominant lethals are obligatory recessive lethals or recessive visible mutations.

A characteristic feature of the mutations is the formation of severe developmental abnormalities (*morphoses*) in offspring. The morphoses are manifest on one side of the body and they are not inherited. The genes were called *ontogenes* because of the specificity of mutation manifestation. Mutations give rise to genomic instability It is expressed as (1) <*delethalization*> of lethals; (2) loss of expression of well-known dominant mutations in opposite homologous chromosome; (3) loss of the X chromosome in meiosis and mitosis (patroclineous sons in the progeny, mosaics and gynandromorphs); (4) formation of visible mutants with complete or incomplete penetrance; (5) formation of chromosomal rearrangements; (6) mass appearance of phenocopies and modifications.

It is suggested that DNA sequences that serve as templates for the formation of the regulatory products directing development course were subject to mutant effect. The sequences are within known genes and outside them. The causes of the dominant expression and incomplete penetrance of the mutations are explained. Ontogenes form the invariant part of the genome; this part contains the sequences that are conserved for organisms of various taxa.

It is suggested that the ontogene is a cassete of cis-alleles. A single allele functions in a particular organism. The existence of «*silent*» cis-alleles makes possible a systemic transformation of the regulatory part of the genome cryptically without exposure to selection. A new species results from «*innovation*», a set of functionally related mutations, not from single mutations.

RIBOSWITCHES: THE OLDEST REGULATORY SYSTEM?

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Riboswitches are a new class of regulatory signals recently discovered by the comparative analysis of bacterial genomes and confirmed in experiment (reviewed in Vitreschak et al. (2004) *Trends Genet.* 20: 44). The regulatory mechanism involves formation of alternative RNA structures dependent on binding of small compounds (amino acids, vitamins and their derivatives, etc). Unlike other RNA regulatory elements, riboswitches are capable of direct binding of small molecules and require no intermediate signal transducers, such as proteins and tRNA. The regulatory structure consists of a relatively large metabolite-binding structure and a regulatory hairpin that modifies gene expression. The hairpin may function as a terminator of transcription or repress initiation of translation by sequestering the ribosome-binding site. An alternative structure forming in the absence of the metabolite consists of parts of the metabolite-binding element and the regulatory hairpin and derepresses gene expression.

Riboswitches have been found in most major groups of eubacteria, as well as eukaryotes (plants and fungi) where they regulate splicing, and some archaea. The structures of the metabolite-binding elements are specific for the metabolite and very highly conserved. At that, not only the secondary structure is conserved, but non-paired nucleotides as well. Recent determination of the X-ray structure of the purine riboswitches has demonstrated that such nucleotides are involved in tertiary interactions and metabolite binding. The regulated cellular process (transcription or translation) is correlated with taxonomy, so that Gram-positive bacteria (Firmicutes) have mainly transcriptional repressors, Gram-negative bacteria (Proteobacteria), translational ones, and Actinobacteria, riboswitches that directly sequester the translation initiation site without an additional regulatory hairpin. There also exist activator riboswitches that are alternative to regulatory hairpins and regulate genes involved in catabolism and export of respective compounds.

The extremely high conservation of riboswitches, their presence in all three domains of life and all major bacteria taxa, the fact that they regulate all major gene expression mechanisms (transcription, splicing and translation), and finally, their ability to bind small molecules directly make them ideal candidates to the role of regulators in the RNA world and thus the earliest known regulatory system. This is joint work with Andrei A. Mironov, Dmitry A. Rodionov and Alexei G. Vitreschak. This study was supported by grants from the Howard Hughes Medical Institute, Russian Academy of Sciences, and Russian Science Support Fund.

POPULATION-GENETIC CONSEQUENCES OF THE ECOLOGICAL CATASTROPHE (CHERNOBYL'S EXAMPLE)

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The "novelty" of ecological conditions caused by human activity is the specific trait of modern time. Chernobyl serves as model for the study of the effects of fast changes in the environment on the ecosystems. We investigated the laboratory lines of mice, exposed to ionizing irradiation in special vivarium near Chernobyl's NPP, voles species, trapped in Chernobyl's zone; cattle herd, reproduced in experimental farm near Chernobyl's NPP and children, which exposed to the same doze of ionizing irradiation but in different mode: acute (in utero) and chronic ones. No increase in the quantity of constitutive mutations (mutant organisms) in investigated genes or chromosomes in analyzed species (cattle and *Rodentia* species) was observed. We revealed the acute decrease in fertility in first cow generation which was born in Chernobyl (in number of calf per one cow per one year). Observed by us the increase of frequencies of cytogenetic anomalies in blood cells of children (14 – 16 years age) who received the doze of ionizing radiation in utero and our data about the cattle fertility decrease, allowed to suppose, that children exposed to low dozes of ionizing radiation in utero, could face reproductive problems in the future. In generations of cattle, disturbance of equiprobable transmission of alleles of a number of molecular genetic markers, increase of heterozygosity and radio resistance were observed. In family analysis the changes of genetic structure in exposed to ionizing irradiation cattle generations the shift of gene pool from typical for specialized parent dairy breed Holstein to that characteristic for the less specialized breeds was revealed (decrease in level of specialization) All these appearances corresponded to a rule of I.I. Shmalgauzen that any change of the environment lead to preferable reproduction of the more primitive forms within a species. Thus, the main problem after the Chernobyl's catastrophe, as well as other ecological changes, lies not in the occurrence of the new mutant organisms, but in the long-term changes of the genetic structure of populations and, accordingly, in the appearance of the new interspecies interactions between the less specialized (marginal) representatives of each species in species communities.

GENETICS AND EVOLUTION (WHAT WE DON'T KNOW)

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During the classic period of genetics, application of genetic methodology to the theory of evolution resulted in formulation of synthetic evolution theory. The essence of the latter was stated as an ultimate laconic scheme by N.V. Timofeev-Ressovsky, in the paper published in the Botanical Journal (1958, T.43, N 3, pp.317-336; in Russian). Skyrocketing of genetics during the following half-century provided new important information for genetic characteristics of the evolution process, revealing fundamental posers to indicate shortage of our knowledge. Some of these posers are as follows:

In regard to the species formation, what is the role of non-canonical autogenous variability (as migratory genetic elements, chimeric genes in cytoplasmic male sterility, etc.)?

The "silent" part of genome – what would be its role in evolution if revealed?

What is the role of major hereditable ontogenetic transformations ("happy monsters"), if regarded to fast shifts of modifier gene frequency values showing significant influence upon trait manifestation and expressivity ?

Fitness of the moment – is it correlated to possibility of evolutionary transformations?

Epigenotype formation – is it important for a species maintenance or/ and for evolutionary transformations?

Are radical environment changes obligatory for evolutionary transformations?

Pattern of a species population structure – is it of any significance for possibility and direction of evolutionary changes?

The major problem probably concerns to understand cell-genome as an integral structure, and a population – as an ecological-and-genetic structure.

EVOLUTION OF MAMMALIAN GENOMES: CYTOGENETICS ASPECTS

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Comparative genome maps record the history of chromosome rearrangements that have occurred during evolution. The rates, types and directions of chromosomal rearrangements as well as phylogenetic relationships can be inferred by comparative analysis of the distribution patterns of conserved segments in different phylogenetic lineages. The successful identification of chromosomal homology between species is fundamental for comparative cytogenetic and genomic analysis. Cross-species chromosome painting, being accurate, efficient, and suitable for genome-wide comparison, has become the method of choice for comparative cytogentics, particaularly for comparing distantly-related species or species with highly rearranged karyotypes. The use of painting to identify regions of chromosomal homology has allowed the transfer of information from map-rich species such as human and mouse to a wide variety of other species. When combined with chromosome banding and gene mapping, comparative chromosome painting can provide the most accurate comparative chromosome maps for species being compared. From a painting analysis spanning some mammalian taxa (Primates, Artiodactyla, Perissodactyla, Carnivora, Lagomorpha, Rodentia and Afrotheria) three distinct classes of synteny conservation have been designated: (1) conservation of whole chromosome synteny, (2) conservation of large chromosomal blocks, and (3) conservation of neighboring segment combinations. This analysis has also made it possible to identify a set of chromosome segments (based on human chromosome equivalents) that probably made up the karyotype of the common ancestor of the Placentalia orders. This approach provides a basis for developing a picture of the ancestral mammalian karyotype and trends of karyotype and genome evolution in all these taxa.

THE EVOLUTIONARY TRENDS IN THE CHANGES OF PHOTOSYNTHETIC APPARATUS IN GENUS TRITICUM L.

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The speciation in the genus Triticum L. is immediately related to alloploidy due to the spontaneous hybridization of 14-chromosome species of Triticum L. (T. beoticum и T. urartu, the ancestors of subgenome A^b и A^u, relatively) and Aegilops and their subsequent evolution. During the evolution the significant structural-functional differences in photosynthetic apparatus of ancient and modern wheat species have occurred. The objective of this study was to investigate the evolutionary trends in the changes of photosynthetic apparatus in genus Triticum L.

In evolution of genus Triticum L. the increase of plant productivity was marked against the decrease of photosynthetic rate per unit leaf area (the so called "paradox of Evans"). One of its reasons is the genetic changes in photosynthetic activity of a single chloroplast caused by adding of Aegilops subgenomes to wheat genome. The other reason is the changes in interior organization of mezophyll: the decrease of chloroplast number per unit leaf area and interior assimilation area in leaf. From one hand these changes were caused by the intensification of plant growth and the formation of a larger leaf area according to nuclear ploidy, that gives more advantages to modern alloploid forms compared to ancient diploid species. From the other hand they have led to the decrease of photosynthetic rate per unit leaf area in polyploids due to the worse conditions for CO_2 diffusion to carboxylation centers in leaf. The study of the structural-functional leaf organization in plants with different genomes have shown the non-sufficient approach in analyses of alloploids from the position of polyploidy as the diverse origin of the genome strongly imprint the formation of photosynthetic parameters. The changes in leaf photosynthetic apparatus of alloploid species were often the same, as in autopolyploids. Mainly it relates to groups of species that originated from one ancestral form. In the majority of cases genome constitution influenced on the structure of photosynthetic apparatus and its functional characters.

RETROPOSON IMPACT ON PRIMATE GENOME EVOLUTION

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Remnants of more than 3 million transposable elements, primarily retroelements, comprise nearly half of the human genome and have generated much speculation concerning their evolutionary significance. However, for real understanding the genetic reasons for hominoid speciation a comprehensive comparison of their genomes is required. It would allow to reveal the genetic basis of revolutionary phenotypic changes in hominids at relatively conserved ape phenotypes and similar rates of the molecular evolution of human and great apes.

Recently, we developed an experimental approach to genome-wide searching of retroelement integration site differences between closely related genomes. The techniques were successively applied for human and chimpanzee genomes comparison aimed on the identification of species specific integration sites of human endogenous retroviruses (HERVs) and related solitary long terminal repeats (LTRs). Based on the results, we concluded: a) as many as 150 HERV insertions differ the human genome from that of chimpanzee; b) discovered human-specific insertions represent members of three distinct phylogenetic groups of HERV-K LTRs suggesting long term parallel retrotranspositions of various master-genes of the HERVs; c) significant part of human-specific LTR integrations are unevenly distributed both along individual chromosomes and among them and often located in close vicinity or even within human genes suggesting their possible involvement in the regulation of the neighbouring genes expression. We confirm regulatory potential of LTR sequences by results of transient expression assay of a reporter gene and by discovering of tissue-specific alterations of the LTRs methylation.

Thus, newly integrated LTRs could serve as alternative promoters, enhancers, parts of locus control regions, and a source of other signals for surrounding genes. Due to their capacity to modulate existed genes activity LTRs are considered as efficient pacemakers in primate evolution. Also human specific insertions of retroelements, in particular LTRs, could be served as a new class of genetic markers for further studying human evolution, gene mapping, population and medical genetics.

KARYOTYPE EVOLUTION IN *NICOTIANA*

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The genus *Nicotiana* (Solanaceae) has 76 species in 13 sections and makes an excellent model system to study plant chromosome evolution and speciation. The base chromosome number is n =12, but the numbers can vary at both the diploid and tetraploid levels. The genus' centre of diversity is S. America, most species occur in South/North America, and one large section, *Suaveolentes*, is found in Australia (and one species in Africa). The Australian distribution of *Suaveolentes* arose as a consequence of recent long-range dispersal. We have embarked on a systematic analysis of karyotype evolution in the genus by combining molecular phylogenies derived from multiple gene sequences and karyotype/chromosome analysis using FISH and molecular biology. We report patterns of chromosome divergence between diploid and polyploid sections and in sections of different ages. We show relationships between rates of speciation and of karyotype divergence.

PROTEIN SYNTHESIS OF YEASTS *CANDIDA* UNDER SOME EXTREMAL CONDITIONS

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Alive organisms are distinguished with high adaptivity to the persistent changing environmental conditions. Even in extreme conditions the organisms survive working out the definite protective mechanisms, particularly – synthesizing new proteins.

In recent years the datas about survival of organisms in extreme conditions have been received. In particular, the existence of some definite proteins has been proved that named as "proteins of thermoshock". These proteins are produced in organisms surviving in conditions of high temperature.

The extreme factors firstly influence on nucleic acids and proteins, which realize the transmission of genetic information and the basic structural and functional characteristics of organism.

The investigation of protein fraction composition of yeasts *C. guilliermondiiWKM U-42* in normal conditions and after nitrogene starvation was realized. It has been shown that the nitrogene starvation lead to decrease of water- and salt-soluble proteins, and to increase of alkali-soluble fraction. So, if the water-soluble protein fraction of non-starved yeasts is 52,3±2,2%, and the alkali-soluble fraction – 24,8±1,3%, then in case of nitrogene starved yeast they are 28,3±0,26% and 51±0,5% accordingly. The protein fractions content of yeasts *C.guilliermondii NP-4* also suffer some changes under influence of X-rays. It has been shown that after X-radiation of these yeast cells the quantity of water-soluble fraction was decreased, and the alkali-soluble fraction – increased approximately in 6%.

We have investigated the protein synthesis of yeasts *C.rugosa WSB-925*. It has been shown that in high temperature conditions the "proteins of thermoshock" with molecular mass of 74 and 79kD are synthesized. These proteins probably carry out the direct protection function as well as have an important role in formation of cell resistance.

ROLE OF MUTATOR ALLELES IN ADAPTIVE EVOLUTION

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The level of genetic variability that maximizes the fitness of population varies with the degree of its adaptation to the environment. It is low when environment is stable and high when environment is unstable and hostile. Since environmental conditions are changing, the adaptation is never permanent. Consequently, because the genetic variability in bacteria is first generated by mutagenesis, it could be expected that populations with high mutation rates would have better chance for successful evolution. Indeed, bacteria with elevated mutation rates are frequently found among natural isolates. Experimental observations and theoretical calculations suggest that there must be positive selection for higher mutation rate in spite the fact that majority of newly generated mutations are deleterious or lethal. Mutator alleles rise to a high frequency through their association with the favorable mutations they generate that counterbalance the load of deleterious mutations. However, when adaptation is achieved, the load of deleterious mutations counterselects high-mutation rates. Therefore, evolution of bacterial populations may happen through alternating periods of high and low mutation rates that provide a remarkable potential for the fine tuning of the rates of generation of genetic variability in the function on the adaptation to environmental conditions.

COMPARATIVE STUDY OF THE EXON-2 OF *DRAS1* GENE IN THE *DROSOPHILA VIRILIS* SPECIES GROUP

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Single nucleotide polymorphism among eleven species (one of them was represented with two subspecies) was revealed by sequencing of PCR-products obtained using the following primers:

TACGATCCCACCATCGAGGA(forward) and TTGTTGCCCACGAGCACCAT(reverse). The length of the polymorphic part of the fragments, which are homologous to exon-2 of *D. melanogaster Dras1* gene, was about 300 bp.

Sequence alignments of the twelve amplified fragments with full genomes of the following species: *D. melanogaster*, *D. pseudoobscura*, *D. mojavensis*, *D. yakuba*, and *D. ananassae*, were completed. This analysis revealed similar rates of mutation accumulation in uncoded regions (introns and uncoded parts of exons) in different species. It was shown that the rates of mutation accumulation for coding regions in exons were dramatically less.

The nucleotide substitutions within the conservative region that coding aminoacid motif DTAGQE, which is typical for *Dras1* gene, are of particular interest. *Dras1* gene refers to the group of monomeric GTP-ases that transduce signals received by cell surface receptors to the MAPK cascade. RAS protein activates molecules affecting cell proliferation. *Dras1* gene is essential for embryo development as loss-of-function mutations of this gene disrupt different developmental processes: axial pattern formation, segmentation, and organogenesis.

RFLP analysis in 11 species of *D. virilis* species group was carried out. The PCR-fragment identified in *D. virilis* was used as a probe labeled with [α-P-32]. Total DNA was digested with restriction endonuclease *Pvu*II. Species hybridization patterns differed from each other as shown with Southern blot hybridization. Polymorphism revealed in restriction fragments as well as in nucleotide sequences may be used as a genetic marker in interspecies hybrid analysis.

DEEPENING OF CHROMATIN DAMAGES DURING THE POSTRADIATING REPAIR PERIOD

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Alive organisms differ with their high adaptivity to extreme conditions of environment, such as radiation, thermoshock, starvation, etc. They develop the certain protective mechanisms, the study of which is rather actual. It is known, that all consequences of action of radiation are caused by damages of chromatin and its components, in particular – of DNA. Therefore it is extremely important to know the mechanism of formation of radiating damages of components of chromatin, their nature and biochemical consequences, and also ways of their reparation and transformations.

The structural damages of DNA of yeasts *Candida guilliermondii* after X-radiation (54000R) was realized by us. It has been shown, that after the 24-hours postradiating incubation of yeast in the synthetic environment promoting the survival of yeast and repair of DNA damages, there are observed the significant changes in the some physicochemical characteristics of DNA: in comparison with non-radiated and radiated DNA the melting temperature was increased, the melting interval was decreased. By elektrophoretic and fluorescent investigations it has been also shown that there are some damages of secondary and primary structures of DNA. Thus, the yeast DNA structural damages become deeper after the postradiating repair period.

THE DYNAMICS OF SUBTELOMERIC REPETITIVE DNA CHANGES DURING EVOLUTION AND AMPHIPLOIDS FORMATION

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The section Sitopsis is divided into two distinct groups: one containing only *Ae. speltoides* and other, *Ae. longissima, Ae. searsii, Ae. sharonensis* and *Ae. bicornis*. This grouping by RAPD analysis is in agreement with the taxonomical classification of the subsections. The evaluation of the divergence of repetitive sequences during evolution has been done on the base of the primary structure, the suborganization of families, the copy numbers and chromosomal localization of two subtelomeric repetitive sequences – Spelt1 and Spelt52. It was shown that the amplification of Spelt1 took place in the ancient progenitor of *Ae. speltoides* that gave rise to the B or G genomes of the polyploid wheats. The data indicates Spelt52 amplification in the lineage of *Ae. speltoides*, and this amplification probably happened before the divergence of the allopolyploid *T. timopheevii* but after the divergence of *T. durum*. In a separate amplification event, Spelt52 copy number expanded in the common ancestor of *Ae. longissima* and *Ae. sharonensis*.

The genomic content of the subtelomeric repeated sequences Spelt1 and Spelt52 was studied by dot-, Southern- and *in situ*-hybridization in eleven newly-synthesized amphiploids of *Aegilops* and *Triticum* and data compared with the parental plants. Spelt1 had reduced copy numbers in the first generation of three synthetic amphiploids and did not change in two others, whereas Spelt52 was amplified in nine amphiploids and did not change in two. In the second allopolyploid generation, Spelt1 copy number did not change further whist there was amplification of Spelt52 in some allopolyploids and decreases in others. Neither allopolyploidy level nor the direction of the cross affected the patterns of change in the newly-synthesized amphiploids.

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THE CHANGES IN THE WHEAT SEEDLINGS CHROMATIN PART REARRANGEMENT UNDER INFLUENCE OF NON-THERMAL ELECTROMAGNETIC RADIATION

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Gene function is subjected to the effects of surrounding chromatin. The nature of these effects may be epigenetic occurring in some cell, but under influence of some external factors these processes can be disrupted. Inside the nucleus there are three structural compaction of DNA. Regions of dense heterochromatin masses scattered throughout the interphase nucleus. Only recently has some understanding of the mechanisms of its formation and propagation been achieved. Heterochromatin is divided into constitutive heterochromatin, containing satellite DNA and facultative heterochromatin, inactive in certain cell lineage but expressed in other lineages. Heterochromatin is involvement in epigenetic silencing phenomena including repression along extended regions of chromosomes and the inactivation of whole chromosomes. The potential of heterochromatin to silence of nearby genes, a phenomenon known as position effect variegation, has been both puzzling and attractive for scientist since its discovery.

In the presented work we study changes in melting parameter of chromatin having different localization in nucleus: euchromatin and heterochromatin under influence of coherent non thermal electromagnetic irradiation on wheat seeds during germination The EHF generator with the range of working frequencies 37,5-53,5 GHz was used as a source of monochromatic radiation of mm-waves. The irradiation was carried out in a distant zone of radiation of the generator.

Our experimental data allows us to expect that influence of coherent non thermal electromagnetic irradiation on wheat seeds during germination lead to the significant changes in some part of heterochromatin and in result to increasing of preservation system of living organisms.

DNA DAMAGE IN SPLEENOCYTES OF DIFFERENT AGE MICE EXPOSED TO IMMOBILIZING STRESS

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The aim of our work was to study the DNA damage in spleenocytes of different age mice exposed to immobilization stress. As the parameters, there were chosen: the level of the DNA single-strand breaks and alkali-labile sites, the amount of the low weight DNA fragments, and the amount of the DNA-protein cross-links. Additionally we determined the spleen weights and total number of spleenocytes. The work was made on the male mice CBA/lac at the age of 80, 100, 140 and 180 days. As the stress factor was used treatment of mice in narrow individual cages with the size of 3.0x5.5x5.5 cm during 24 hours.

It was shown that the stress-exposure leads to a statistically significant decreasing of the total number of spleenocytes in all treated mice groups and this effect was more expressed in young mice (80 days old).

To summarize the results of stress-factor influence on the spleenocyte chromatin structure of the different age mice we can point out that:

1. The amount of the low weight DNA fragments in response to stress-factor was the highest at the age of 80 days (1,7 –fold exceeding the level of control);
2. The level of DNA-protein cross-links in response to the stress exposure was the highest at the age of 100 days (2,4 – fold exceeding the control level);
3. It is not observed any significant increasing of the amount of DNA single-strand breaks and alkali-labile sites in response to stress exposure in all age groups.

In conclusion we can consider that with the age the mouse reactivity to the action of immobilization stress is decreasing. It is possible, that observed phenomena is caused by the age changes in the mouse neuro-humoral status. Probably that with the increasing of the age of exposed animals till the age comparable with the mouse lifetime we would observe the opposite picture (the increasing of animal sensitiveness to stress).

THE PROCESSES OF FREE-RADICAL OXIDATION IN PHYLOGENETIC DEVELOPMENT OF VERTEBRATES

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Free-radical lipid oxidation is a persistent process in intact membranes. The intensity of these process is formed by heterogeneity of lipid content of biomembranes (namely by unsaturation of fatty acids) in one hand and by work of antioxidant system of organisms (fermentative and not fermentative), which is regulate this process in other hand. The level of free radicals underlie of the so named "superweak shining" of tissues or chemiluminecsence (ChL). The intensity of ChL is a qualitative index of free radical's presence in the system, and different methods of enhanced ChL let to give a quantitative characteristic of lipid peroxidation.

We isolated lipid fractions from the brain, heart, liver and muscle of vertebrates: crucian carp (*Carassius carassius*), marsh frog (*Rana ridibunda*), caucasian agama (*Stellio caucasicus*), and nonpurebred white rats by the method of Keits. The tissue homogenate (20mg/ml in Tris-HCl buffer, pH=7.4) and lipid fractions have been radiated by UV-lamp (MEDICOR, Q-439, Budapest); exposition 2,5 min, distance to quivette 5 cm. Chemiluminescence intensity was determined on a quantometric devise, equipped with FEU-139 photomultiplier.

Lipid peroxidation was studied in nervous tissue of vertebrates of different classes using chemiluminescence (CL) and spectrophotometric measurement of malonic dialdehide (MDA) concentration and superoxide dismutase (SOD) activity. Free radical-mediated oxidation processes decrease in the row:fishes>amphibians>reptiles>mammals. This phenomenon may be related to the increase degree of saturation in fatty acids of lipids in this evolutionary row. This fact is also confirmed by the decrease in SOD activity accompanying philogenetic changes in lipid peroxidation processes.

Fotoinduced cemiluminescence (FChL) was studied in homogenates and lipid fractions from tissues of representative of four classes of vertebrates. Levels of homogenate's luminescence after UV-radiation increases in row of poikilotherm vertebrates: and slightly decrease in homogenates of mammal's tissues. Intensities of FChL of lipids from tissues of vertebrates are distinguish not to a marked degree, what, probable, testify about key role of protein components of cell membranes as emitters of quantum during UV-induced ChL.

THE ROLE OF PRION DOMAINS IN EVOLUTION

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Prion (from proteinaceous infectious particles) is a protein isoform that is able to convert the normal form of the same protein into prion form. This term was proposed by S. Prusiner in 1982 to explain the unusual properties of pathogen transmission observed in the cases of some neurodegenerative diseases in mammals. Examples of the "prion diseases" include "mad cow disease", or bovine spongiform encephalopathy (BSE), sheep scrapie disease, and human Creutzfeldt-Jacob disease. Importance of prion diseases was emphasized by the possibility of BSE transmission from cows to humans. Prion capability is not restricted to PrP and can be found in the other proteins. Moreover, discovery of the yeast prions [*PSI*$^{+}$], [*URE3*] and others makes it clear that PrP represents just an extreme case of much more common phenomenon. Experiments using yeast and fungal systems confirm that prion-like phenomena are widespread, and identify the major components of the cellular machinery that modulates prion formation and propagation.

The prion concept reveals a new mechanism of inheritance, which operates at the level of the structural organization of proteins. Possibly, the prion formation is a pathological process, while conservation of prion-forming potential in evolution is due to some adaptive functions played by PFDs (prion forming domains) in certain conditions. As misfolded and potentially aggregating proteins are usually accumulated during aging, it is an intriguing possibility that aging could promote prion-like pathologies. Indeed, some aggregation-related diseases (*e.g.*, Alzheimer's disease) in humans are frequently associated with advanced age. An alternative model suggests that prion formation by itself could be an adaptive process, so that certain prions are responsible for the emergence of the adaptive traits. Various models, explaining the biological roles and evolutionary conservation of prion-forming processes, and relating them to the certain controversial aspects of the theory of evolution are discussed.

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MEMORIAL

ON THE PREHISTORY OF MOLECULAR BIOLOGY

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It was x-ray crystallography that made, in early 1950s, a decisive contribution to the deciphering of the structure of DNA. But for the first time x-ray crystallographers (Astbury and Bernal) and geneticists (Timoféeff and others) jointly discussed the problem of the chromosome and the gene in Klampenborg in April 1938, on the first of the meetings initiated by Timoféeff-Ressovsky. While visiting Niels Bohr, Timoféeff admired the scientific style of Bohrs Kreis. Back home, he started discussions of the nature of gene mutation and the structure of the gene. They resulted in 1935 paper by Timoféeff, Zimmer, Delbrück, which later has been discussed by E. Schrödinger in his 1944 *What is Life?* Brilliant scientist and a charming personality, Timoféeff became the moving spirit of the new discipline in mid-1930s. In TZD he has formulated the central problem of "convariant reduplication" which permitted to consolidate efforts of experts in genetics and x-ray crystallography, cytology and electron microscopy, cell physiology and embryology, chemistry and biochemistry, theoretical and experimental physics, with the end to lay the bases of what he provisionally called "biophysics". Timoféeff's project to make cooperation more efficient, by having four meetings during two years, 1938-1939, found financial support from the Rockefeller Foundation. While drafting 1938 annual report, RF director of natural sciences Warren Weaver entitled the section, on the Klampenborg and Spa conferences, "molecular biology". The 3rd conference scheduled for Melrose (near Edinburgh), August 31 – September 1, 1939, became impossible because of WWT. In war-time Germany Timoféeff summed up his view of the new science in *Biophysics*, vol. 1 (co-authored by Zimmer). Written in 1944, the book was published in Leipzig in 1947, and had an interesting history in America. In September, 1945, a *donos* made by Nuzhdin, a Lysenkoite who visited Berlin, resulted in the arrest of Timoféeff, who had to spend several months in Karlag concentration camp. Joliot Curie sent a letter to L.P. Beria, and Timoféeff was released from the camp. In August, 1948 his genetics was outlawed. (Buzzati-Traverso and Cavalli dedicated their 1948 *Teoria dell'urto*... to Timoféeff, "amico e maestro", with hope that he will possess a possibility to continue his work.) Still there remained his radiobiology and biophysics. His standing was announced by a 1949 article accompanied by cartoons ordered by Stalin himself, which presented his negative personal attitude towards both Timoféeff and the RF. In 1950, while prisoner at a *sharashka* Timoféeff was nominated the Nobel prize for his research in biophysics/molecular biology.

SHORT PAPERS

by Young Scientists

GENETICS

CHANGES IN THE LEVEL OF 8-OXOGUANINE IN DNA AND LIVER CELLS OF OXYS RATS WITH INHERITED OVERGENERATION OF FREE RADICALS

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Reactive oxygen species (ROS), including superoxide, hydroxil radical, hydrogen peroxide, and singlet oxygen, are generated in cells by ionizing radiation, oxidizing agents and through metabolic processes [1-3]. An important biomarker of oxidative DNA damage is 8-oxoguanine (8-oxoG) formed by exogenous and endogenous ROS attacking nucleic acids. Formation of 8-oxoG in cellular DNA was found to be associated with such important biological processes as mutagenesis, carcinogenesis, aging and several aging-related diseases [4].

Rats of the OXYS strain bred at the SB RAS Instiurte of Cytology and Genetics provide an unique model for studies of the mechanisms of oxidative DNA damage and of the relationships between the intensity of mutagenesis and development of degenerative pathologies. This strain is characterized by hereditary overproduction of ROS, oxidative modification of lipids and proteins, and, consequently, the development of pathologies similar to human ROS-dependent diseases such as malignant tumours, cataracts, cardiomyopathies, emphysemas, disruption of long-term memory, etc. [5]. Thus, an investigation of the age- and strain-dependent dynamics of 8-oxoG levels are of interest for understanding the mutagenesis/pathogenesis relationships.

Materials and Methods

We have applied several immunostaining techniques using monoclonal anti-8-oxoG antibodies to follow age-dependent dynamics of 8-oxoG levels in DNA from the liver of OXYS rats and Wistar rats, the latter used as a control strain. High sensitivity and specificity of monoclonal anti-8-oxoG antibodies [6] make them a perfect reagent for detecting oxidative DNA damage in cells. Immunological techniques present therefore an optimal approach for 8-oxoG detection *in situ*. We used this technique to determine the relative levels of 8-oxoG in the liver and lung of 2- and 18-months-old OXYS and Wistar rats. Using immunohistochemical tissue staining, we have also analyzed the relative amount and distribution of 8-oxoG in the liver of 2-, 6-, 12- and 18-months-old OXYS and Wistar rats.

The data on the 8-oxoG content in rat liver and lungs DNA are presented in Fig 1A and Fig 1B, respectively. It was shown that in both strains the level of 8-oxoG in DNA from lung was 1.7-2.0-fold higher than in liver DNA for 2-month-old animals, and 1.3-1.7-fold higher for 18-months-old animals. The level of oxidative DNA damage in the liver of 2-months-old OXYS rats was 2.4-fold higher ($p<0.01$), and in the liver of 18-months-old OXYS rats, 1.5-fold higher ($p<0.05$) than in Wistar rats of the matched age. The level of oxidative DNA damage in lungs of 2-months OXYS rats was 2-fold higher ($p<0.01$), and in liver of 18-months, 1.7-fold higher ($p<0.05$) compared with Wistar rats. Mutations in DNA showing up in RNA and protein abnormalities accumulate with age. Therefore, the observed age-dependent 2–3-fold difference in DNA oxidation between OXYS and Wistar rats can be an important factor explaining accelerated somatic cell senescence in OXYS rats. Using indirect immunofluorescence, we have observed the highest level of oxidative DNA damage in liver cells from 18-month old OXYS rats. The mean values and their 95% confidence intervals for 2-, 6-, 12-, and 18-months old OXYS and Wistar rats are shown in Table 1 and in Fig. 2. DNA damage in 18-month old OXYS rats was 12.5-, 2.1-, and 1.4-fold higher than that of 2-, 6-, and 12-months old OXYS rats, respectively. Interestingly, the changes in the 8-oxoG-levels in liver nuclei of OXYS rats had an approximately linear age-dependent profile, whereas in Wistar rats, the lesion accumulation accelerated with age.

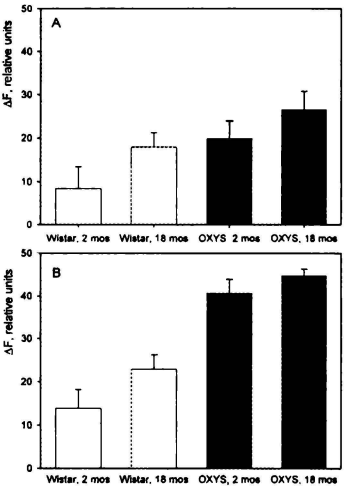

Figure 1. Oxidation of guanine in DNA samples from liver (A) and lungs (B) OXYS and Wistar rats. Mean ± S.D. are shown

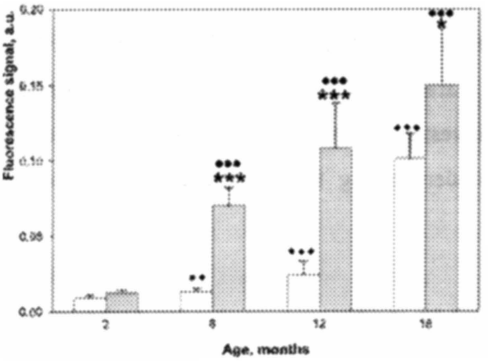

Figure 2. *Increase in 8-oxoG signal in Wistar and OXYS rat liver with age.*
Mean signal level and upper margin of 95% confidence interval (calculated by bootstrapping, see main text) is shown. Albino, Wistar rats, gray, OXYS rats. *Asterisks* indicate statistically significant interstrain differences at $p>0.95$ (*) and $p>0.999$ (***). *Diamonds* indicate statistically significant differences in the Wistar strain between 2-month-old animals and animals of other age groups at $p>0.99$ (♦♦) and $p>0.999$ (♦♦♦). *Circles* indicate statistically significant differences in the OXYS strain between 2-month-old animals and animals of other age groups at $p>0.999$ (●●●). Statistical significance of the differences was calculated from Mann–Whitney rank sum test

Table 1. Relative levels of 8-oxoG in Wistar and OXYS rats of different age

Age, months	Wistar		OXYS	
	mean	95% confidence *interval*	mean	95% confidence *interval*
2	0.019	0.015–0.024	0.023	0.019–0.026
6	0.023	0.018–0.030	0.042	0.033–0.052
12	0.027	0.022–0.034	0.060	0.044–0.079
18	0.056	0.045–0.070	0.080	0.058–0.105

In liver cells of 18-month old Wistar rats we have detected a 11.2-, 7.8-, and 4.2-fold increase in the 8-oxoG level, compared with 2-, 6-, and 12-month old Wistar rats, respectively. Clear interstrand differences were observed at each age: The level of 8-oxoG in liver nuclei of OXYS rats was 1.3-fold higher than in Wistar rats at 2 months, 5.4-fold higher at 6 months, 4.5-fold higher at 12 months, and 1.5-fold higher at 18 months of age. These differences were statistically significant. Thus, the most pronounced interstrand differences were observed in 6- and 12-months old rats.

Conclusions

A quantitative immunofluorescent method was developed for analysis of a mutagenic product of oxidative stress, 8-oxoguanine, in animal DNA. Higher levels of guanine oxidation were found in DNA from rats of OXYS strain characterized by premature aging. Lungs were shown to be one of the main target organs for high 8-oxoG accumulation.

Acknowledgements

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THE EVOLUTIONARY TRENDS IN THE CHANGES OF PHOTOSYNTHETIC APPARATUS IN GENUS TRITICUM L.

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The speciation in the genus Triticum L. is immediately related to alloploidy due to the spontaneous hybridization of 14-chromosome species of Triticum L. (T. beoticum and T. urartu, the ancestors of subgenome A^b and A^u, relatively) and Aegilops and their subsequent evolution. During the evolution the significant structural-functional changes in photosynthetic apparatus of ancient and modern wheat species have occurred. The objective of this study was to investigate the evolutionary trends in the changes of photosynthetic apparatus in genus Triticum L.

Materials and Methods

The objects of our study - 22 species from the genus Triticum L. and 4 species from the genus Aegilops L., of diverse origin, genome constitution, level of ploidy, totally – 125 samples, comprising groups: *Aegilops* L. 2n=14 (genomes B^{sp}, B^L, D), *Triticum* L. 2n=14 (genomes A, A^b), 2n=28 (A^uB, A^bG), 2n=42 (A^uBD, A^bGD) и 2n=56 (A^bA^bGG). Photosynthetic rate was determined radiometrically, leaf mezostructure by [1], according to the typology of cells, described earlier [2], growth rate – by the changes in cumulative area of leaves on the main shoot [3].

Results and Discussion

In each subspecies of the modern alloploid wheat species we marked the increase of productivity 2-4 –fold that was achieved by the formation of a large assimilating area compared to ancestral forms and aegilops species. This was due to the rise of each leaf area (2-2.5-fold) and growth rate (40-60%), as the number of leaves and their longevity did not change. Photosynthetic rate per leaf area and mass unit decreased to 1.5-2 times. It was caused by the decline of the photosynthetic activity of a chloroplast by 30-50% in alloploids (genomes A^uB, A^bG, A^uBD, A^bGD, A^bA^bGG) compared to diploid species (2n=14) with genomes A и D due to the introgression of the aegilops subgenomes. This fact does not explain the decrease of photosynthetic rate in the transition from tetraploid to hexa-and octoploid levels. This might be the result of the changes in the conditions for CO_2 diffusion to carboxylation centers in leaf of modern species caused by the structural rearrangement of leaf mesophyll during the evolution of genus Triticum L. The study of the phototrophic tissues structure in wheat and aegilops leaves in relation to genome constitution revealed that the enlargement of leaf surface in modern wheat

species was caused by the increase of cell volume (1.3-fold) and their number in the leaf according to the rise of chromosome number in the nucleus. These effects were caused by more intensive cell division and elongation during ontogenesis. The increase of cell volume did not influence on the plastid-to-cytoplasm relations in cells, as the number of chloroplasts increased proportionally to cell surface. This trend caused the decline of cell number and chloroplast number per unit leaf area 1.2 – 1.5-fold. The interior leaf surface that participates in the conductance CO_2 to the carboxylation centers, is formed of the cumulative plasmalemma and outer chloroplast membrane surface. The decline of cell and chloroplast number per leaf unit area in each ploidy leveled to the decline of cumulative inner assimilation surface and as a result – worse conditions for CO_2 diffusion and lower photosynthetic rate in alloploids compared to ancestral species.

Conclusion

During the evolution of genus Triticum L. the increase of plant productivity was marked against the decrease of photosynthetic rate per unit leaf area (the so called "paradox of Evans"). One of its reasons is the genetic changes in photosynthetic activity of a single chloroplast caused by the introgression of Aegilops subgenomes to wheat genome. The other reason is the changes in interior organization of mesophyll: the decrease of chloroplast number per unit leaf area and interior assimilation area in leaf. From one side these changes were caused by the intensification of plant growth and the formation of a larger leaf area according to nuclear ploidy, that gives more advantages to modern alloploid forms compared to ancient diploid species. From the other they have led to the decrease of photosynthetic rate per unit leaf area in polyploids due to the formation of the worse conditions for CO_2 diffusion to carboxylation centers in leaf.

The study of the structural-functional leaf organization in plants with different genomes shows the non-sufficient approach in analyses of alloploids from the position of polyploidy as the diverse origin of the genome strongly influence on the formation of photosynthetic parameters. The changes in leaf photosynthetic apparatus of alloploid species were often the same, as in autopolyploids. Mainly it relates to groups of species that originated from one ancestral form. In the majority of cases genome constitution influenced on the structure of photosynthetic apparatus and its functional characters.

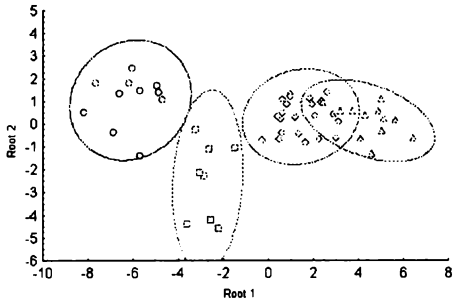

Fig.1. The discriminant analyses of diverse ploidy species
○ Aegilops, 2n=14; □ Triticum, 2n=14;
◇ Triticum, 2n=28; △ Triticum, 2n=42

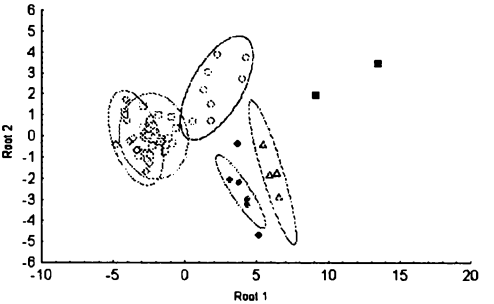

Fig..2. The discriminant analyses of diverse wheat genotypes .
■ B^{bic}, ◆ B^{L}, ● B^{sp}, △ D, ○ A, □ $A^{u}B$, ◇ $A^{u}BD$

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SPONTANEOUS ADDITIONAL SYNTHESIS OF DNA AND FORMATION OF CHROMOSOME ABERRATIONS

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A number of recent studies point out to the nonrandom character of allocation of chromosome aberrations (CA) along the length of a chromosome. Besides, there are clear experimental data which allow a suggestion that the main reason of exchange chromosome aberrations (ECA) formation is not a distortion of mutagen induced DNA repair but a regular process in cellular nucleus which initiates recombination processes which resulted in ECA formation [1].

Such a process was predicted about 30 years ago by a group of Russian geneticists by the name of Spontaneous Additional Synthesis (SAS) of DNA [2]. An amount of experimental data in favor of this hypothesis was obtained after that, however a SAS is still a hypothetic process, the main features of which are not well studied yet.

We have found in our own preliminary research on animal (human lymphocytes) and plant (Crepis capillaris and wheat) cells that incorporation of 5-bromo-2'-deoxyuridine (BrdU) at DNA in G0, G1 and the border of G1 and S-phases as well as in S-phase itself results in similar radiosensitizing effect on the CA criteria [3]. It should be noted that we used the BrdU as a marker in our experiments, because it incorporates only at DNA molecule. Thus we were faced with a fact that incorporation of BrdU at minor fractions of DNA, for example, in G0 and G1-phases led to sharply expressed radiosensitizing effect analogous to the same in S-phase.

Materials and Methods

In the present work we conducted cytological analysis of a character of incorporation of BrdU at human lymphocytes DNA as well as the cytogenetic research of the peculiarities of modifying effect of the Etoposide - an inhibitor of Topo II, on a character of a radiosensitizing effect of BrdU in PHA–stimulated human lymphocytes. To do this we studied a result of combined action of BrdU and Etoposide in GO, G1, S and G2-phases of a cellular cycle. Duration of each treatment of cells was equal to 4 hours: 0-4 hrs in G0, 20-24 hrs in G1, 32-36 hrs in S and 44-48 hrs in G2- phase. At the end of each treatment cells were irradiated with gamma-rays of Co^{60} (4 Gy). A character of BrdU incorporation in G1 (10-24 hrs) and S-G2 -phase (34-48 hrs) has been studied by means of differential chromosome staining technique [4]. We have also conducted

cytogenetic analysis of radiosensitizing effect of combined action of BrdU and Etoposide on human lymphocytes.

Results and Discussion

The analysis of differentially stained chromosomes shows that BrdU incorporation at DNA in different phases of cellular cycle has a specific character. In G0, G1 and G2 - phases BrdU incorporates at separate locuses of chromosomes and the staining occurs on the R-type. On the other hand in S-phase incorporation of BrdU takes place in accordance with the course of DNA replication process and chromosomes themselves were stained on harlequin type (Fig 1).

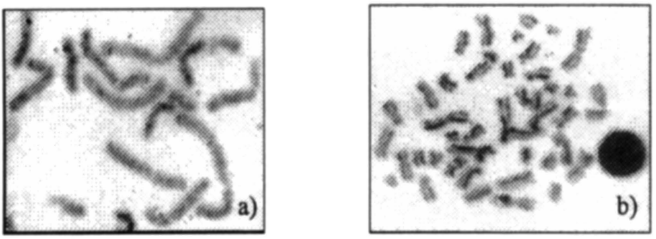

Fig 1. Differentially stained chromosomes of human lymphocytes after their incubation with BrdU in G1 (a) and S-G2-phase (b)

The cytogenetic analysis of combined action of BrdU and Etoposide has shown a specific character of the modifying influence of Etoposide on radiosensitizing effect of BrdU depending on the phase of a cell cycle (Table 1). It turned out that DNA sequences which included in BrdU in G2-phase were much more sensitive to combined action of BrdU and Etoposide on all four studied criteria, whereas G0- and G1- cells have expressed practically a similar character

Table 1. Cytogenetic analysis of combined action of BrdU and Etoposide on human lymphocytes

Phase/Treatment conditions		Aberrant cells (in %)	CA per one cell	Exchange CA per one cell	DSBs per one cell
G0	BrdU+irradiation	54,0	1,2+/-0.2	0.3 +/- 0.1	0.8 +/- 0.1
	Thymidine+irradiation	29,5	0.4+/-0.1	0.10 +/- 0.04	0.3 +/- 0.1
	BrdU+Et	23,0	0,6/-0,1	0.10 +/- 0.04	0.4 +/- 0.1
	BrdU+Et+irraditation	58,8	1,2+/-0,1	0,24+/-0,07	0,8+/-0,1
	Thymidine+Et+irradiation	40,7	0,8+/-0,1	0, 2+/-0,06	0,5+/-0,1
G1	BrdU+irradiation	61,0	1,5+/-0,2	0,4+/-0,1	0,9+/-0,1
	Thymidine+iiradiation	29,5	0,6+/- 0.1	0,13 +/- 0,04	0,4+/- 0.1
	BrdU+Et	42,1	1,4+/-0,4	0,3+/-0,1	0,9+/-0,2
	BrdU+Et+irraditation	58,8	1,1+/-0.2	0.3+/-0.1	1,1+/- 0.2
	Thymidine+Et+irradiation	36,7	1,0+/-0,3	0,2+/-0,1	1,0+/-0,3
G2	BrdU+irradiation	38,0	0.6+/-0.2	0.1 +/- 0.04	0.3 +/- 0.1
	Thymidine+iiradiation	36,0	0.5+/-0.1	0.06 +/- 0.02	0.3 +/- 0.1
	BrdU+Et	42,3	0.8+/-0.2	0.10 +/- 0.06	0.5 +/- 0.1
	BrdU+Et+irraditation	88,9	4,5+/-0,7	0,44+/-0,16	2,0+/-0,4
	Thymidine+Et+irradiation	66,7	1,3+/-0,3	0,2+/-0,1	0,8+/-0,2

of their sensitivity to radiosensitizing action of BrdU. It doesn't matter whether this action was combined with Etoposide or not. On the contrary, an incubation of lymphocytes with BrdU and Etoposide in G2 resulted in more than two times increasing of all studied criteria.

Conclusion

First of all, our analysis shows that BrdU incorporation outside of S-phase occurs at minor DNA fraction, which presents a very insignificant part of genomic DNA. Correspondingly we can conclude that the radiosensitizing effect of BrdU is determined not by the amount of incorporated BrdU but localization at a chromosome of those DNA sequences which include in BrdU during SAS of DNA in mentioned periods of cell cycle.

It proved to be also that Ethoposyde affects specifically upon the character of radiosensitizing effect of BrdU in G0, G1 and G2- phases. An analysis of data obtained in the present study allows us to propose that BrdU incorporates in G2 phases at DNA fractions which are placed at the nuclear matrix attachments cites or very closely to them: BrdU+Etoposide treatment of G2-cells in combination with irradiation resulted in significant increasing of DSB and ECA.

Acknowledgements

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MOLECULAR-CYTOGENETIC INVESTIGATION OF COMPLEX CHROMOSOMAL ANOMALIES IN THE MEDICAL-GENETIC SERVICE OF ARMENIA

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METHODS of conventional cytogenetics are applied in Armenia already for 30 years. However, cytogenetic diagnosis of complex cases stayed outside of the possibilities of conventional karyotyping. It concerns the etiology of marker chromosomes, structural and microstructural chromosomal anomalies, some cases of chromosomal mosaicism [1]. Therefore, it became important to supplement the methods of conventional cytogenetics with the molecular-cytogenetic method FISH (fluorescence *in situ* hybridization). In the medical-genetic service of Armenia FISH has been applied with our participation since 2002 year [2,3]. FISH application allows essentially to increase the quality and reliability of results of cytogenetic diagnosis and permits to realize additional retrospective investigations of the patients, whose karyotypes, previously analyzed by the methods of conventional cytogenetics, continued to remain unspecified.

Materials and Methods

We have realized prenatal and postnatal analysis of 174 patients, directed to the medical-genetic service at Research Center of Maternal and Child Health Protection in the period 2000-2004. In the 168 cases performed postnatal and in 6 cases – prenatal investigations. Investigated patients included the following groups: with multiple inborn developmental defects; with anomalies of sexual development; with burdened hereditary and obstetrical anamnesis. From 168 postnatal investigations, 38 cases were selected for molecular-cytogenetic analysis of complex chromosomal anomalies (fig. 1, fig. 2). Analysis of 26 cases was performed in collaboration with Institute of Medical Genetics, University of Zurich. 8 cases are already included in European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations (ECARUCA). In 6 cases FISH was applied for prenatal analysis. Metaphase chromosome preparations were obtained from PHA-stimulated lymphocyte cultures. Chromosome analysis was carried out on the G- and C- banded metaphases. Commercial (Vysis, USA; Cytocell Technologies, U.K.) and homemade DNA-probes were used. BAC clones of interest have been obtained from the University of Bari, Italy. Whole Chromosome Painting, Centromere Enumeration Probes, Locus Specific Identifier and Subtelomere specifi DNA-probes were introduced in the work. After counerstaining by DAPI II (Vysis, USA) slides were interpreted by three filter-set fluorescent microscope (Zeiss, Germany) and analyzed by software ISIS (MetaSystems).

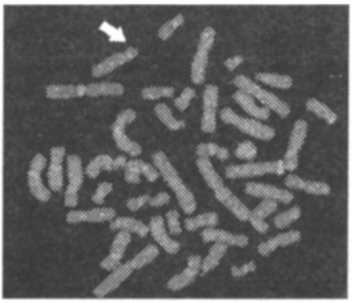

Figure 1. Cri-du-chat syndrome. Karyotype: 46,XX.ish del(5)(p15.2p15.2)(D5S721-,D5S23-).Used DNA-Probe: 5p15.2(D5S721 D5S23)/5q31 (EGR1)

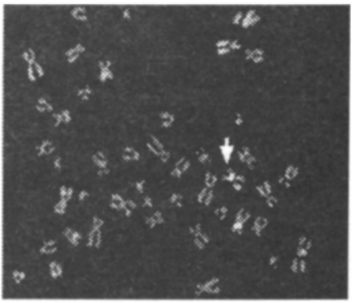

Figure. 2. Williams-Beuren syndrome. Karyotype: 46,XX. ish del(7)(q11.23q11.23)(ELN-). Used DNA-Probe: 7(D7Z1) alpha-satellite Control Probe/7q11.23

Results and Discussion

Investigation of patients was performed in 3 stages: general clinical investigations, conventional cytogenetic investigations and molecular-cytogenetic analysis. In the table 1 presented the results of cytogenetic investigations. From the 168 postnatal cases, analyzed by conventional cytogenetics, in 38 complex cases (23%) diagnosis needed in the specification.

Table 1. Results of cytogenetic diagnosis of chromosomal anomalies

Investigated groups	Quantity of cases analyzed by the methods of conventional cytogenetics	Quantity of FISH-investigations
autosomal anomalies	100 (59,5 %)	8
numerical	84	1
structural	8	4
numerical+ structural	8	3
mosaic forms	4	2
anomalies of sex chromosomes	38 (22,6 %)	13
numerical	27	2
structural	5	4
numerical+ structural	6	7
mosaic forms	9	7
sex reverses	8 (4,8 %)	6
marker chromosomes	3 (1,8 %)	2
mosaic forms	2	1
chromosomal variants	13 (7,7 %)	3
normal karyotype but deviation in clinical description	6 (3,6 %)	6
prenatal investigations	-	6
Sum. total	168	44

In the table 2 are presented indications to FISH application and the results of molecular-cytogenetic investigations.

Table 2. Results of molecular-cytogenetic diagnosis of chromosomal anomalies

Indications to FISH-analysis	Quantity of FISH-investigations	Results of FISH-investigations			
		Coincidence of diagnoses	Specification of diagnoses	Change of diagnoses	Unspecified diagnoses
IC	5 (13 %)	3	-	-	2
SSCA	9 (24 %)	4	5	-	-
IC with SSCA	19 (50 %)	9	6	4	-
DLPM	5 (13 %)	4	1	-	-
Sum total	38	20 (52,6 %)	12 (31,6%)	4 (10,5 %)	2 (5,3 %)

IC - identification of chromosomes; SSCA - specification of structural chromosomal anomalies; DLPM - determination of low percentage of mosaicism.

Conclusion

On the base of cytogenetic investigation of largest group of patients we can conclude that in the quarter of cases of complex chromosomal anomalies the cytogenetic diagnosis, obtained on the base of conventional karyotyping, needed in further specification. Only in the half of these cases FISH-investigations confirm results, received on the base of conventional cytogenetics. In the other cases diagnosis was specified or changed. The data received as a result of introduction of molecular-cytogenetic method FISH are important for the medical-genetic service of Armenia.

Acknowledgements

The author is grateful to Professor Arbert Schinzel (Institute of Medical Genetics, University of Zurich) for the significant help in the introduction of molecular-cytogenetic investigations to the medical-genetic service of Armenia.

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MULTICOLOR FISH TECHNIQUES AS POWERFUL TOOLS TO STUDY CRYPTIC CHROMOSOMAL ABERRATIONS IN ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL)

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Abnormal karyotypes have been reported in up to 80% of children and 70% of adults with ALL, many of which are extremely complex [1]. Molecular genetic and molecular cytogenetic approaches have demonstrated that a small percentage of apparently normal karyotypes may be cryptic versions of known recurrent translocations, generated by submicroscopic insertions or more complex rearrangements [2]. Therefore, the purpose of this study was to disclose cryptical and complex structural aberrations in a series of ALL patients with previously normal karyotypes by multicolor FISH techniques.

Materials and Methods

Bone marrow samples of 16 karyotypically normal (according to routine banding cytogenetics) patients with ALL were studied. Seven patients had T-ALL, 5 patients with ALL affected B lineage, and four patients did not have any phenotypical information. A cytogenetic analysis was performed on non-stimulated cultures of bone marrow samples according to standart protocols. We applied M-FISH, mMCB (multicolor chromosome banding)[3] and subCTM-FISH (i.e. 24 chromosome-specific probe sets consisting of subcentromeric, subtelomeric and whole chromosome painting probes) [4] by labeling of corresponding BAC, PAC plasmid DNA and microdissection derived WCPs. Multicolor FISH was performed according to standard protocols. At least 10 metaphases were analyzed in each case. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature [5].

Results and Discussion

We detected known abnormalities as well as novel and rare rearrangements. The results of the study are presented in Table 1.

We found novel cryptic and complex aberrations in five cases. To the best of our knowledge revealed rearrangements ins(5;7)(q21;p14p22)t(3;7)(p21;q11.23), t(7;17)(p22;q22), del(4)(q31), (5;7)(q31;p12) and t(5;6)(q23 or q31;q23 or 24), t(4;18) (see Fig1) are not yet described in previously data [6,7]. At six patients were shown very complex chromosomal abnormalities. Altogether we found deletions of 4q31, 5q34, 5q21, 7q11.23, 9q34.3, 9p24, 11p15.5, 11q25, 12p13, 13q34, 17q25 (see Fig1). Some of them have been already detected in haematological malignancies [6,7].

This pilot-study demonstrate an unexpected high rate of cryptic chromosomal aberrations of 75 % of ALL-cases. Confirmation of FISH results by MCB, M-FISH and mMCB probes, subCTM probe set allowed to find partner chromosomes, including in rearrangements, and to describe breakpoints. Using different combination of FISH techniques with routine cytogenetic analysis becomes essential condition to discover new aberration and chromosomal breakpoints, potentially contain genes, involving in leukemogenesis .

Acknowledgements

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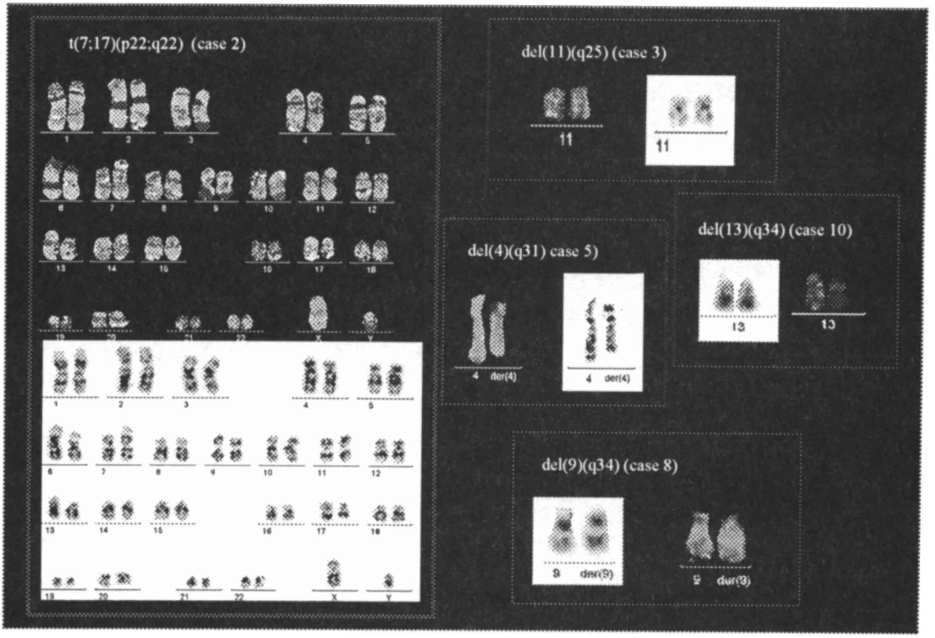
Table1. Details and summary of FISH results of studied ALL cases

Patient	*Age(yrs)/sex*	*Diagnosis*	*FISH results*
1	8/M	Pre-T-ALL	46,XY,+21,ins(5;7)(q21;p14p22)t(3;7)(p21 ;q11.23), t(9;22)(q34 ;q11) t(?;14)(?;q32?)
2	42/M	T-ALL	46,XY, t(7;17)(p22;q22?),del(12)(p13.33)
3	65/F	B-ALL	46,XX, del(11)(q25?), t/del(17)(q25), del(18)(p?)/dup(18)(q11)
4	23/F	B-ALL	46~100
5	57/F	Pre-B-ALL or c-ALL	46,XX, del(4)(q31), del(5)(q21)/del(5)(q11.2)/r(5q),del(9)(p24?)
6	19/M	ALL	46,XY
	19/M	ALL-Rec.	46,XY,del(12)(p13.33)
7	19/F	pre-B-ALL,Ph+?Rec	46,XX, del(11)(p15.5)
8	45/F	T-ALL,VK	38~46,XX, del(9)(q34.3)
9	96264	ALL	Normal
10	30/M	T-ALL in CR	46,XY, der(4)t?,del(13)(q34)
11	23/M	T-ALL	46,XY
12	22/M	T-ALL after Ch.Th.	46,XY
13	51/F	pre-pre-B-ALL rez.	46~89
14	21/M	T-ALL VK,remission	45,XY,-19
15	17/F	acute leukemia	45,XY,der(4)?t(4;18)?, der(18)?t(4;18)?,mar1, mar2
16	?/F	ALL	46~47,XX,+8, der(4)? t/del(4) (p16.3?), del(12)(p12),del(5)(q34), t(5;7)(q31;p12), t(5;6)(q23 or q31;q23 or q24),

Figure 1.



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ESTIMATES OF GENOTOXIC EFFECT BY THE ANALYSIS OF MALE GAMETOPHYTE GENERATION OF VINES

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Introduction

The process of in situ indication of genotoxicity requires well-founded selection of indicator species to obtain a reliable indicator of the genotoxic potential in the examined locality or region.

One of advantages in using pollen grains as bioindicators of mutagencity is the high sensitivity conditioned by the haploid state, when all lethal mutations affecting the development of pollen are immediately evident (Micieta and Murin, 1995). The pollen abortion assay reflect deletions in virtually any part of the genome. However, the greatest advantage is that the species live in the polluted environment under evaluation, and this makes possible to evaluate the effectiveness of ecological factors including pollution components (Constantin,1984).

For such research are very convenient the sorts of Vitis vinifera with low level of sterility. Some minimal sterility of pollen is detected even in normal functional anthers. It can be the result of abnormal distribution of chromosomes in meiosis. Lyakh (1995) presented interesting hypothesis about the evolutionary consequences of decrease or absence of concurrence between microgametophytes. In that case in the population arrive many new non-typical forms, that can be positive for the preservation of the species in the changing conditions of the environment.

Male gametophyte generation of vines can be applied as bioindicators of genotoxicity (Ma, 1979). The pollution of the environment with technogenic factors can change the evolutionary formed complexes of adaptive reactions. In situ indication of genotoxicity requires well-founded selection of indicator genotypes to estimate genotoxic potential in the examined locality or region (Micieta and Murin,1995).

The reasons of formation of abortive pollen can be different. They can include the regulatory role of tapetum, RNA, i-RNA, intensity of all synthetic processes. Pederson et al (1987) mentioned the difference in the gene expression on the different stages of formation and functioning of the pollen. In the species with three-cell pollen the genes can be expressed on both stages, and in two-cell pollen most of the genes are expressed during the pollen formation.

Penetration of different types of irradiation (UV, gamma, X-rays) varies in species, depending on the size and shape of the pollen grain, and the thickness of the pollen wall (Gilles, Prakash, 1987). Low levels of normal pollen bring to low fertilization and abnormal seeds development.

One of mentioned approaches, allowing to estimate the genotoxic action of environmental factors, is the test for the definition of plants pollen grains fertility. Flowering plants have been used as bioindicators of mutagenicity, phototoxicity and genotoxicity of environmental pollutants. Pollen grains fertility parameter for any kinds of plants including fruit trees, allows to estimate gametocide effects of environmental mutagens. Thus, it is reasonable to use those kinds of plants that proceed in the investigated environment sporophyte and gametophyte cycles of development.

Materials and Methods

The complex analysis of male reproductive system of vines growing on distance of 3-5 km from the Armenian Nuclear Power Plant (ANPP) near the settlement Metsamor, in comparison with the control point on distance more than 30 km from ANPP is realized. Investigated 10 sorts of vines are Charentsi, Meghrabuyr, Nerkarat, Burmunk, Shahumyani, Kishmish black, Kishmish white, Ararati, Arakseni, Hadisi. Biometric parameters of pollen are investigated by the method of the acetocarmine preparations analysis. In the fertile grains the cytoplasm is colored dark carmine-red. The sterile pollen grained are not stained or are stained non-uniformly. The data obtained on the basis of analysis of large quantities of pollen grains (about 10000 in each variant).

Results and Discussion

The results presented in Table provide evidence for a high level of pollen fertility in investigated sorts of vine. The highest level of pollen fertility discovered for sort Nerkarat (in control $98.84 \pm 0.11\%$, in experiment $97.62 \pm 0.15\%$). The data on sorts Charentsi, Meghrabuyr, Nerkarat, Burmunk, Kishmish black, Arakseni, Hadisi are shown the fertility level more than 87% in both points of experiments. Analysis of the male gametophyte of vines confirmed a higher quality of the pollen population.

Table. Properties of pollen fertility in vines in different points of growth

Sort of vine	Pollen fertility, %	
	Metsamor	Control point
Charentsi	95.36 ± 0.21	95.16 ± 0.21
Meghrabuyr	90.60 ± 0.29	90.80 ± 0.29
Nerkarat	98.84 ± 0.11	97.62 ± 0.15
Burmunk	96.60 ± 0.18	97.69 ± 0.15
Shahumyani	89.96 ± 0.3	72.36± 0.45
Kishmish black	98.0 ± 0.14	97.34 ± 0.16
Kishmish white	87.67 ± 0.33	84.90± 0.36
Ararati	69.55 ± 0.46	96.8 ± 0.18
Arakseni	87.70 ± 0.33	96.92 ± 0.17
Hadisi	97.03 ± 0.17	94.17 ± 0.24

Conclusion

The obtained results demonstrate high fertility of pollen of investigated sorts of vines growing around the area of ANPP, that significantly not differs from the control point. Thus, the influence of the ANPP on the male generative system of the investigated sorts of vines is not revealed.

The further monitoring of pollen fertility is necessary at the different plants species, growing around the area of ANPP, for the definition of their suitability for bioindication of action of environment factors.

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THE STUDY OF PHENOMEN ON FASCIACIA ON THE ARABIDOPSIS THALIANA

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Fasciation is a term used to describe a variety of developmental abnormalities in the shoot system [1]. These include distortions in phyllotaxy and broadening, flattening and in extreme cases, bifurcation of the stem. Fasciation represents a breakdown in the pattern of organogenesis and has been associated with meristematic enlargement. [2] The study of fasciacia might reveal how meristem structure and function are established and maintained in normal plants. The study phenomenon of fasciacia also supports elaboration of methods of rising crop.

Materials and method

Seeds mutants 90 (clavata pod) and yellow-green mutants 12.6.15, V-76, 58/15 locus Flavi1, mutant 568/5 locus chrolina-5 and mutant 130 locus clavovilidis was used in experiment [3]. The seeds were placed at 4°C for one month and then seeds were sown in a soil. Soil: 60% sand, 40% turf. All plants were grown under 600 ft-candles of constant cool white fluorescent light at 15°-20° C. Seeds were sown at a maximum density of one seed per five cm^2.

Results

We crossed mutant 90 with green-yellow mutants. The plants in the F_1 have been wild type. We analyzed F_2 generation. (Tab.1) The phenomenon fasciacia of stem was observed among plants class green with clavata pods, owing to the fact that we supposed fasciated plants were heterozygotes (Photo). The plants F_2 generation was grown up and their progeny (the F_3 generation) was harvested from each F_2 plant separately to give a collection of F_3 families. We have (plants F3) lines 14 with different expressivity and penetrance. (Tab2) The plants with phenomenon fasciacia of stem have been positively correlated between fasciacia of stem and the quantity pods and quantity of seeds in the pods. (Tab3)

Conclusion

The fasciated plants are heterozygots. The fasciated plants have been positively correlated between fasciacia of stem and the quantity pods and quantity of seeds in the pods. The fasciated plants have more pods and quantity of seeds in the pods than mutant 90.

Tab. 1

The F_2 segregation in crosses between mutant 90 and yellow-green plants.

Cross	Total	Wild type	Green, cla	Yellow, normal	Yellow, cla	X^2	P 9:3:3:1
90x12.6.15	156	105	31 (23)	15	5	12,74	>0,05
90xV-76	70	49	14 (6)	11	0	7,33	<0,05
90x568/5	116	91	19 (10)	1	5	43,33	>0,05
90x58/15	130	93	30 (12)	4	3	27,65	>0,05
90x130	126	80	27 (3)	12	7	7,21	<0,05

Note: in bracket - number of fasciated plants

Tab. 2

Analyse penetrance in F_3.

Progeny in crosses between				
90x12.6.15	90xV-76	90x58/15	90x130	90x568/5
A1 75,47%	B1 52,75%	C1 76,74%	D1 12,28%	E1 24,71%
A2 42%	B2 55,26%	C2 66,67%	D2 0%	E2 96,43%
A3 61,82%	B3 24,39%	C3 100%		E3 50%
A4 0%		C4 66,67%		

Tab3

Calculation of quantity characteristics in F_3.

Mutants	Height sm.	Quantity Pod	Quantity seeds in pod	Width of stem mm.
Mutant 90	30,5±2,2	90,6±9,4	49,5±3,2	1,2±0,02
A1	23,5±1,6	156,5±10	75,8±4	3,4±0,1
A2	26,8±1,2	148,7±9,5	69,7±3,2	3,5±0,2
A3	25,7±1,1	147,2±8,2	70,6±3,1	3,6±0,2
B1	21,1±0,8	160,2±11	72,3±4,1	4,2±0,4
B2	20,3±0,7	155,6±9,3	68,7±3,1	4,4±0,2
C1	15,6±0,3	151,4±9,7	65,2±2,8	4,1±0,1
C2	22,2±1,4	148,3±8,4	63,7±2,5	3,9±0,3
C3	21,4±1,7	146,2±7,6	69,7±3,2	3,7±0,2
D1	23,1±1,3	142,3±7,4	50,2±3,1	1,4±0,06
E1	25,2±1,5	140,4±7,5	49,5±2,8	3,2±0,05
E2	21,5±1,4	142,3±8,2	55,7±3,3	3,4±0,2
E3	22,7±1	144,6±8,6	57,4±3,4	3,5±0,3

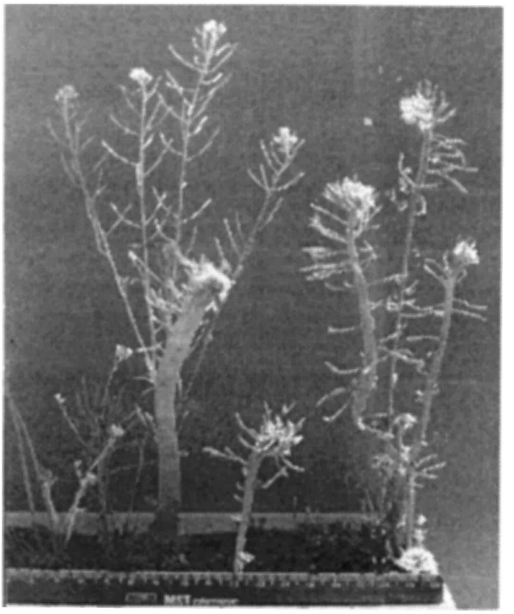

Photo. Fasciated plant and not fasciated plant

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GENOME INSTABILITY OF THYROID CELLS OF PATIENTS SUFFERING FROM PAPILLARY THYROID CANCER IN DIFFERENT REGIONS OF BELARUS

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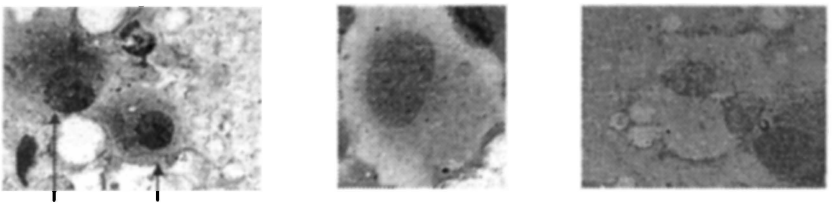
At present the cytogenetic effects in somatic cells are the important parameter of the level of human genetic damages under the effect of ionizing radiation.

Materials and Methods

Objects of the given cytogenetic research were patients (PC) who at the moment of Chernobyl accident were under 20 years old and lived in the areas of Belarus with various radioactive contamination levels. Autopsy thyroid material of people from *Minsk* who died for the reasons not associated with thyroid diseases was used as the control. Cytogenetic analysis of thyroid cells *in vivo* was carried out by *the interphase method of the aberrant chromosome analysis* [1]. Cells with micronuclei (MN) (Figure 1. b, c) and aberrant cells with bridges (Figure 1. d) were considered by light microscopy on smear-impressions of removed tumour and nontumour thyroid tissues of PC patients.



a) b) c) d)

Figure 1. Thyroid cells: a) normal; b),c) with MN; d) with a bridge

Results and Discussion

The analysis of the cytogenetic damage level in thyroid tissues (Table 1) has shown that the level of cells with MN and bridges in tumour and nontumour tissues of PC patients from all examined areas was 3-5 times as high as in the control. The presence of markers of radiation exposure - cells with bridges and with plural aberrations was noted in 53 % of the examined persons, in 33 % they were registered in both tissues of PC patience but not in the control. The presence of the high level of aberrant cells and cells with plural aberrations not only in tumour, but also in nontomour tissues of these patients points to a mutation process course in the entire thyroid tissue of PC patients and allows the assumption of external or internal radiation exposure of the whole thyroid tissue in these people. From the data presented in Table, it follows that the highest level of MN and aberrant cells with bridges in nontomour tissue was registered in

patients from the *Brest* region (0,89%±0,06 %). It exceeded the levels of genetic damages observed in tissue of patients from *Gomel* and *Minsk* regions (P<0.05). In tumour tissue, the highest level of cytogenetic damages was observed in patients from the *Gomel* region (1,19±0,06%). The level of cytogenetic damages in patients from the *Minsk* region did not differ in tomour and nontumour tissues and was 0,74 %. The significant difference in the yield of MN and aberrant cells with bridges in the tumour tissue was noted between *Gomel* and *Minsk* regions (P<0.05), the *Brest* and *Minsk* regions (P<0.05).

Table 1. The level of cytogenetic damages in tumour and nontumour thyroid tissues of PC patients

Thyroid tissue	Location	Number			
		examined people	cells		
			examined	with MN and bridges (x±Sx)	with plural aberrations (x±Sx)
Nontumour tissue	Gomel.region	22	23004	0,73±0,05*	0,07±0,03
	Brest.region	20	19244	0,89±0,06*	0,06±0,02
	Minsk region	9	8541	0,74±0,09*	0,05±0,03
	Average			**0,79±0,07***	**0,06±0,03**
Tumour tissue	Gomel.region	22	24523	1,19±0,06*	0,10±0,02
	Brest.region	20	21482	1,04±0,06*	0,08±0,02
	Minsk region	9	9639	0,74±0,08*	0,08±0,04
	Average			**0,99±0,07***	**0,09±0,03**
Autopsy tissue (the control)	**Minsk**	**13**	**75500**	**0.21±0.02**	**0**

Note: * - *Significant difference from the control (P<0.01)*

Distinctions between regions in the rate of the mutational process can directly be associated with ecological conditions there. So, for the *Gomel* region, it is typical as for the situation with the highest radioactive contamination after Chernobyl accident and more auspicious conditions with iodine provision in comparison with the *Brest* and *Minsk* regions. Expressed iodine endemia observed in the *Brest* area on the one hand promotes nonuniform irradiation of thyroid, reaching a doze tens of times exceeding the average one in its separate sites, on the other hand, increases a dynamic and proliferative activity of thyroid gland with accompanying goitre and other benign processes in thyroid. Thus, real dozes for thyroid microsites even under equal radioiodine taken by an organism, and in some cases with equal exposure for rate thyroid gland of the persons living in goitre endemic regions, can exceed by an order and more the total doze for the whole gland and be more than in people from nonendemic regions [2]. It in turn makes the contribution to the expression of cytogenetic damages in thyroid cells. Probably, this can explain a higher level of chromosome aberrations in nontumour tissue of

patients from the *Brest* region in comparison with the level noted in nontumour tissue of patients from the *Gomel* region.

It is known that the irradiation doze plays a great role in determination of the life-span of the damaged cell (before its division). Since at low dozes of thyroid irradiation cells do not die, and can enter a lot of divisions, not only maintaining the aberrations, but also giving rise to new cells with the same damages and as thyroid is a poorly proliferative tissue, it is possible to assume that not only new aberrations occur as a result of numerous division of less irradiated cells, but also these damages are accumulated [3]. This can explain the fact of the presence of greater number of radiation exposure markers - cells with bridges in thyroid tissue of patients from the *Minsk* region in comparison with other regions and the control. Their quantity in tissue of patients from the *Minsk* region is 5 times as high as in the control ($P<0,05$) and above 2 times, than in the *Gomel* and *Brest* regions. Number of cells with plural chromosome aberrations was observed approximately with identical frequency in thyroid tissue of all examined persons from different regions of Belarus.

Conclusion

Thus, the cytogenetic analysis of thyroid tissues of PC patients has revealed the high level of cytogenetic damages not only in tumour, but also in nontumour tissues of patients from all three regions of Belarus. Cytogenetic changes in the form of cells with one, two and more MN and bridges, indicating the presence of external or internal effect on the whole thyroid tissue of PC patients were noted in both tissues of the examined patients. And, undoubtedly the cytogenetic damage level was higher there where the most inauspicious ecological situation was in the *Gomel* region where the maximal ratio between the fall-out density of ^{131}I and that of ^{137}Cs [4] was registered, and *Brest* where the combination of goitre endemia and the radiation factor have caused an additive effect of both factors on thyroid cells and on the whole gland.

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ALTERATIONS OF 5S rRNA GENES IN *TRITICUM-AEGILOPS* ALLOPOLYPLOIDS

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Introduction

The genomic organization of nuclear genes coding for 5S ribosomal RNA was described for many diploid and polyploid *Triticeae* species. This genes are organized as one or several arrays of tandemly repeated elements. Each repeat consists of a highly conserved 120 base pair (bp) coding region and a polymorphic nontranscribed spacer of about 200-400 bp. According to the spacer length 5S rDNA repeated units were divided into two subfamilies *5S DNA-1* and *5S DNA-2* (1, 2). It was shown that in a number of natural allopolyploids the entire arrays containing 5S rDNA of one of the progenitors have been completely lost or replaced by the genes of another progenitor (3). However, in cases of natural allopolyploids it is difficult to estimate the timing of such a reorganization since most of them have a long history and unknown origin. The synthetic allopolyploids represent convenient model for study of genomic changes induced by allopolyploidy itself. Using this model different genomic alterations involving various DNA sequences were revealed (4). The objectives of the research were to determine if the changes in 5S rDNA organization are induced by allopolyploidy and to define the stage of polyploid formation when such a reorganization could take place. We used as a model the newly synthesized *Triticum* and *Aegilops* allopolyploids.

Materials and methods

The newly synthesized allopolyploids (S_1-S_5) *Ae.umbellulata* TU04 × *Ae. sharonensis* TH01, *T. urartu* TMU38 × *Ae. tauschii* TQ27, *T. dicoccoides* TTD20 × *Ae. tauschii* TQ27 and their parental plants was kindly supplied by Dr. Feldman from Weizmann Institute of Science (Rehovot, Israel). Total genomic DNA was extracted from young leaves of individual plants (4). PCR amplification, cloning, sequencing, Southern-blot hybridization were described in (5). As a probe for Southern-blot hybridization we used α-^{32}P labelled clone pTa794 which contains a fragment of the 5S rDNA isolated from common wheat, *T. aestivum* L. (1).

Results and Discussion

To reveal possible changes we compared PCR patterns of allopolyploids and corresponding parental plants. The PCR analysis was done using specific primers designed to amplify the 5S rDNA repeating unit. All the studied species, except *T.urartu*, produced two PCR fragments of about 400 and 500 bp, whereas *T.urartu* produced one prominent fragment of about 300 bp. The allopolyploids TU04 × TH01 and TTD20 × TQ27 as well as their parents produced two PCR fragments. The PCR pattern of TMU38 × TQ27 showed lower intensity of the fragments from *Ae.tauschii* than the corresponding parental fragments. The amplification of a control parental DNA mix yielded fragments of similar intensity from both parents (Fig. 1). The hybridization pattern of allopolyploid TMU38 × TQ27 contains DNA fragments from both parents, but the fragments derived from *T.urartu* was more intensive. The data of PCR analysis were supported by Southern-blot hybridization using different restriction enzymes. The described PCR and RFLP-patterns was held in all analyzed self-pollinated progenies (S_2 – S_5) compared with parental species. For further analysis of 5S rDNA sequences we cloned and sequenced the total PCR products of allopolyploid TMU38 × TQ27 and it's parents. Totally, 14 clones belonging to a long (~500 bp) and short (~ 400 bp) subfamilies were sequenced. For comparative study of primary structure we downloaded all the published 5S rDNA sequences of *T.urartu* and *Ae.tauschii* from databases (Table 1) and aligned the sequences with Clustal W program.

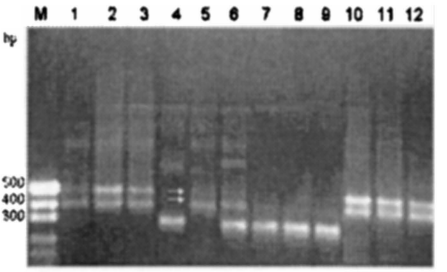

Fig.1 PCR analysis of 5S rDNA. The 5S rDNA units were amplified, products were separated in 1% agarose gel and hybridized with α-^{32}P labelled pTa794
1-*Ae.sharonensis* TH01; 2-*Ae.umbellulata* TU04;
3- TU04xTH01 (S_1); 4 - *T.urartu* TMU38; 5 - *Ae.tauschii* TQ27; 6 - DNA mix (TMU38/TQ27);
7, 8, 9 - TMU38xTQ27 (S_2, S_3, S_5); 10 - *T.dicoccoides* TTD20; 11, 12- TTD20xTQ27 (S_2, S_3). Bands that showed weak intensity in the TMU38xTQ27 are indicated by arrows.

Table 1. 5S rDNA sequences of allopolyploid *T.urartu* x *Ae.tauschii* and corresponding parental species.

Genome	Overall number of DNA sequences	Number of DNA sequences[1] of different length		
		*5S DNA-1** 350 bp	*5S DNA-1* 400-450 bp	*5S DNA-2* 450-500 bp
T.urartu × *Ae.tauschii*	9'	8'	-	1'
T.urartu	25+4'	15+4'	-	10
Ae.tauschii	2+1'	-	1	1+1'

*- subfamily specific for *T.urartu* (A genome)

[1] - i.e. full monomeric units

' - cloned DNA sequences isolated in present research

DNA sequences isolated from TMU38 × TQ27 were closely related to analogous sequences from *T.urartu* (95% homology between consensus sequences). Thus, the relative amount of short 5S rDNA sequences, specific for *T.urartu*, really increases in allopolyploid TMU38 × TQ27.

Conclusion

We used the three newly synthesized *Aegilops* and *Triticum* allopolyploids as a model system to study 5S rRNA genes organization in the early generations after polyploidization. Using PCR analysis, Southern-blot assay and comparing primary structures of fourteen 5S rDNA sequences cloned from allopolyploid *Triticum urartu* (TMU38) × *Aegilops tauschii* (TQ27) and corresponding parents we found the changes in 5S rRNA genes organization in the allopolyploid, namely, the increasing of relative amount of 5S rDNA sequences, specific for *T.urartu*. Thus, we showed that quantitative reorganization occured early in polyploid formation and held in the next generations.

This work was supported by grants from INTAS (01-0537).

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HSP70 OF D. MELANOGASTER ARE "HOTSPOTS" FOR P ELEMENT INSERTIONS

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Introduction

The 70 kDa family of hsps is a major system of cellular response to stress in nearly all organisms (Zatsepina et al., 2001). Recent studies have shown that promoter regions of these genes in *D. melanogaster* sometimes are disrupted by transposable elements (TE) (Lerman et al., 2003). Such insertions may play an important role in microevolution of *hsp70* genes and in organism adaptation to local thermal environment. In this study we investigated the frequency of P element-containing construction (*EPgy2*) insertions into the *hsp70* genes using *D. melanogaster* model system. Our results show that promoter regions of *hsp70 Aa* and *Ab* are "hotspots" for *EPgy2* insertions.

Material and methods

***Drosophila* stocks**: *D. melanogaster* strains, containing *EPgy2* insertions, were obtained from the Berkeley Drosophila genome project from university of California, Berkeley. All flies were cultured at 25^{0}C on a yeast, molasses and agar medium. **Genetic crosses:** we used genetic crosses (Fig.1) to generate strains that may have the additional copy(s) of the starting element *EPgy2*. Local transpositions from the starting element *EPgy2* were generated in the F_1 males with *Δ2-3* transposase source. The new insertions were recovered in the F_2 males with elevated eye pigmentation.

	Female		Male
F_0	EPgy2	x	*Δ2-3*
F_1	w/w; +/+	x	*EPgy2/ Δ2-3*
F_2	w/w; +/+	x	*EPgy2**
		↓	elevated eye pigmentation
			Stocks

Figure1. Genetic crosses

Southern blot analisis: genomic DNA was preparated as described by (Zatsepina et al., 2001). Each DNA sample was digested with BamHI/HindIII endonucleases, separated by agarose gel electrophoresis, transferred to the Hybond N+ membrane. Hybridization was carried out as described by (Zatsepina et al., 2001) with radioactively labeled XhoI/BamHI-fragment complementary to 5'-end and BamHI/SalI-fragment complementary to 3'-end of *hsp70 D. melanogaster*. **PCR screens**: the polymerase chain reactions were performed for 30 cycles. We employed primers from 5'- and 3'-ends of P element as described by (Timakov et al., 2002), primers from 5'- and 3'-ends of *hsp70 genes* of *D. melanogaster* with in outward and inward orientations, from *CG18347* and from *aurora*.

Sequencing: to identify the *EPgy2* insertion sites we used the direct sequencing of the PCR products with appropriate primers. Sequencing was performed in the centre "Genome" of EIMB RAS (http://www.genome-centre.narod.ru/).

Results and discussion

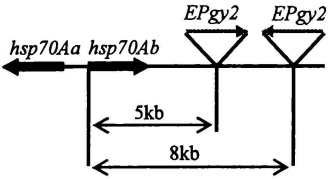

Figure 2. Location of the starting element *EPgy2* in two stock strains *D. melanogaster*

We investigated the frequency and localization of P element-base construction transpositions in two deletion strains of *D. melanogaster*, which contain the starting element *EPgy2* located at 5 and 8 kb distance from *hsp70Ab* (Fig.2). Local transpositions were produced by special genetic crosses (see above). *EPgy2* contains mini-white as a marker (Hugo J. Bellen et al., 2004) and flies carrying an additional copy of this construction showed elevated eye pigmentation. We have found that overall frequency of *EPgy2* transposition was 7,5% (562/7400 males). To detect insertions in *hsp70* genes we used the method of Southern blot hybridization. DNA digestion with BamHI/HindIII endonucleases and subsequent hybridization with 5'- and 3'- end of *hsp70* allowed to identify insertions in different copies (six total) of *hsp70* genes (Fig. 3A).

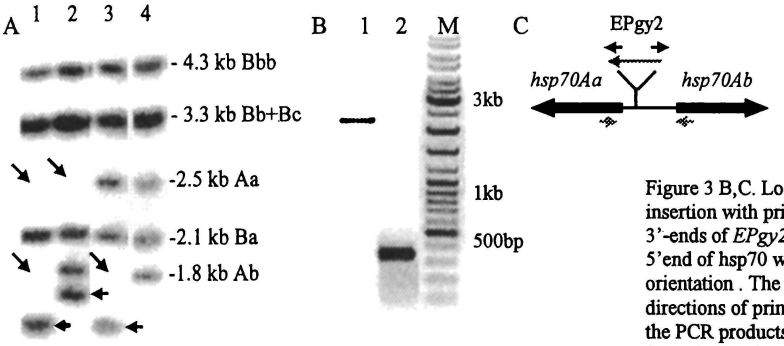

Figure 3A. Hybridization with 5'-end of *hsp70*. Changing of banding pattern in Southern hybridization due to insertion of EPgy2 is marked with arrows.*EPgy2* insertions in *hsp70Aa, Ab* (lane 1), *hsp70Aa* (lane 2), *hsp70Ab* (lane 3);

Figure 3 B,C. Localization of *EPgy2* insertion with primers from 5'- and 3'-ends of *EPgy2* and primers from 5'end of hsp70 with an outward orientation . The arrows indicate the directions of primers (5' to 3') (C); the PCR products that were produced from these primers (B – lane 1, 2). Molecular size standards are shown on the right

The frequency of *EPgy2* transposition into *hsp70* genes is 8,2% (31/375). The genomic location and orientation of insertion *EPgy2* were confirmed by PCR. We scanned the genomic region near starting element by using several PCR reactions with primers to 3'- and 5'-ends of *EPgy2*, and primers to promoter, structural and 3'-flanking regions of *hsp70* genes. Results of one of these reactions are depicted in the Figure 3B, C. Our analysis of first construction (starting element located at 8 kb distance from *hsp70Ab*) has shown that: *EPgy2* inserts in opposite;

EPgy2 inserts in promoter region of *hsp70* (within 44 - 256bp interval) preferentially at positions -112 and -113 bp upstream from transcriptional start; the majority of transpositions were detected in *hsp70Aa* (87%) and the rest in *hsp70Ab* (13%); no local insertions were found in the coding region or in 3'-flanking region; no local insertions were found in *hsp70Ba, Bb, Bc, Bbb* (87C locus). Herein we present localization (upstream from transcriptional start, bp) and frequency (%) of *EPgy2* insertions in *hsp70Aa, Ab* genes: -112, -113bp (58%), -151 (12,9%), -256 (6,4%), -58 (6,4%), -44 (3,2%), -56 (3,2%), -160 (3,2%), -190 (3,2%), -153 (3,2%).

Analysis of second construction (starting element located at 5 kb distance from *hsp70Ab*) confirmed our results on highly non-random distribution in the process of transposition.

Conclusions

High frequency of P element-base construction insertions into *hsp70* promoters indicate that these regions are hot spots for P element transpositions. The target preference of such insertions could be explained if the *EPgy2* predominantly inserts into the "open" chromatin containing an active promoter of *hsp70* genes. Thermotolerance experiments revealed significant differences in the survival of strains with insertions in *hsp70* loci in comparison with the original strain. Basing on thermotolerance experiments exploring the transgenic strains we speculate that such insertions may play an important role in the microevolution of *hsp70* genes and in organism adaptation to thermal environment.

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THE PROTO-ONCOGEN C-KIT LOCALIZATION ON THE B-CHROMOSOMES OF CARNIVORA

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B-chromosomes are small, supernumerary chromosomes found in certain species, in addition to the standard complement of A-chromosomes. It is generally believed [1, 2] that most B-chromosomes lack major genes, this hypothesis has not been rigorously tested [2].

Here we report the localization of C-KIT proto-oncogene on B-chromosomes of two canid species – red fox (*Vulpes vulpes*) and raccoon dog (*Nyctereutes procyonoides*) by FISH. It is the first finding of a known autosomal gene on the mammalian additional chromosomes. We detected the C-KIT presence on all the canid Bs investigated and revealed unexpected conservancy between sequenced regions of canine C-KIT gene and fox B-chromosomal C-KIT copy. These findings argue in favour of potential role of B-chromosomes in the genome and give new insights onto their origin and evolution.

Materials and Methods

Slide and probe preparation. Metaphase chromosomal spreads were prepared from primary fibroblast and peripheral blood lymphocytes cultures and bone marrow as described previously [3, 4, 5].

Fox and raccoon dog B-chromosome specific probes were obtained by flow sorting and microdissection, as described [3,4].

FISH. Fluorescence in situ hybridization was performed using a standard protocol [3, 5]. Canine BAC clones were labelled by NICK-translation kit (Invitrogen, CA).

Southern and dot blot hybridization. DNA of fox, dog and Chinese raccoon dog were isolated from spleen by standard procedures [6].

Hybridization of fox, dog and raccoon dog DNA was performed on membrane Hybond-N (Amersham Pharmacia Biotech, UK).

Semiquantitative PCR analyses. The modified method of the semiquantitative PCR [7] was used.

Results

A BAC clone containing full sequence of the canine C-KIT gene was used to localize this gene on dog and fox chromosomes. C-KIT was assigned by FISH, as expected, to homologous regions on canine chromosome 13q22-23 and red fox chromosome 2p12 Unexpectedly, FISH also revealed additional signals on B-chromosomes of the red fox (Figure).

To investigate C-KIT localization in genomes of other canids with and without Bs we hybridized the C-KIT probe onto chromosomes of Chinese raccoon dog, Japanese raccoon dog and arctic fox (another canid species that lacks Bs). The hybridization results demonstrate that the C-KIT probe was localized to the proximal part of the long arm of Chinese raccoon dog chromosome 6, the proximal part of Japanese raccoon dog chromosome 2q and arctic fox chromosome 11p13. In both Chinese and Japanese raccoon dog signals were also revealed on the distal part of all Bs.

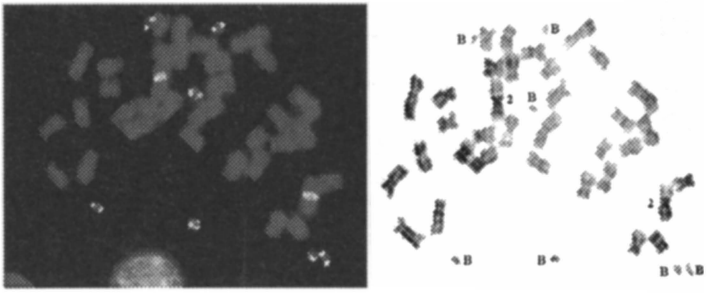

Figure Localization of C-KIT probe onto G-banded chromosomes of red fox

The presence of autosomal genes on the B-chromosomes would favour the theory of an autosomal origin of additional elements. If B-chromosomes bearing C-KIT originated from a large autosomal segment, they might contain some other genes near to the C-KIT sequences. We localized C-KIT neighbouring genes on fox chromosomes and not detect it on Bs.

Ten C-KIT exons from the fox B-chromosome library (4, 5, 6, 7, 8, 11, 12, 17, 18, 21) were sequenced and the exon-intron boundaries were observed as was expected.

To estimate the correlation of B-chromosomes and C-KIT copy number we performed Southern and dot hybridization with dog, fox, and raccoon dog genomic DNA, and semi-quantitative PCR. The positive correlation between C-KIT copy number and the number of Bs was observed.

Conclusion

If the B-chromosomes of the raccoon dog and fox have originated from the karyotype of a putative common ancestor, they have become lost in all other extant 25 Canidae species lacking Bs. In the 12.5 MYA since the fox and raccoon dog first diverged, their karyotypes have become highly rearranged. In this situation, one would expect the supernumerary chromosomes usually degenerate via the mechanism of Muller's Ratchet [8], and the C-KIT sequences would not have been conserved for such a long time. However, comparison of fox C-KIT exons amplified from B-chromosome library and canine C-KIT sequence revealed very little variation. If the presence of the C-KIT gene on B-chromosome is not beneficial for the host cell we would expect an accumulation of multiple mutations in its coding region. So far the sequence data has failed to confirm it.Further experiments should show if B-chromosomal and autosomal copies are

identical, if B-chromosomal C-KIT genes are expressed, and if C-KIT genes are present in other mammalian B-chromosomes. It can be concluded, however, that the detection of the same gene on the Bs of two different species argues against a current view that B-chromosomes represent parasitic elements of certain genomes.

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LOCUS-SPECIFICITY OF PARTICIPATION IN GENETIC DIFFERENTIATION OF MOLECULAR-GENETIC MARKERS

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The importance of hexaploid wheat forced the search of markers, which could be suitable for genetic identification of varieties. At now the detailed genetic maps of each from three homeology chromosomal complement (A, B and D) was composed; the variety-specific singularity of genetic structures on such genetic-biochemical systems, as storage proteins was described. However the inter-varieties differences on the numbers of genetic-biochemical systems emerged very rarely. Moreover, there are absent the differences on electrophoretic mobility between enzymes, genes of which are three-time repeated and localized in homeologous chromosomes. So, on our data, between 16 wheat enzymes one zone of activity was observed in electrophoretic gels for 7 enzymes; 2 zones – for 8 ones, and only 1 enzyme was presented by 3 zones of activity. The relation between polymorphism of structural genes and biochemical function of their products was observed [1]. The new generations of molecular-genetic markers, such as microsatellite loci or DNA fragments flanked by them (ISSR-PCR markers) allowed receiving the polyloci, high polymorphous spectra. However their interpretation required the preliminary researches. In our researches the three wheat varieties (Kiev awned, Yatran' 60 and Mironovskaya 30) was investigated with the use of ISSR-PCR markers.

Materials and Methods

We used the method proposed by Zietkiewicz E. et al., 1994 [2] for PCR-amplification of DNA fragments, flanked by microsatellite repeats (ISSR-PCR), with the use as primers dinucleotide repeat - (AC)9T and two three nucleotide repeats - (AGC)6T and (GAG)6C. DNA was isolated from etiolated seedlings with a STAB buffer. The mix for PCR contained in all cases 50 ng of DNA, 15 pmol of each primer, 2,5 mcl 10 x buffer (700 mmol/l TRIS-HCL, pH 8,8 at +25oC, 170 mmol sulfate ammonium, 1,7 mg/ml BCA, 0,3 mmol/l Mg2Cl), on 200 mcmol/l desoxinucleoside triphosphates, and also on 1,5 U of Taq polymerase ("Bion", Moscow). PCR was carried out in volume 25 mcl in thermocycler "Eppendorff" (Germany). ISSR–PCR amplification products in 1 ×TBE buffer were separated by electrophoresis in a 2% agarose gel 10 cm in length with addition of ethidium bromide and following testing of them under ultra-violet light.

obtaining of any amplification product (amplicon), but similar to it sequence (AC)9T in PCR produced 7 amplicons, the similar ones in investigated varieties (Figure **1).**

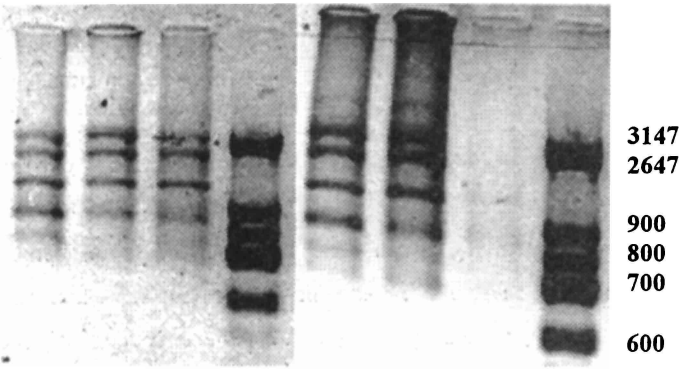

Figure 1. The spectra of amplification products, receiving with the use as primer in PCR the sequence (AC)9T on the DNA, extracted from etiolated seedlings of wheat varieties Kiev awned (K), Yatran' 60 (Y) and Mironovskaya 30 (MIR). M – molecular weight marker. The "minor" zones, which were sensitive to PCR conditions, marked by arrows

The primers (AGC)6T and (GAG)6C allowed to receive 32 amplicons, 12 from them were polymorphous between varieties (Figures 2, 3).

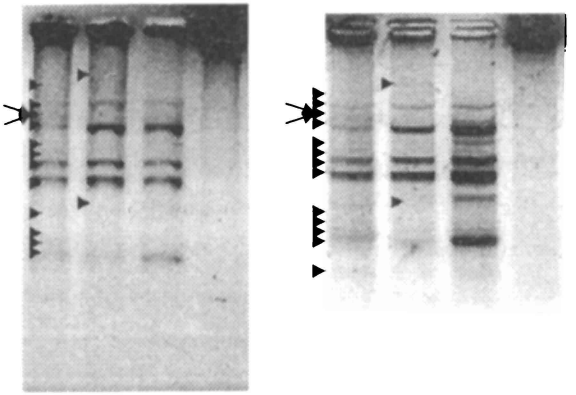

Figure 2. The spectra of amplification products, receiving with the use as primer in PCR the sequence (AGC)6T on the DNA, extracted from etiolated seedlings of wheat varieties Kiev awned (K), Yatran' 60 (Y) and Mironovskaya 30 (MIR)

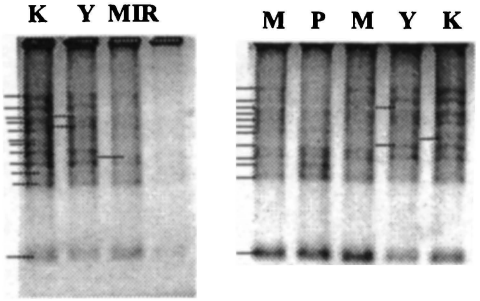

Figure 3. The spectra of amplification products, receiving with the use as primer in PCR the sequence (GAG)6C on the DNA, extracted from etiolated seedlings of wheat varieties Kiev awned (K), Yatran' 60 (Y), Mironovskaya 30 (MIR), Panna (P)

All spectra included zones, which were less sensitive to different conditions of amplification ("major" zones) and those, which not always reproduced at the repeated analyses ("minor" zones, for example, Fig 1). With the use of ISSR-PCR markers the genetic phenomena was described, when with the using of 7 primers in ISSR-PCR in self-pollinated wheat variety the 49 polymorphous from 131 loci in 20 individual plants were observed [3]. Polymorphism of amplicon's spectra was related with the specificity of nucleotide sequence, which was used as primer. For example, the using of similar primers (AG)9C and (GA)9C lead to forming of very different amplicon's spectra [3].

Conclusion

The obtained data testified the variability of evolution rate from one microsatellite locus to another ones and the absence of common regularities even for microsatellites with similar nucleotide sequences. Thus, the structural genes, microsatellite loci, their inverted repeats in hexaploid wheat essentially differed from each other on a genetic variability.

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RADIOBIOLOGY

CHROMOSOME ABERRATIONS IN HUMAN BLOOD LYMPHOCYTES AFTER THE INFLUENCE OF SPACE FLIGHT FACTORS

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Analysis of chromosomal aberrations in human blood lymphocytes is widely used as a method of biological indication and biological dosimetry. Space radiation, consisting primarily of high-energy charged particles, is very penetrative, and shielding can reduce, but not eliminate, crew exposure to ionizing radiation, and the interaction of heavy nuclei of space radiation with the spacecraft shielding can produce particles, which are more effective in causing biological damage. Although physical dosimetry is performed during space flights, biological dosimetry reflects general reaction of biological objects to irradiation, and it can be used as a prognostic indicator. And it is more important to assess biological damage rather than to calculate the dose value itself. Also, very little is known about the possible action of microgravity on radiation damage repair and modifying effects of other space flight factors.

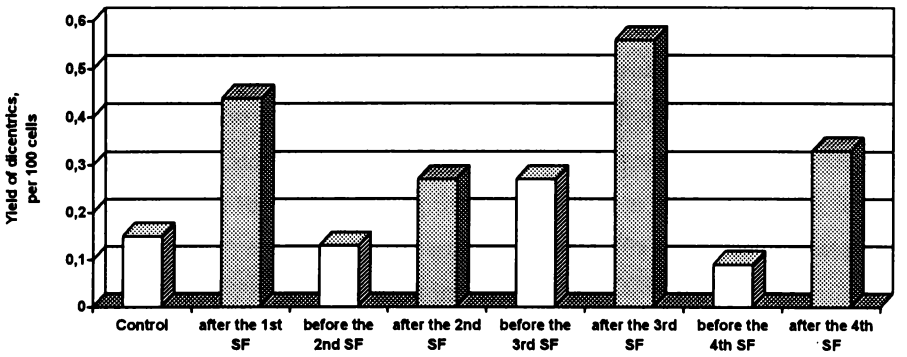
Materials and Methods

Blood cultivation and analysis of chromosomal aberrations were carried out according to the standard technique (Moorhead et al., 1960). Blood was taken 1-2 month preflight and one day postflight. Statistical analysis was performed using Fisher's exact criterion and t-Student criterion. Individual doses of cosmonauts were measured with the use of thermoluminescent detectors ID-3M. Beside the experiment with cosmonauts two more unique on-ground experiments were also carried out on volunteers, in which separate factors of space flights were simulated. The first of these experiments was a model of weightlessness simulated by antiorthostatic hypokinesia. The second one, coded SFINCSS-99, was a model of a real space flight without radiation and microgravity factors.

Results and Discussion

Cytogenetic studies of cosmonauts' blood lymphocytes after space flights on MIR station. A total of 21 cosmonauts were examined. The cosmonauts' background yield of dicentrics and centric rings, being the markers of radiation exposure, exceeds that for Moscow region residents. It is possible that this could be a result of more frequent medical X-ray exposures the cosmonauts underwent during pre-flight physical examinations. Cytogenetic examination of cosmonauts before repeated space flights showed that the mean frequency of dicentrics and centric rings reduces nearly reaching the background level. The frequency of chromosome aberrations increases after space flights at the expense of dicentrics and centric

rings. In particular, the yield of cells with dicentrics and centric rings rises up to 0.42 after flights exceeding the pre-flight level by approximately 3 times. At the same time the yields of acentrics and chromatid aberrations do not change. The dynamics of the yields of dicentrics and centric rings in before and after space flights is illustrated in Fig. 1. As a whole a wave-shaped trend can be seen. In other words, there is an increase in the number of chromosomal aberrations after the first space flight as compared with the background level, then it decreases over the 2.5-year inter-flight period increasing again after repeated flight etc. It is connected with accumulation of chromosomal aberrations induced by space radiation during space flights and following natural elimination of damaged cells from circulating blood over the inter-flight period.



F

igure 1. Yields of dicentrics in cosmonauts' lymphocytes before and after space flights

In order to assess individual doses received by the cosmonauts, a calibration curve (Gorbunova et al., 2000) was used showing the incidence of dicentrics and centric rings per cell as a function of dose. Using biodosimetry individual residual doses the cosmonauts received over a flight were estimated to be from 0.02 to 0.32 Gy. The doses were assessed without taking into account a correction for dose rate, that could certainly affect the biological effectiveness of ionizing radiation. Physical doses measured with the use of thermoluminescent detectors varied from 0.024 to 0.095 Gy (Akatov et al., 1998). Cytogenetic data were distinguished by a great individual variability. A regression analysis showed that there was a weak correlation between physical dosimetry data with biological assessments of residual doses over a flight ($p=0.16$). Thus, analysis of unstable aberrations can be used as a biological indicator, and only in some cases as an additional method of dose assessment, for such doses that are encountered by cosmonauts during their space missions.

Long-term weightlessness simulated with antiorthostatic hypokinesia. A total of 13 healthy volunteers, stayed in simulated weightlessness (SW) for 60 and 120 days, were examined. They were broken into several groups, depending on certain preventive measures applied to them. Antiorthostatic hypokinesia (bed-rest regime with bed tilted lengthways by -6°)

was the model of zero gravity conditions. After a 60-day stay in simulated weightlessness with the use of preventive measures the cytogenetic analysis did not show any significant change of the frequencies of chromosomal aberrations. The frequency of acentric aberrations was found to increase by two times in group, which underwent the exposure of SW for 120 days. This increase might be related with an acute gravitational stress. Also the total yield of chromosomal-type aberrations in this group rose as compared with the background level. Other types of chromosomal aberrations was not found to change significantly.

SFINCSS-99 experiment. The subjects were 11 healthy people, who stayed isolated in hermetically-closed space for 240 or 110 days. The conditions of this on-ground experiment simulated a real space flight except for microgravity and radiation environment: isolation and closed space, environmental parameters, basic flight operations, nutrition, hygiene and sanitary conditions and possible difficult situations. Cytogenetic analysis was performed before, in the middle of and after isolation. As a whole the frequencies of acentrics and chromatid-type aberrations tend to increase with time of isolation. And this increase was statistically reliable almost for all terms, beginning from 55 days. Thus, the more the time of isolation, the higher the frequency of chromosomal aberrations. The likely reason is the processes of lipid peroxidizing in the organism which undergoes a psychological stress. These processes are known to occur following the stress.

Conclusions

1. The influence of space radiation results in a statistically significant increase in the yield of dicentrics and centric rings (up to 0.43±0.05 per 100 cells) as compared with the pre-flight level. The frequencies of acentrics and chromatid aberrations do not change after space flights.
2. Individual doses of cosmonauts were biologically estimated to be from 0.02 to 0.32 Gy over a space flight. A weak correlation was found between biological and physical individual doses.
3. During the inter-flight period of about 2.5 years the frequency of chromosomal aberrations reduces reaching the background level due to elimination of damaged cells. The number of chromosomal aberrations increases again after repeated flights.
4. The influence of simulated weightlessness for 120 days results in a statistically significant increase in the yield of chromosomal-type aberrations. No change in the yield of chromosomal aberrations was found in the group, stayed in simulated weightlessness for 60 days.
5. A statistically significant increase in the frequency of acentrics and chromatid-type aberrations was found in volunteers participated in modelled on-ground long-term space flights.

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CONDITIONAL DOMINANT LETHALS IN DROSOPHILA AND THE REMOTE CONSEQUENCES OF RADIATION IN HUMAN

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According to current concepts, the cause of the remote consequences of irradiation in humans (malformations, cancer, and leukaemias) is gene impairment. However, it is difficult to explain the appearance, expression, and inheritance of this pathology by damage of the classical (Mendelian) genes. It is also unclear why radiation damage of classical gene gives rise to genomic instability. Using *Drosophila melanogaster*, we developed an experimental procedure for producing mutations in the regulatory genes that control development (mutations in *ontogenes*). The produced mutations show unusual features resembling the pattern of genetic pathology that arises as a long - term consequence of radiation.

Materials and Methods

Mutations in *ontogenes* [1] (over 100 mutations in the X, autosomes 2 and 3) were induced by gamma - irradiation of *Drosophila melanogaster*, using the experimental procedure for their identification (Fig. 1).

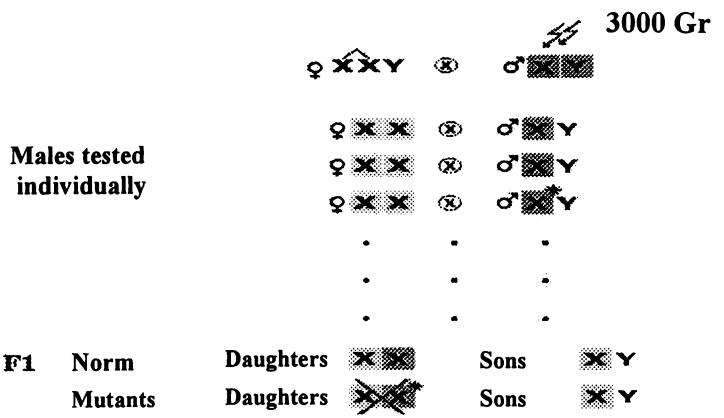

Figure 1. Recovery of mutations in the ontogenes in the *Drosophila melanogaster* X chromosome

The presence of mutations in a male was determined by the absence of daughters in the offspring of crosses to *yellow* females (Fig. 1, Table). On formal grounds, the mutations are facultative

dominant lethals (FDL): manifest as dominant lethals in the female and not manifest in the males. We developed 4 experimental procedures to produce FDLs [2].

Results and Discussion

The produced mutants loss the lethality under the effect of numerous genetic factors: the sex of the mutant, the presence of chromosomal rearrangement in the genome, the cross direction, and others. For example, substitution of *yellow* females by those of other strains results in loss of the lethal effect of mutations: daughters arise among the offspring of the mutant male (Table).

Table

Proportion of daughters among the progeny of the mutant male in crosses to females of different laboratory stocks

Number of male stock	Females of laboratory stock:					
	yellow		*Berlin wild*		*Barnaul*	
	Total number of progeny	Proportion of daughters	Total number of progeny	Proportion of daughters	Total number of progeny	Proportion of daughters
1	191	0.00	–	–	91	0.02
2	435	0.00	7	0.17	143	0.03
3	180	0.00	18	0.17	146	0.04
4	293	0.00	–	–	135	0.01
5	303	0.02	–	–	143	0.19
6	283	0.02	212	0.31	138	0.03
9	529	0.04	128	0.29	303	0.28
11	409	0.06	223	0.38	178	0.22
26	89	0.01	100	0.22	111	0.23
27	93	0.00	84	0.08	112	0.10
29	61	0.00	64	0.20	113	0.26
30	115	0.00	102	0.33	135	0.17
31	83	0.00	87	0.08	129	0.07
32	117	0.00	140	0.34	80	0.30
33	90	0.00	149	0.28	48	0.27
34	110	0.00	99	0.24	117	0.27

A characteristic feature of the mutations is abnormal development (morphoses) among mutant offspring. These are developmental defects (Fig. 2) of the "plus – tissue" or the "minus – tissue" type. They are not heritable, but the mutation causes the appearance of offspring with morphoses in every generation. We believe that the FDLs are mutations in the regulatory genes that control development (in the *ontogenes)*. It proved that mutation in an ontogene cause genomic destabilization. The destabilization is manifest as:1) mass formation of modifications;

2) appearance mutations with complete or incomplete penetrance; 3) disturbed chromosome distribution in meiosis and mitosis etc.

a) b) c)

Figure 2. Morphoses in strains with mutations in ontogenes: **a)** formation of the seventh leg; **b)** head doubling; **c)** abdomen rotation by 180°

The produced mutations showed unusual features characteristic of long – term pathology after radiation exposure. Thus, the penetrance of the mutations is incomplete; they can appear the offspring of the first generation of the radiation – exposed parent. Their inheritance does not conform to the Mendelian pattern. The mutation cause mass appearance of malformations. They also initiate genomic instability with multiple forms of manifestation. One form of the manifestations is the appearance of point and chromosomal mutations. We believe that mutations in the regulatory genes that control development (mutations in *ontogenes*) may be the causes of high mass of pathologies arising after radiation exposure.

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METHOD OF BIOCHEMICAL -BIOPHYSICAL INDICATION AND ORGANISMS' "LIFE'S QUALITY" FORECAST IN CONDITIONS OF RADIATION POLLUTION

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Responses of living organisms to any natural stress factors are formed due to changes of genome activity regulation in replication processes, the repair, directed on translation. And in these processes, as well as in reactions of specific irritants reception, the big role play conformational changes of DNA and the protein being an obligatory stage of their synthesis and functioning [1], and permolecular basic medium-forming clusters: waters and membrane lipids [2]. In turn these processes underlie adaptive potential of a living organism and cause, together with activity of first echelon of protective systems - antioxidants, its change "life's quality" at action of different stress factor of environment.

The purpose of work is the development of the biochemical-biophysical integrated approach of an estimation of organisms' "life's quality" in polluted environments which includes cytological, biochemical and biophysical methods of an estimation of stress factors action on a homeostasis of growing cells of various organisms.

Materials and Methods

Simultaneous definition of inclusion ^{3}H-thymidine in DNA, ^{14}C-leucine in synthesized fibers and mitotic index of growing cells for an estimation of differential processes activity of replicative and reparative DNA synthesis, translations of fibers with $\tau_{1/2}$> 24 hours, and also definition of low-molecular (LMAO) maintenance and activity of high-molecular antioxidants (SOD) are carried out. All characteristics are estimated in relative units in relation to the control. The biophysical component consists in registration of secondary radiation parameters (S) of cellular culture in an aquatic environment, describing a degree of structurization and conformational alteration in system water clusters of mediums - permolecular biopolymer structures of cells.

The method is approved on yeast culture *Cuf liver* at action on them of radiating stress factors, for example, at reception of a total absorbed dose by them γ radiation 100÷350 μRentgen (μR). For creation of a background γ-quantum 243Am in a range of total dozes from 100 up to 350 μR has been used.

Results and Discussion

Results of consequences of γ-radiation influence in three dozes are introduced on tab.1.

Table 1. Cytology-biochemical and biophysical characteristics of Cuf livere culture after influence γ-radiations. (normalized in relation to the control)

radiation dose, μRentgen	mitotic index	^{3}H-thymidine inclusion	^{14}C-leucine inclusion	Superoxide dismutase activity	Content of low-molecular antioxidant	Area of secondary luminescence
0	1,0	1,00	1,0	1,0	1,0	1,00
100	0,9	1,3	1,3	0,8	1,1	1,17
250	0,9	0,8	0,8	1,0	1,0	1,40
350	0,8	1,2	1,2	0,8	1,3	1,22

Primary results allow one to calculate in relative unit processes speed of replicative *(k_{repl})* and reparative *(k_{rep})* synthesis of DNA, and also translation *(k_{trans})*, the total activity and efficiency of genome, and also a degree of conformational changes, and structures of DNA, proteins and water clusters (table. 2)

Table.2. Used factors calculation formulas

k_{tga} (general genome activity) = [^{14}C-leucine inclusion]$_N$ + [^{3}H-thymidine inclusion]$_N$.	k_{repl} (replication) = [mitotic index]$_N$
k_{gst} (general genome stability) = k_{aop} + k_{rep}/ k_{gga}	k_{trans} (translation) = [^{14}C-leucine inclusion]
k_{prod} (productivity) = (k_{trans} + k_{repl}) / 2	k_{cc} (conformational changes) = [S secondary luminescence]$_N$
k_{aop} (antioxidant protection) = (SOD activity + [LMAO]) /2	k_{rep} (reparation) = [^{3}H-thymidine inclusion]$_N$ - [mitotic index]$_N$

Values of the coefficient (table. 3.) testify that dependences of genome functional activity and stability, as well as characteristics of conformation transposition and structure degrees of biopolymers and water clusters, from a doze γ-radiations, nonlinear.

Table 3. Calculated values $k_{коаз}$, $k_{трансл}$, $k_{репл}$, $k_{реп}$, $k_{коаг}$, $k_{уст}$, $k_{прод}$, R and biophysical characteristics of cells of yeast *Cuf livere* after influence γ-radiations (normalized to the control)

radiation dose, μRentgen	k_{aop}	k_{trans}	k_{repl}	k_{rep}	k_{tga}	k_{gst}	k_{prod}	Area of secondary luminescence	R
0	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00
100	0,92	1,25	0,85	1,42	1,17	1,07	1,05	1,17	1,12
250	1,00	0,78	0,88	0,94	0,873	1,04	0,83	1,40	0,86
350	1,01	1,23	0,84	1,39	1,15	1,11	1,04	1,22	1,15

The main of them, connected to protein synthesis, DNA replication, the total activity, genome efficiency and stability achieve a maximum at a total doze 100 μR and show the tendency to presence of the second maximum at a doze 350 μR. At the same time, the maximum of conformational intensity of all water clusters falls at a doze 250 μR, witch resulting in increase of structure degree. (tab. 3).

Apparently, at this doze the resonance between parameters of the given form ionizing radiation and own frequency characteristics of fluctuations water dissipative structures is reached. As provides the maximal structurization of water, it is similar to increase in intensity of a geomagnetic field, characteristic for small indignations which cause an increase in a degree of structure of water by 2 times [3].

Table. 3. Reorganizations water clusters depending on γ-radiations

radiation dose, μRentgen	replication contribution	translation contribution	Reparation contribution	Water structures contribution
100	1,0	0,07±0,01	0,05±0,01	0,16
250	1,0	0,07±0,01	0,05±0,01	0,42
350	1,0	0,07±0,01	0,05±0,01	0,22

Due to that, the degree "reception" of γ-radiations by cells (more precisely structures of their aquatic environment) rises. The latter, apparently, is the reason of decrease, at a doze 250 μR, activity of DNA reparation, as a consequence of genome efficiency and a parameter of "life's quality" ($R = k_{prod} * k_{gst}$)

Conclusion

The research shows efficiency of the suggested method at an estimation of "life's quality"of organisms in conditions of radiation pollution connected with a change of stability and activity of cellular genome in processes of replicative and reparative synthesis of DNA, translation, and also with induced conformational reorganizations not only of biopolymers, but also of cluster structures of water.

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CYTOGENETIC ANOMALY DYNAMICS IN BLOOD CELLS OF HOLSTEIN CATTLE IN CONDITIONS OF THE RADIONUCLIDE CONTAMINATION

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The influence of chronic low doze ionizing irradiation on the mutation spectra is investigated insufficiently, in particularly, in dynamics. In this relation the comparative analysis of cytogenetic anomaly frequencies in peripheral blood cells of Holstein cattle breeding in territories with high level of radio pollution after Chernobyl's accident was carried out (experimental farm "Novoshepelichi": contamination by Cs-137 ≈ ~200 Ci/km^2; "Vladimirovka" - ~100 Ci/km^2). The archive collection of blood smears of animals from farm "Vladimirovka" was accumulated from different cattle groups in the same age (near 2 years) and in the same season (in winter) in different years after Chernobyl's accident (1987 – 1990 yy).

The cattle group from experimental farm "Novoshepelichi" was presented by parent's generation (F0), born in "pure" zones and founded the experimental herd in farm "Novoshepelichi", and F1, F2 generations, born from parent's generation in "Novoshepelichi". In summary, the experimental exposed herd included 17 parents, 96 animals F1 and 50 ones F2 (first and second generations born in conditions of chronic influences of low doze of ionizing irradiation).

Materials and Methods

The number of erythrocytes with the micronuclei (EM), binuclear leukocytes (BL), leukocytes with the micronuclei (LM) and apoptosis cells (A) in smears of cattle peripheral blood was ivestigated (Figure 1). Quantity of cells with the cytogenetic anomalies was calculated on the same preparations in the cells with saved cytoplasm in ‰. Statistical reliability of the differences between group was evaluated with use of Student criterion (tS).

Results and Discussion

In cattle of farm "Vladimirovka" at 1988 y the number of the cytogenetic anomalies was authentic above, than in animals at 1987 y (tabl.1): EM – 5,1±0,3 against 3,4±0,2 ($P<0,01$); BL – 2,4±0,3 against 1,4±0,1 ($P<0,01$); and LM – 5,8±0,1 against 3,0±0,3 ($P<0,001$). The frequencies of all characters in cattle at 1989y were authentic below, than in animals at 1988y: EM – 3,7±0,3 ($P<0,01$); BL – 1,7±0,1 ($P<0,05$); LM – 4,6±0,4 ($P<0,05$). In 1990y the authentic increase of these characters in comparison with ones at 1989 y was again observed: EM - 4,6±0,1 ($P<0,01$); BL – 2,5±0,2 ($P<0,01$); LM – 5,0±0,2 ($P<0,05$). The frequency of apoptotic cells decreased in animals in 1990y - 0,7±0,1. Thus, the frequency changes of EM, BL and LM in

animals from farm "Vladimdrovka" in different years after Chernobyls accident had non-linear character: it increased in 1988y in a comparison with 1987y, then decreased in 1989y and again increased in 1990y.

Table 1. The frequency erythrocytes with the micronuclei (EM), binuclear leukocytes (BL), leukocytes with the micronuclei (LM), apoptotic cells (A) and metaphase plates frequencies (MI) on 1000 cells in animals from farm "Vladimdrovka" in 1987-1990yy

Years	Number of animals	EM	BL	LM	A
1987	11	3,4±0,2	1,4±0,1	3,0±0,3	0,9±0,2
1988	18	5,1±0,3	2,4±0,3	5,8±0,1	0,9±0,2
1989	10	3,7±0,3	1,7±0,1	4,6±0,4	0,9±0,3
1990	30	4,6±0,1	2,5±0,2	5,0±0,2	0,7±0,1

The analysis of the same cytogenetic anomalies of cattle from farm "Novoshepelichi" was carried out also. The herd was subdivided to parent generation (which was born before Chernobyls accident) and three generations, which born in conditions of increased ionizing irradiation.

The frequency LM in parent generation (F0) was 4,5±0,4 ‰, that was authentically above, than in first (F1) 2,4±0,4 ($P<0,01$), second (FII) 3,0±0,3 ($P<0,01$) and three (FIII) 1,5±0,4 ($P<0,001$) animals generation (tabl.2). It allowed to suppose, that the chronic low-dose ionizing irradiation induced the increase of cytogenetic anomalies in parent's generation, but in offspring we observed the process of population-genetic adaptation and the increase the number of radio resistant animals, which were born in conditions of ionizing irradiation. However, it is interest to note that in different generations the frequency of all cytogenetic anomalies had non-linear dynamic also: it decreased in FI in comparison with F0 generation, then increased in FII and decreased again in FIII generation (tabl.2).

Table 2. The frequency erythrocytes with the micronuclei (EM), binuclear leukocytes (BL), leukocytes with the micronuclei (LM) in parent generation Holstein breed and also F1, F2, F3 generations from experimental farm "Novoshepelichi"

Number of animals	Generation	EM	BL	LM
6	0	5.0±1,0	8,0	4,5±0,4
15	1	4,5±0,3	5,4±0,5	2,4±0,4
12	2	5,9±0,5	5,3±1,0	3,0±0,3
3	3	5,0±0,6	6,0±3,0	1,5±0,4

Conclusion

Thus, our results testified the non linearity dynamics of cytogenetic anomaly frequencies as in the various years after Chernobyl accident in cattle groups as in the various generations of animals which was born before and after accident. The similar non linear dynamics of cytogenetic anomaly frequencies in human blood cells was described also [1], and, perhaps, it lead to complexity of data interpretation about consequences of chronic low irradiating dose influences on the cytogenetic anomaly frequencies.

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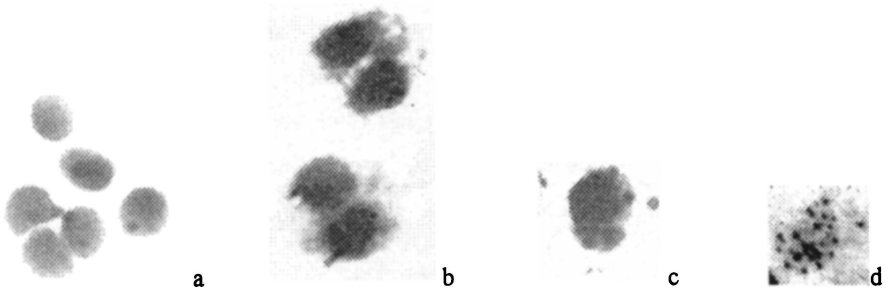

Figure 1. The erythrocytes with micronuclei (a), binucleated leukocytes (b), mono-nucleated leukocyte with micronuclei (c), apoptotic cell (d)

THE COMPARATIVE EVALUATION OF VASCULAR AND NEURAL RESPONSES OF CHILDREN'S POPULATION LIVING ON ECOLOGICALLY SATISFACTORY, RADIATING AND RADIATING-TOXIC TERRITORIES

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The state of health of the children's population of Russia is in a disastrous position and puts a serious problem of search of the reasons and mechanisms of such growth of disease level of children. The probable reason of such changes is the sharp modification of structure of a medium (radioactive, toxic and chemical, stressor and socially generated effect), accompanied by answer-back adaptive tension of immune, nervous, endocrine systems of an organism of children and teenagers. The regularities of such responses are not deciphered and require the search of relations between these negative modifications and effects of the factors, and revealing of «hierarchy» of effects with a condition of a medium and mechanisms of answer-back responses.

Materials and Methods

For an evaluation of a degree of strength of adaptive systems of an organism of children we investigated vascular and neural parameters of children 12-13 years (pubertate age), living on ecologically satisfactory, radiating and radiating - toxic territories of Bryansk region. We inspected 304 children (152 boys and 152 girls).

For an evaluation of a condition of vegetative system the VSD-responses and dermographism were investigated. Neural responses were evaluated by rephlexometria that is determination of time of sensomotoric response. The velocity of sensomotoric response on the light of various colour and sound of various tones (with accent on the given responses and combinations of responses - simple and complicated of sensomotoric response) was investigated. The inadequate responses (inappropriate to the given situation) were evaluated as the false answers.

Table 1. Vascular response of 12-13 years children living on ecologically satisfactory, radiating and radiation-toxic territories

	Territories of Bryansk region		
Parameters	**Ecologically satisfactory (M±m)**	**Radiating (M±m)**	**Radiation-toxic (M±m)**
Hyperreactios of dermographism (m), %	16,2±0,21	59,2±0,85	73,0±0,34
Hyperreactios of dermographism (f), %	14,0±0,17	64,3±0,98	75,7±0,41
Frequency of VSD-reactions (m), %	18,3±0,24	37,7±1,06	52±1,08
Frequency of VSD-reactions (f), %	26,5±0,36	49,1±1,01	64±1,11

The distinctions are statistically authentic ($p<0,05$)

Table 2. Neural reactions (rephlexometria) of 12-13 years children, living on ecologically satisfactory, radiating also radiation-toxic territories

	Territories of Bryansk region		
Parameters	**Ecologically satisfactory (M±m)**	**Radiating (M±m)**	**Radiation-toxic (M±m)**
Simple sensomotoric reaction (m), m/c	197,3±4,68	231,0±5,87	256,9±6,53
Combined sensomotoric reaction (m), m/c	276,8±7,24	355,2±8,76	409,4±8,97
Frequency of false reaction (m), %	14,2±0,19	38,5±0,98	80,0±1,52
Simple sensomotoric reaction (f), m/c	223,2±5,91	241,5±6,02	254,3±6,38
Combined sensomotoric reaction (f), m/c	302,0±7,83	376,4±8,54	412,6±9,02
Frequency of false reaction (f), %	21,8±0,47	58,3±1,31	78,9±1,45

The distinctions are statistically authentic ($p<0,05$)

Results and Discussion

Vascular responses of children living on ecologically satisfactory territories irrespective of their sex and of directedness of deviations from norms are expressed within the limits of usual for children of this age frequency. On the territories with an increased radiating hum noise, but without toxic effects of a medium the parameters are increased on 20-50 % with more expressed deviations in group of the girls. The most expressed violations of regulation of vessels tonus, both at a level of capillary responses (dermographism), and at a level of large magistral vessels

(vegeto-vasculare distonic responses) are registered on territories with combined radiation and toxic effects. The outcomes are indicated in Table 1.

Neurological responses of children actually repeat (on a character of relations with a condition of a medium) their vascular responses. Least frequently sensomotoric deviations from norms meet in groups of children from ecologically pure areas. In radiating areas and, especially, in radiation – toxic areas, the responses « expanding of time» of the answer on the irritator and frequency of inadequate (false) responses sharply increase. The indicated data specify the tension of functions of vegetative and central nervous systems on territories with an increased radiating hum noise with maximally expressed responses when combined radiation – toxic effects of a medium act. The outcomes are indicated in Table 2.

Conclusion

The sharp strain of a medium and entering of the radiating factor into its structure entails the tension of adaptive systems, including central and vegetative nervous systems. The expression of tensions is related to a potency of technically-generated hum noise of radiating effects.

Acknowledgements

I express my gratitude for a management of this theme to my scientific adviser Prof. Mikhalev V.P. and for solution of complicated methodical problems to Dr. Inozemtsev V.A..

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RADIOSENSITIVITY OF CHROMOSOME APPARATUS OF VOLES FROM ALIENATION ZONE OF CHERNOBYL'S ACCIDENT

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Traditionally small-sized rodents were used for bioindication of territory pollution by different gene toxic agents. Investigations of cytogenetic anomaly frequencies in somatic cells in connection with ionizing irradiation in the last 50 years were widely conducted on the people, plants, small-sized *Rodentia* species and others. The high individual variability in tested species in the same conditions, and also the absence of precise relations between quantity of cytogenetic damages in somatic cells and dozes of ionizing irradiation were the singularity of the accumulated data. It is not exclude, that one of cause of this result may be relate with both the peculiarities of species karyotype and morphology of individual chromosomes.

Materials and Methods

The different vole's species were trapped in Chernobyl's zone with various levels of radio nuclide pollution (20 – 100 - 1000 Ci/km2). Species differed by number of acrocentric and metacentric chromosomes in the karyotypes: *Microtus arvalis* (2n=46, Fna=84), *Microtus subarvalis* (2n=54, Fna=54), *Clethrionomus glarealus* (2n=56, Fna=56), *Microtus oeconomus* (2n=30, Fna=56), *Microtus agrestis* (2n=50, Fna=54). The preparations of bone marrow cells of representatives of vole's species were obtained by standard technique without colchicine. It was analyzed 9 cytogenetic characters in bone marrow cells such as the frequency aneuploidy (which was evaluated in two variants: general aneuploidy (A1) and aneuploidy (A2) on one chromosome (2n±1)), polyploidy (PP), the frequency of metaphase plates with chromosome aberrations (CHA), interchromosome fusion on a type of Robertsonian translocation (RB), with asynchronous separation of centromere chromosome region (ASCR) (in %). Quantity of metaphase plates (MI) in 1000 cells, binuclear leukocytes (BL) and leukocytes with the micronuclei (LM) calculated on the same preparations in cells with saved cytoplasm (in ‰). Statistical reliability of between group differences was evaluated with use of Student criterion (t_S).

Results and Discussion

In the zones with high-level radio pollution in all species of voles the increase of the mitotic activity and related with it the little decrease of metaphase frequency with asynchronous fission of centromeres (ASCR) were observed (tabl. 1).

In the zones with low-level radio pollution the specters of cytogenetic parameters had significant species peculiarities. For example, at the *Microtus arvalis* the frequency of aneuploidy cells was higher then at the other species. At the Clethrionomus *glarealus* the frequency of metaphases with interchromosome fusion on the type of Robertsonian translocation were met more often then at the *Microtus arvalis* (Fig.1*)*.

In the cytogenetic anomaly specters of animals from Chernobyl's zone with high-level radio pollution the increased frequencies of those cytogenetic anomalies which were unstable in the zones with low-level radio pollution (*Microtus arvalis* – aneuploidy, the voles with acrocentric autosomes – centric fusion of the chromosomes) were observed. The high frequency of chromosome inversions in group of little-sized chromosomes in *Microtus agrestis* (about 30% of metaphases) was revealed.

The participation of individual chromosomes in mutation spectra of some vole's species was analyzed. The group of biggest chromosomes involved into aneuploidy with high frequency in the *Clethrionomus glarealus* but the same chromosomes participated with low frequency in asynchronous separation of centromere regions. However, in cells of *Microtus oeconomus* the chromosomes 10 and 14 preferable involved into aneuploidy and asynchronous separation of centromere regions.

Table 1. Spontaneous and induced mutation spectra in different species of voles

N anim	N met.	frequency of metaphases						on 1000 lymphocytes		
		A1	A2	PP	RB	CHA	ASCR	MI	BL	LM
Razezzhee (<5 Ci/km^2) *Microtus arvalis*										
15	948	44,4±5,1	8,6±2,8	0,9±0,5	0,1±0,5	2,5±0,6	16,5±4,9	4,5±0,9	5,0±0,8	3,0±0,4
Chistogalovka (>500 Ci/km^2)										
9	784	52,7±8,3	17,9±4,4	0	0,4±0,4	3,.6±0,8	3,7±0,7	10,0±0,6	7,9±0,3	6,8±0,5
Lelev (~ 20 Ci/km2) *M. rossiaemeridionalis*										
3	170	36,3±13,9	5,0± 3,4	3,4± 3,4	31,6± 5,9	2,0±2,0	0,7±0,7	3,6±2,8	3,4±1,3	2,4±0,8
Nedanchichi (<5 Ci/km^2) *Clethrionomus glarealus*										
4	97	33,7±6,0	9,0±3,5	14,0±3,5	1,5±0,5	1,2±0,7	6,2±3,6	3,2±0,6	3,5±0,6	5,5±1,5
Red forest (~1000 Ci/km^2)										
3	252	33,7±0,9	5,0±2,1	3,7±3,7	5,7±3,3	7,3±3,4	2,3±1,9	10,3±1,9	7,0±1,0	9,3±1,9
Lelev (~ 20 Ci/km^2) *Microtus oeconomus*										
4	370	21,2±6,4	4,2±1,4	1,7±0,8	0	2,7±0,9	12,7±3,3	3,7±0,2	8,0±2,1	4,5±0,9
Like Deep (500 Ki/км^2)										
6	579	23,0±5,0	12,5±4,7	0	0	5,.0±0,9	1,8±0,7	9,8±0,6	7,.2±0,4	5,.5±0,6
Red forest (~ 1000 Ci/km^2) *Microtus agrestis*										
2	124	25,0±8,5	3,9± 3,6	0	16,1± 11,9	9,7±1,4	21,7±11,6	1,5±0,5	1,8±0,2	2,8±0,2

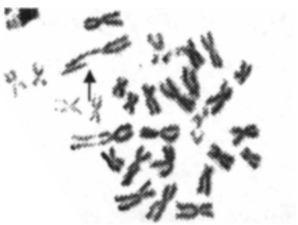
In cells of *Microtus arvalis* the group of smallest chromosomes was preferable involved in aneuploidy, but the group of big chromosomes participated in it very rare. However the group of big chromosomes participated preferable in the asynchronous separation of centromere regions.

Conclusion

Our dates shows that spontaneous spectra (at the low-doze radio pollution) of cytogenetic anomalies in bone marrow cells are characterized by the species-specific traits in the voles both on predominance of cytogenetic anomalies (centric fusion – for species with predominance of acrocentric chromosomes, aneuploidy – for the *Microtus arvalis*, asynchronous separation of centromere regions – for the *Microtus arvalis* and *Microtus oeconomus)* and on involving into anomalies of the individual chromosomes also. In the Chernobyl's zone the animals with constitutive chromosome aberrations did not revealed. The chronic ionizing irradiation did not lead to appearance of the unusual cytogenetic damages. In these conditions the speeding of the cell proliferation in different vole's species was marked and also the increase of the frequency of those cytogenetic anomalies, which had species-specific particularities in investigated vole's species.

Microtus arvalis, aneuploidy, 47 chromosomes

Clethrionomys glareolus, interchromosome fusion on the type of Robertsonian translocation



Microtus oeconomus, chromatide break with deletion of fragment

Microtus

Figure 1 Cytogenetic anomalies in different vole's species

SEQUENCING ANALYSIS OF MUTANT ALLELE CDC28-SRM OF PROTEIN KINASE CDC28 AND MOLECULAR DYNAMICS STUDY OF GLYCINE-RICH LOOP IN WILD TYPE AND MUTANT ALLELE G16S OF CDK2 AS MODEL

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Nanoseconds long molecular dynamics (MD) trajectories of the CDK2/ATP complex were analyzed. The MD simulations of substitution CDK2-G16S (CDC28-G20S) shows a conformational change of CDK2 structure resulting in the moving of the G-loop away from ATP and a new rearrangement of amino acids in the T-loop.

Introduction

The cyclin dependent kinases (CDKs) are a subfamily of serine/threonine-specific protein kinases. The enzymes catalyze a transfer of the gamma-phosphate of adenosine triphosphate (ATP) to a protein substrate. CDKs are crucial regulators of the timing and co-ordination of eukaryotic cell cycle events. Transient activation of these kinases at specific cell stages is thought to trigger the principal transitions of the cell cycle, including DNA replication and entry into mitosis. In yeast, both transitions are controlled by single CDK (CDC28 in *Saccharomyces cerevisiae* [3]). In human cells, cell cycle events are governed by several CDKs. CDK4-cyclin D is necessary for passage through G1, CDK2-cyclin E is necessary for the transition from G1 to S phase, CDK2-cyclin A is necessary for progression through S, and CDC2-cyclin B is necessary for the transition from G2 to M phase. Two of these proteins CDK2 and CDC2/CDK1 have long been studied extensively [1-7]

Results

We simulated the CDK2-G16/ATP of the wild-type structure and compared the conformational changes with CDK2-S16/ATP structure. For CDK2-G16 and CDK2-S16 structures we have also generated a number of the animation movies to display the real-time dynamical motions. We concentrate on the positional changes between the ATP, residue 16 and T-loop.

If to make the comparison of the pictures of the initial and final states of wild-type CDK2-G16/ATP structure, it is easy to see that there is not a visually large difference between these two structures. Moreover, the position of the amino acid residue 16 does not change within 1 million time step MD steps in comparison to that at the initial configuration. So, for the wild-type protein we found that it keeps its conformation stable relatively to the original state.

Regarding the CDK2-S16 variant, a completely different picture of the dynamics and conformational changes has seen. First of all, the amino acid residue 16 moves dramatically far from the ATP location site. In comparison to the wild structure, the distance between the position S16 and ATP, as it seen from the snapshots, increases of about 2-2.5 times at average. At the same time, such movement results on a relative shift of the T160 residue and the whole T- and G-loops positions. On the one hand, it is obvious that the increase of the ATP-S16 distance influences on the picture of the hydrogen bonds formation involving the ATP and G-loop. On the other hand, the mutation induced CDK2' conformational change results in an interhelical protein movement, covering a phosphorylation point (viz. T160).

Discussion

The G-loop enables protein kinase to adopt a wide range of backbone conformations. The significance of this domain is demonstrated by the fact that substitution of the glycine residues in the G-loop, particularly the first and the second glycine (GxGxxG) with either alanine or serine results in a dramatic decrease in cAPK activity. The functional importance of the G-loop has been described in detail for cAPK [8-11], but its importance for CDK regulation has not been yet discussed. It is believed that the G-loop catalytic function – that is, correct ATP binding and *alignment – is the same as its function in cAPK, but it exhibits a new inhibitory function for* CDK [12]. We have obtained the mutation *cdc28-srm* that posseses a pleotropic manifestation in yeast cells. Analyses have shown that this mutation is localized in the glycine-rich loop (G-loop) and is a substitution of the third glycine with serine.

The crystal structure of inactive monomeric CDK2 showed that the T-loop (residues 147-153) would block access of substrates to the active site and that ATP would bind with the wrong geometry for efficient catalysis. Binding to cyclin A simultaneously moves the T-loop of CDK2 away from the substrate binding cleft and repositions of the G-loop (residues 11-18), so that they can interact properly with the ATP phosphates. This complex has a low but detectable activity. Less dramatic changes occur in the structure of the CDK2/cyclin A complex following activation by phosphorylation of T160 [13]. The phosphorylation of T160 might relieve a proposed steric interference between the substrate and the unphosphorylated T-loop and help to organize an acidic patch containing E162, E208 and D235 by repositioning E162, a T-loop residue. The serine of the peptide substrate is hydrogen bonded to the ATP gamma-phosphate oxygen, to the catalytic aspartate D127, and to the conserved lysine K129 [14].

MD simulation analysis shows that in CDK2-S16/ATP the distance between ATP and the residue 16 in the G-loop dramatically increased. The shift is equal 5 Å. It's known that the G-loop was changed during the early stage of inactive (CDK2/ATP), partly active (CDK2/cyclin

A/ATP), and fully active (pT160-CDK2/cyclin A/ATP) CDK2 simulations in comparison with its conformation as found in the crystal structures [12]. The G-loop moves away from the ATP phosphate moiety binding site after the interaction of CDK2 with cyclin A and again after CDK2/cyclin A/ATP complex phosphorylation at the T160 site. The shift of the G-loop is equal to 3.5 Å (CDK2/cyclin A/ATP) and 8.6 Å (pT160-CDK2/cyclin A/ATP) in comparison with the G-loop position found in the CDK2/ATP system. It's interesting that we observe this shift in the mutant allele CDK2-G16S/ATP as well.

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THE PHENOMENON OF GENOMIC INSTABILITY IN THE CHILD'S BODY EXPOSED TO PROLONGED RADIATION AT SMALL DOSES AND HEALTH STATE

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In addition to an increased frequency of chromosome aberrations, some researchers have resently reported an increase in general somatic morbidity and in neuropshychical dysadaptation, and a tendency to chronization of diseases in children living on territories contaminated with radionuclides after the accident at the Chernobyl NPP [1]. There is a considerable body of published data about a remote effect of radiation –induction of genomic instability in the offsprings of repeatedly divided cells exposed to radiation at medium and high doses [2,3]. In connection with the fact that destabilization of genome may lead to the above – mentioned states, the aim of the present work has been to assess the reality of this new genetic phenomenon in children exposed to low-intensity radiation at small doses.

Materials and Methods

Cytogenetic examination was carried out in 90 children, constant residents of territories with radioactive contaminations (Novozybkov district, Bryansk region, 16 – 18 Ci/km^2, ^{137}Cs) exposed to radiation at different stages of ontogenesis: postnatal-irradiated in 1986 (32 subjects), uterine-irradiated in 1986 (15 subjects), born after the accident in 1987-1992 (19 subjects) and in 1994-2000 (24 subjects) from irradiated parents. In 15 children (1986-1998) genomic instability was tested in 3 cell generations using ^{137}Cs γ –irradiation of blood lymphocytes in vitro. The control group was composed of children living in contamination – free regions (16 subjects). The children were examined in the Federal Children's Scientific-Practical Center of Antiradiation Protection (prof. L.S. Baleva) to which they were delivered with various pathologies in the state of health. Cytogenetic preparations, analysis of chromosome aberrations (CA) and sister chromatid exchanges (SCE) were made according to the commonly accepted procedure. Differential staining of sister chromatids with 5 -BDU was used.

Results and Discussion

In all examined groups of children exposed to radiation at different stages of ontogenesis the frequencies of one or other type of radiation-induced CA were significantly increased as compared to the control. No significant intergroup differences in the frequencies of aberrant genomes were revealed although the calculated average doses of technogenic irradiation from long-lived radionuclides accumulated by the children were different (Fig. 1). Analogous results were also obtained in comparative analysis of the frequencies of other cytogenetic parameters. The absence of dependence of the expression of dysgenomic effects

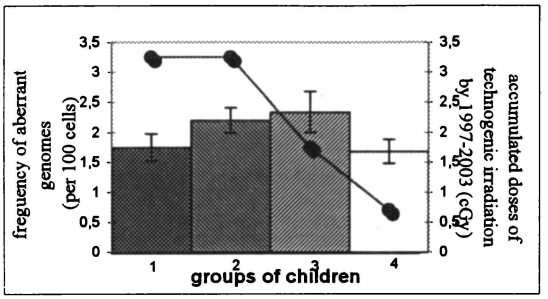

Fig.1. Doses (•) and frequencies of aberrant genomes (■) in children living on territories contaminated with radionuclides: 1 - children born in 1982-early 1986; 2- children born in 1986 (intrauterine irradiation); 3- children born in 1987-1992; 4- children born in 1994-2002

———— - control group

on the level of accumulated doses may be an indicator of both individual radiosensitivity and radiobiological peculiarities of the action of low-intensity radiation at small doses on the organism of children. One of such peculiarities seems to be the transgeneration phenomenon of genomic instability in children of irradiated parents.

In the irradiated children the average frequencies of exchange aberrations of the chromosomal type after testing irradiation in vitro (10 cGy and 100 cGy) were significantly higher than in the control group ($p<0,02$) suggesting increased radiosensitivity of somatic cell genomes in those children (table). An accelerated growth (estimated by the coefficients of linear regression) of the frequencies of paired fragments+centromeric breaks in 3 successive mitoses of intact and in vitro irradiated (10 cGy) lymphocytes of the children exposed low-intensity radiation as compared with the children of the control group points to induced postradiation chromosomal instability occurring along with reduplication of chromosome aberrations and their preservation in succeeding cell generations. The level of reciprocal SCE in lymphocytes of the 3rd mitosis (in contrast to lymphocytes of the 2nd mitosis) turned out to be significantly higher in the children from the contaminated territories as compared to the children of the control group ($p<0,001$),

Table. Frequencies of exchange aberrations of the chromosomal type in metaphases of the 1st mitosis of lymphocytes of examined children

Examined children	Dose (cGy)	Echange aberrations of the chromosomal t ype (frequency per 100 cells)
Control (5 subjects)	0	0
	10	0,33 ± 0,09
	100	8,35 ± 0,39
Born in 1986 (intrauterine irradiation) (5 subjects)	0	0,32 ± 0,09
	10	0,83 ± 0,14
	100	15,36 ± 1,39
Born in 1987-1991 (5 subjects)	0	0,50 ± 0,17
	10	1,16 ± 0,25
	100	17,19 ± 2,07
Born in 1994-1998 (5 subjects)	0	0,16 ± 0,16
	10	0,82 ± 0,33
	100	14,18 ± 2,46

which is due to the appearance of genomes with a large number of SCE (fig. 2) and also points to induced genomic instability.

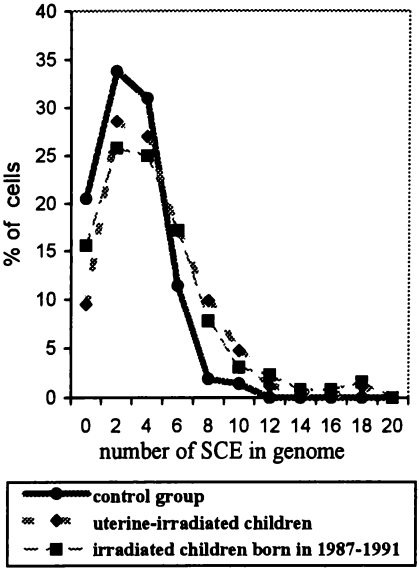

Fig.2. Distributions of lymphocytes of the 3rd mitosis depending on number reciprocal SCE

No essential differences were revealed in the average frequencies and in the distributions of SCE in intact and in in vitro irradiated lymphocytes of the 2nd and 3rd mitoses both in irradiated children and in the control group ($p>0,05$). A wide range of interindividual variability of the frequencies of dysgenomic effects (DE) was observed in intact and irradiated in vitro lymphocytes of the children from radionuclide-contaminated territories. In most of irradiated children with pronounced DE combined abnormalities in the immune system were noted. No significant differences were revealed for CA frequencies depending on the structure of morbidity. In children having disturbances of the central nervous system only the frequency of chromatid fragments was higher than in the group of children having infective-allergic diseases. The highest sensitivity to testing irradiation and most pronounced expression of DE in lymphocytes of the 3rd mitosis were observed in 5 children having the following pathologies: disturbances of the central nervous system, chronic gastritis, chronic pneumonia, extremity malformation.

Conclusion

The data obtained suggest the reality of induction of genomic instability in a growing organism exposed to prolonged low-intensity radiation and the necessity of further studies of the relationships between individual peculiarities of the expression of genomic instability and the health of children.

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SOMATIC GENE MUTATIONS IN PATIENTS WITH BENIGN TUMORS LIVING IN RADIATION CONTAMINATED REGIONS

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Radiation-induced somatic cell mutations are likely to be the principal cause of cancer risk elevation after radiation exposure. Therefore, estimation of frequency of cells with gene mutations has been suggested to be a useful method for cancer risk assessment in irradiated individuals [1,2]. One of the methods for estimation of somatic mutagenesis level is determination of mutant cell frequency at T-cell receptor (TCR) locus. Although mutations at this locus are not directly related to carcinogenesis, they are likely to reflect probability of cancer-associated mutations. To confirm this suggestion we evaluated the TCR-mutant cell frequency in group at high risk in respect to oncological diseases. The aim of this study was to research level of somatic mutagenesis in women with benign tumors of reproductive system living in radiation contaminated regions in comparison with control healthy individuals.

Materials and Methods

139 persons were investigated and divided in two groups. The first group consisted of 97 patients with myoma who had been living in Novozibkovskiy region of Bryansk oblast during 18 years since the moment of the Chernobyl accident. The residents were 0-30 years old at the moment of the Chernobyl accident. Mean ^{137}Cs density in this region was 799kBq/m^2. The second (control) group included 42 age-matched unexposed healthy individuals.

Flow cytometry was used to evaluate the frequency of peripheral blood lymphocytes bearing mutations at the TCR locus as it as described earlier in details [3]. Number of variant (or TCR-mutated) lymphocytes is determined by means of enumeration of the CD4+(positive) cells lacking the CD3 antigen on their surface. Studies performed by the inventors of the assay, S. Kyoizumi and associates from RERF (Hiroshima, Japan), have demonstrated that the absence of the CD3 on the CD4+ cells was mainly due to alterations of the TCR underlain by the mutations of the genes encoding the TCR polypeptides. Following these findings, the test has been termed the "TCR assay" [4]. Such mutations occur in mature lymphocytes after the passage through the thymus, as cells with defective TCR are eliminated in the gland by apoptosis and cannot enter the bloodstream.

Results and Discussion

The mean frequency of the TCR variant cells was significantly higher in patients with myoma (n=97) than in controls (n=42): $(5,3\pm0,5)\cdot10^{-4}$ vs $(4,0\pm0,2)\cdot10^{-4}$ on average ($p<0.05$,

Mann-Whitney test). 18 patients (18,6%) had the TCR–mutant cell frequencies exceeding the 95% confidence interval in control group ($>7{,}0 \cdot 10^{-4}$). The frequencies of mutant cells in other patients corresponded to those in control group.

Data on the frequency of the TCR variant cells in the inhabitants of radiation-contaminated regions and age-matched control donors are presented in Table 1.

Table 1. Frequency of the TCR mutant cells in unexposed control donors and residents of Bryansk oblast contaminated with radionuclides as a result of the Chernobyl disaster

Study groups	Number of persons	Age at the moment of analysis, yr		TCR mutant cell frequency, x 10^{-4}	
		Range	Mean	Range	Mean±SD
Residents with myoma 18 years after the Chernobyl accident	97	20-65	44	1,1 -52,9	5,3±0,5[a]
Unexposed control donors	42	40-50	45	1,0-10,0	4,0±0,2

[a] $p<0.05$ as compared to unexposed controls by t-test

Number of mutations is known to increase with radiation dose at any genetic locus tested. Therefore, elevated TCR-mutant cells frequencies in an individual may imply higher probability of the occurrence of cells harboring gene mutations at loci that eventually may cover critical oncogenes and tumor suppressors. It was plausible to suggest that individuals with elevated TCR-mutant cells scores might belong to a high-risk group potentially prone to the development of neoplasm. As an argument demonstrating such reasoning, one may mention results of a study performed in Scandinavia [5]. During a long-term epidemiological investigation, cancers were found to occur more often in a cohort of individuals with elevated level of chromosomal aberrations. Similar investigations of cancer incidence in the individuals with elevated rates of gene mutations have not been published so far. At the same time, data on high and early onset of cancer in patients with inherited genome instability syndromes (ATM, Bloom's syndrome, etc.) who also have elevated frequency of spontaneous gene mutations are well known [6,7]. The rationale of the hypothesis was indirectly demonstrated in our work: resident of Bryansk oblast with benign tumors of reproductive system had statistically significant higher levels of variant cells than individuals from the control groups.

Conclusion

The significant elevation of the TCR-mutant frequency was observed in the certain proportion of persons with benign tumors of reproductive system who belonged to high risk

cancer group. Our results confirm that the TCR-method may be used for individual assessment of long-term health consequences after the irradiation. Individuals with elevated TCR-mutant cell scores might belong to high-risk group potentially prone to the development of neoplasm and need more thorough medical observation than the rest of population.

Acknowledgements

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INFLUENCE OF IRRADIATION ON DROSOPHILA LIFE SPAN IN CORRESPONDENCE TO GENOTYPE

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The analysis of the modern literature shows, that *Drosophila melanogaster* is convenient object for research of apoptosis role in various natural and induced processes in an organism, both due to the high level of scrutiny, and due to high conservatism of apoptosis in evolution [1]. However attempts to carry out complex research of the role of apoptosis genes in life span and ageing regulation with use of this classical modelling object till now were not undertaken. Postmitotic condition of somatic imago tissues interferes with regeneration of cellular populations and evidently should show a prospective role of apoptosis in ageing on this laboratory object [2]. Researches on experimental ageing and life expectancy differ duration, therefore *Drosophila*, having short life cycle (2 weeks) and small life span (about 80 days) is the most convenient object for similar researches. As complex experiments with a role of apoptosis genes in ageing it was not carried out, therefore results will represent the high fundamental interest.

Material and methods

Drosophila* strains.** Wild type strain ***Canton-S. Strains with red-ox system defects: ***4015*** (*$Sod^{n1}red^{1}$/TM3, $Sb^{1}Ser^{1}$*), ***4018*** (*Idh^{F} Sod^{F} Pgm^{6tr} Ald^{2}/TM3, Idh^{S} Sod^{S} Pgm^{4tr} Sb^{1} Ald^{4} Ser^{1}*). DNA repair mutants: ***rad54*** (*$okr^{A17\text{-}11}$ cn bw/CyO*), ***mus209*** (*$mus209^{B1}$b pr cn/CyO*), ***mus210*** (*$mus210^{G1}$/CyO*), ***4236*** (*w^{-};mei-41^{D5}/Basc;cn^{1} bw^{1}*), ***mei-41*** (*w mei-41^{D5}/w mei-41^{D5}*). Lines with apoptosis deregulation: ***1576*** (*Df(3L)H99,kni(ri1)pp/TM3,Sb1*), ***618*** (*th^{1}*), ***5053*** (*th^{4}/TM6C, Cu^{1} Sb^{1} Ca^{1}*), ***12093*** (*$y^{1}w^{*}$; P{w^{+mC}=lacW}th^{j5C8}/TM3,Sb^{1}*), ***11041*** (*$y^{1}w^{67c23}$; P{w^{+mC}=lacW}l(2)k11502^{k11502}/CyO*), ***11179*** (*cn^{1} P{$ry^{+t7.2}$=PZ}Dcp-1^{02132}/CyO; ry^{506}*), ***10390*** (*y^{1} $w^{67}c^{23}$; P{w^{+mC}=lacW}Dcp-1^{k05606}/CyO*), ***3E4*** (*w^{1118}; $p53^{E4}$/TM3 Actin-GFP Ser^{1}*) and ***3E8*** (*w^{1118}; $p53^{E8}$/TM3 Actin-GFP Ser^{1}*).

Strains *3E4* and *3E8* were gently provided by Dr. Jongkyeong Chung (Korea Institute of science and Technology).

Experiment conditions. The flies were kept at 25±1^{0}C and 12 h light on yeast meal (Ashburner, 1989). The chronic irradiation was made on ^{226}Ra source. The absorption doze per generation (from embrio to imago, 12-14 days) was 60 cGy.

Results and discussion

The lifespan of all no irradiated mutant strains under investigation was lower than in wild type strain *Canton*-S (tabl. 1-2). Chronic low doze gamma-irradiation (60 cGy per generation) on pre-imago stages led to lifespan increasing in the most cases. The expressiveness of hormesis was depended on genotype. The difference between lifespan of irradiated individuals with compare to control in lines, heterozygous for antioxidant defense (*Sod* mutants) or apoptosis (th^{5C8}, $Dcp\text{-}1^{k05606}$, *dArk*), was higher that in wild type strain (*Canton-S*). As well the median life span after irradiation at these strains was higher that in no irradiated *Canton-S*. We propose that in sensitive to apoptosis induction strains (heterozygous *Sod* mutants and mutants on *IAP*) the increasing of life span is caused by radio-induced elimination of weakened cells with low repair capacity, which has the highest aging speed. In strains with mutations of proapoptotic genes (*1576, 11041, 11179, 10390*) the irradiation led to elimination of superfluous cells, having the effect similar to antineoplastic treatments. In strains with DNA repair defects the hormesis was less expressed that in *Canton-S*. At those strains the radiation induces genomic instability and increased level of somatic mutagenesis that reduce life span hormesis.

Homozygous individuals on *Sod* were hypersensitive to irradiation and have significant decreasing of life span after irradiation (tabl. 1).

Irradiation of strains, homozygous and hemizygous on *mei-41* (but not heterozygous), led to accelerated aging (*mei-41* females and *4236* males, tabl. 3). Well-known, that in humans ATM, homolog of Mei-41 protein, is the key sensor of DNA damage. Possibly, the activation of upstream levels of genome stability maintenance system in $mei\text{-}41^{D5}$ homozygous and hemizygous individuals not occurs, as a result the number of somatic mutations increased and the aging speed accelerated. ATM sensing radio-induced alterations in chromatin and than phosphorilate some effectors such as transcription factor P53. In males and females with *p53* defects (table. 3) we also revealed reduced lifespan after irradiation. It is because of involvement of P53 in sensing of damaged DNA, DNA repair and apoptosis.

Thus not irradiated Drosophila strains with defects of DNA repair, antioxidant protection and apoptosis have higher speed of ageing, than a wild type line *Canton-S*. At the same time the irradiation results in change of life span depending on the line genotype. The low doze chronic irradiation (60 cGy per generation) led to significant increasing of the life span in strains with the mutations of apoptosis genes *grim*, *hid*, *reaper*, *Dcp-1*, *dArk*, *th*, *Sod*. In some cases the level of the life span exceeded that in intact strain *Canton-S* (*Sod*, *th*, *Dcp-1*, *dArk*). Possibly, this is due to radio-induced elimination of the radiosensitive cells that will be subjected to accelerated aging.

Table 1

The influence of irradiation on lifespan in wild type strain, mutations of DNA repair genes and red-ox system

Strain	M	$\bar{X} \pm \Delta m$	90 %	min	max	N
Canton-S (Control)	39.0	35.6±0.6	48	4	60	299
Canton-S (60 cGy) *	44.0*	44.2±1.2	63	14	65	133
Canton-S (10 Gy) *	36.0	33.6±0.7	48	6	62	373
4015 (Control)	28.0	29.3±0.6	45	7	54	217
4015 (60 cGy) *	41.0*	38.8±1.2	54	6	62	170
4015 (10 Gy) *	22.0*	23.5±0.9	37	4	51	182
4015 (30 Gy) *	22.0*	21.4±1.0	32	4	40	116
4018 (Control)	64.0	62.4±1.6	81	2	88	94
4018 (60 cGy) *	49.0*	48.7±1.0	72	3	81	186
rad54 (Control)	35.0	35.5±0.8	48	7	60	197
rad54 (60 cGy) *	34.0	36.3±1.0	55	5	64	184
rad54 (10 Gy) *	19.0*	20.7± 1.9	38	5	49	112
rad54(30 Gy)	33.0	31.3± 2.3	46	6	46	27
mus209 (Control)	32.0	31.5±0.6	40	6	52	168
mus209 (60 cGy) *	33.0**	33.1±0.8	45	6	59	173
mus209 (10 Gy) *	33.0	30.6±1.2	42	6	49	86
mus209 (30 Gy) *	39.0*	35.2±1.6	44	7	48	52
mus210 (Control)	27.0	23.9±0.5	29	6	42	187
mus210 (60 cGy) *	31.0*	27.8±0.8	40	4	46	201
mus210 (10 Gy) *	19.0*	18.0±1.0	26	5	33	93
mus210 (30 Gy) *	22.0*	20.7±1.1	30	7	36	60

* $p<0.001$; ** при $p<0.05$ (in first column – by Kolmogorov-Smirnov test, in second – by Gehan-Breslow-Wilkoxon test); M – median lifespan (days); $\bar{X} \pm \Delta m$ – medium lifespan with the standard error; 90% - time of 90% deaths; min and max – minimum and maximum lifespan; N — sample value; f – females, m - males.

Table 2

The influence of irradiation on lifespan in apoptosis defective lines

Strain	M	$\bar{X} \pm \Delta m$	90 %	min	max	N
1576 (Control)	24.0	25.3±0.8	40	4	60	214
1576 (60 cGy) *	34.0*	31.6±1.0	46	4	64	187
1576 (10 Gy) *	23.5	23.9±1.2	35	4	40	59
1576 (30 Gy)	26.5	23.0±1.0	34	6	34	87
12093 (Control)	33.0	32.9±1.0	50	4	72	214
12093 (60 cGy) *	41.0*	38.9±1.3	57	8	70	118
12093 (10 Gy) *	24.0*	22.0±1.8	33	5	42	64
12093 (30 Gy) *	32.0	30.1±1.3	42	5	56	74
5053 (Control)	27.0	26.9±0.6	41	6	62	338
5053 (60 cGy) *	39.0*	40.7±1.1	57	8	68	311
5053 (10 Gy) *	34.0*	30.9±0.7	48	6	62	349
10390 (Control)	20.0	20.8±0.4	29	6	43	334
10390 (60 cGy) *	49.0*	44.5±1.6	63	4	73	307
10390(10 Gy) *	38.0*	36.9±1.2	55	7	63	322
11179 (Control)	25.0	25.4±0.6	36	3	49	257
11179 (60 cGy)*	29.0*	30.3±1.1	45	7	64	130
11179 (10 Gy)*	28.0**	28.7±1.0	43	6	63	173
11041 (Control)	27.0	26.2±0.8	41	6	50	215
11041 (60 cGy) *	45.0*	39.4±1.7	56	4	58	138
11041 (10 Gy) *	17.0*	16.8±0.9	27	4	35	198

Table 3

The influence of irradiation on lifespan in strain with DNA damage sensing defects

Strain	M	$\bar{X} \pm \Delta m$	90 %	min	max	N
4236m (Control)	49.0	50.3±0.9	71	8	78	177
4236m (60 cGy) *	43.0*	42.1±0.9	60	3	85	212
4236f (Control)	45.0	45.1±1.5	71	10	79	131
4236f (60 cGy)	43.0	39.9±1.0	57	8	70	183
mei-41^{D5}*f* (Control)	36.0	33.8±1.2	50	6	64	144
mei-41^{D5}*f* (60 cGy)*	29.0*	28.1±1.5	50	4	61	117
3E4f (Control)	54.0	48.8±1.4	69	5	83	268
3E4f (60 cGy)	49.0**	46.9±0.9	66	4	75	282
3E4m (Control)	30.0	31.3±0.7	47	4	64	246
3E4m (60 cGy)	26.0**	28.6±0.7	46	4	68	254
3E8f (Control)	46.0	40.4±1.5	58	5	65	243
3E8f (60 cGy)	40.0*	35.8±1.0	54	4	61	264
3E8m (Control)	29.5	29.6±0.8	48	4	65	267
3E8m (60 cGy)	30.0	28.7±0.7	46	4	58	276

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INDUCTION OF DNA POLYMORPHISM IN THE OFFSPRING OF MICE EXPOSED TO γ-IRRADIATION IN DOSE RANGE FROM 1 Gy TO 3 Gy

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Studying of DNA variability arising as a result of an irradiation is represented to one of the most interesting and important directions of radiating biology and genetics. A number of authors consider increase of a level of DNA polymorphism, in particular polymorphism hyper variability non-coding loci, as display genomic instability. A plenty of works is published, in which for an estimation of genetic effects of an irradiation the RAPD-analysis (random amplified polymorphic DNA), giving an opportunity multi loci scanning hyper variability sites of DNA was used. In the present work the task put to investigate the effects of the chronic and acute irradiation shown in offspring of irradiated mice, by RAPD-analysis. Taking into account distinctions of spermatogenesis stages in sensitivity to ionizing irradiation, the estimation of DNA polymorphism arising as a result of irradiation pre-meiosis and post-meiosis cells was included.

Materials and Methods

Mice BALB/c were irradiated in doses 1-3 Gy on gamma-unit GYPOS (dose rate- 4,5 Gy/min, source- Cs-137). Within the second and third weeks and after three months after an irradiation, male have crossed with females the same line. Thus, the sperm participated in fertilization, irradiated, in the first case, at a stage of spermatides, in the second - at a stage stem spermatogonia. For isolation of DNA from a liver and amplification were used commercial kits Diatom DNAprep™ and GenePak PCR Core™ (Izogen Company). The program of amplification consist from 35 cycles: Td-95 ^{0}C, 25 s, Tm - 42 (cycles # 1-4), then 58 ^{0}C (cycles # 5-35), 5 s, Ts- 74 ^{0}C, 80 s. For PCR programmed thermostat PT-48 (TDL Company) were used. Products of RAPD were separated into 1,5 % agaroze gel. Analysis of offspring patterns carried out on the basis of comparison with parental patterns, on purpose to registrate new, "not parental" bands. Results of the analysis were processed statistically. For RAPD a series of five primers, consisting of 20 nucleotides everyone were used.

Results and Discussion

A definite dependence from doses was found for all five primers, used in our study. In all five cases we observed a rise of level of polymorphism in irradiated groups. In cases of irradiate of 1 and 3 Gy difference in degree of polymorphism RAPD-markers of spermatides and

spermatogonia were insignificant. Results from different primers were various. A figure 1 demonstrates the average levels of polymorphism of all five primers. The common tendency takes place, and this difference from the stages of spermatogenesis is maximum with a dose of 2 Gy.

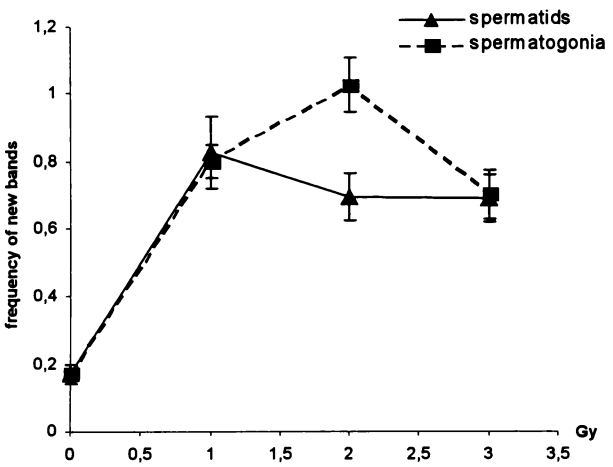

Fig 1. A dose – effect relationship for the frequency of new bands in the offsprings of mice irradiated at pre-meiosis and post-meiosis stages of spermatogenesis

RAPD-test is wide applied as method of estimation induction of polymorphism, side by side with using other markers of polymorphism DNA [1-5]. Dose-depend increase of level of polymorphism was discovered in diapason of the accumulated doses from 25 sGy to 50 sGy. Acute irradiation in dose of 0,5-2 Gy also leads to increase of level of polymorphism DNA in offspring. Results, obtained by RAPD correspond to those, obtained by estimation polymorphism using other marker loci, such as micro- and minisatllite loci [1, 2, 4]. Consequently our results do not contradict to data of other researchers in sphere of application RAPD-assay as marker of induction of polymorphism and manifestation of genomic instability. However in present study dose dependence for polymorphism DNA on field relatively high doses (1-3 Gy) was investigated. Besides this in most published studies data, obtained by amplification with one random primer were uses, whereas in our study number of five random primers was used. For our opinion using number of primers allow to give more complete estimation changes of genome, aroused irradiation. Figure 1 demonstrates generalized dose dependences for both stages on average level of polymorphism of RAPD-markers obtained by all five primers. But for everyone primer, curves «doze - effect» strongly differs from submitted on fig. 1, both for spermatides, and for spermatogonia. The reason of such distinctions can be that various sites of genome a during growth and divisions of a cell participate in a different degree. On the literary data, the least genetic radiosensitivity show pre-meiosis cells whereas spermatides and sperms have the greatest radiosensitivity. In our experiment, the essential difference between sensitivity of stages was registered only at an irradiation in a doze 2 Gy, thus spermatides were less sensitive, than spermatogonia (Fig 1).

Conclusion

In the present work radiogenetical sensitivity of the sexual cells, which are at stages of spermatides and stem spermatogonia, was analyzed. The application of the methodical approach adequate to a problem provides detailed analysis. The doze dependent increase of levels of polymorphism both for pre-meiosis, and for post-meiosis stages of spermatogenesis was registered. Thus authentic difference of stages was showed only at a doze 2 Gy.

Distinction of curves "doze - effect" for each primers can be explained to that various sites of genome are packed in a different degree, and the degree of their packing during growth and divisions of a cell varies differently. Hence, decrease or increase of levels of polymorphism can be explained a various degree of shielding by histones these sites the fibers responsible for packing of a genetic material

Acknowledgements

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THE STUDY OF CHROMOSOME DAMAGES IN THE HUMAN CELLS IRRADIATED BY THE THERAPEUTIC PROTON BEAM

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The therapeutic proton beam was based on the phasotron in LNP and has been used for a long period for radiation therapy (Mytsin G.V. et al, 2002). Peculiarities of the proton energy deposition and the Bragg peak at the end of the particles path allow us to form an optimal dose in the tumour tissues.

The main object of investigation was the study of peculiarities of chromosomal aberrations' formation and the estimation of efficiency of the therapeutic proton beam with initial energy of 170 MeV and in the Bragg peak region.

Materials and Methods

The chromosome aberrations in the human cells (on a model of blood lymphocytes) were studied after irradiation by 170 MeV proton beam prepared for therapy of patients on the entrance and at the Bragg peak region, that corresponds to the irradiation of normal tissues along the beam path and tumour tissues directly. The energy of the Bragg peak protons is the spectrum from 0 to 30 MeV. The spectrum of LET stretches up to ~ 100 keV/mkm. The unstable chromosome aberrations were analysed by the metaphase method.

Results and Discussion

Protons and γ-rays induce the chromosome aberrations in the lymphocytes. As it has been seen (Fig.1a), the frequency of aberrant cells increases linearly from dose up to ~ 4,5 Gy of 170 MeV protons and γ-rays and to 3,5 Gy of the Bragg peak protons (to 90 % of the aberrant cells). Total number of chromosome aberrations increases exponentially from the doses (Fig.1b).

High efficiency of the Bragg peak protons has been shown. RBE values were ~ 1,25 (at the dose interval of 1-4 Gy) as it was estimated at these two tests, while the protons with energy of 170 MeV did not differ from the γ-radiation. Our data are in a good agreement with the results obtained by the authors (Vitanova A. et al, 2002). Also the fraction of cells with numerous aberrations (3 and more) exceeded ~3 times (27% and 10%). The high level of exchange chromosome aberrations (up to 75%) with a predomination of dicentrics (~50 %) was revealed in the analysis of different aberrations.

As the proton beam dose to a tumour is formed from several directions (up to 7), the damage of cells in surrounding tissues along protons path will decrease. So after the dose of 3

Gy about 80% of the tumour cells obtain damages, but in surrounding healthy tissues it will not exceed 10% (Fig.1a).

Conclusion

Our investingation confirms a high efficiency of proton beams for use in radiation therapy.

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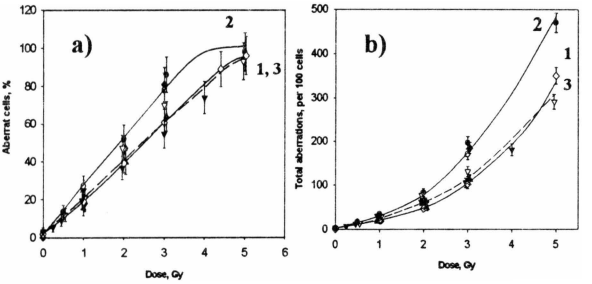

Figure 1. Dose-dependences of aberrant cells frequency (a) and total number of chromosome aberrations (b) after irradiation by 170 MeV protons (1), the Bregg peak protons (2) and γ-rays (3)

RADIATION-INDUCED BYSTANDER EFFECT – MODERN EFFECT OF LOW DOSE RADIATION. CRITICAL REVIEW ON BASIS OF LITERATURE AND OWN DATA

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Radiation-induced *Bystander effect* (BE) is a phenomenon when irradiated cell(s) affect unirradiated neighbouring cells. This phenomenon was observed by radiobiologists in 60th [1-3]. It became widely popular to study after a paper published in 1992 [4], demonstrated increased level of sister chromatid exchanges after irradiation of small part of Chinese hamster cell population by alpha-particles. There are some specific features of BE known from the literature. It were shown that irradiation of small part of cell population (or even one cell through nuclei or cytoplasm) could lead to enhanced level of chromosome, chromatide aberrations, apoptosis, micronuclei frequency, mutations, transformation, cell death, and also to adaptive response, genetic instability and changing of genes expression. BE was shown after low or very low dose irradiation (1 cGy or lower) as well as after high doses. It is interesting that BE does not relate to phenomenon called low-dose hypersensitivity and induced resistance [5]. BE can be induced by high and low LET radiations. BE was demonstrated on many cell lines *in vitro*. There are also few papers in which *in vivo* effect was shown. DSB repair deficient cells demonstrated higher effect. There are at least two different mechanisms of bystander signal transfer known: through microtubes (gap-junction) [6, 7] or through intercellular media [8].

Materials and Methods

Primary human fibroblasts AG01522B cultured in αMEM media as describe in [9] were used in the experiments. Three methods were used to induce bystander signal. The first, microbeam irradiation of 200-400 cells of neighbouring population in the same dish with 1-10 He^{3+} particles. The microbeam facility in Gray Cancer Institute [10] was used. The second, media transfer from 10^4 cells irradiated by 2 Gy of X-rays after 1, 2 or 24 hours after the irradiation. The third, co-culture of 10^4 cells irradiated by 2 Gy of X-rays seeded into membrane inserts with 1μm pores. Micronuclei (MN), γH2AX foci formation, and colony forming efficiency of unirradiated cells were used as endpoints. In addition RNAi method was used to knock out DNA PKcs to find the effect. Scoring has been done in coded dishes.

Results and Discussion

BE was not observed in our experiments (Figure). Moreover additional studies with human glioma T98G cells (Table) did not show any effect after hundreds of cells irradiated with 5 He^{3+} particles. Conditions of experiment were the same as described in papers [11], [12] where the effect was found; some additional experiments were done with variation of time after irradiation and BE was not seen. High variability of MN frequency was observed in both cell lines but in the range of cellular responses been seen in above mentioned papers.

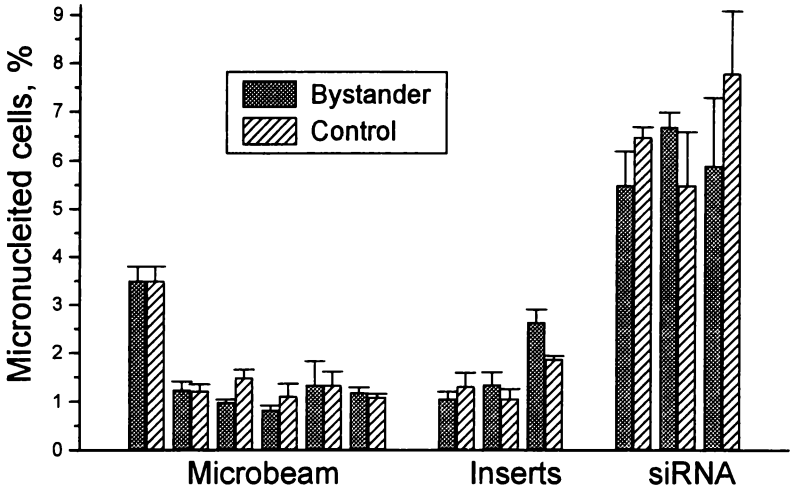

Figure. Results of bystander experiments in AG01522B cells. Each bar represents results of one experiment. Errors are variations between dishes in one experiment

In paper [6] inhibition of gap-junction interaction in monolayer by Lindane neutralised the effect. In paper [11] BE is shown at irradiation of non-contacting cells and the signal should be transferred through media in this case. In the both studies the same cell line, similar protocols and micronuclei frequency as endpoint were used. The scoring were performed in non-coded dishes which could affect the researchers with BE findings. We have not observed difference between control and population with hundreds irradiated neighboring cells (Figure, microbeam) but the difference should be statistically significant according to results [11].

Table. Results of bystander microbeam experiments with T98G cells

Time after irradiation, h	72	67	27	32
Control, % cells with MN	17 ± 1	12.3 ± 0.6	10.6 ± 0.6	6.6 ± 0.6
Bystander, % cells with MN	15 ± 2	13 ± 3	10.6 ± 0.9	6.8 ± 0.5

The controversial results of BE were observed on plating efficiency. It was increased after irradiation of small part of population by alpha-particles [13], in other studies it was

decreased [7, 14]. However different cell lines were used in these experiments. In our study we did not see any change of plating efficiency. BE could be induced by ROS (reactive oxygen spices) or by NO (nitric oxides). It was shown in the experiments with different scavengers. If it is right there should be dependence on irradiation dose in case ROS/NO are induced by radiation or on number of irradiated cells in case the cells produce the ROS/NO but it is not observed. In addition there is no dependence on distance from irradiated cell.

There is not known a lot about mechanism(s) of BE despite of huge amount of experimental data: a) BE is induced not in all cell lines and not by all cells; b) laboratory investigations are sometime controversial and not always agreed with epidemiological findings [15]; c) the nature of bystander signal is still unclear. We suggest that there could be many different mechanisms related to effect which is called "Bystander Effect" now.

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RADIOECOLOGY

OPPORTUNITIES FOR USE OF LECCINUM AS BIOMONITORS OF RADIOACTIVE AND CHEMICAL POLLUTION IN WOOD COMMUNITIES

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Introduction

Now use of fungi for bioindication is limited by the chemical analysis of heavy metals and radioactive elements in fruit bodies. The essential restrictions are caused as sporadic of fruit bodies' production and small conformity between the amount of polluting substances in sporocarps and their quantity in the soil.

The problem in the search of species-biomonitors, capable to change morphological attributes under influence of polluting substances, will open new opportunities in the use of fungi for estimation of environmental condition. Potential biomonitors fungi should satisfy the following conditions: to have wide geographical distribution, to possess high variability depending on ecological conditions and forming ectomycorrhizas.

The genus *Leccinum* S.F. Gray (*Boletales*, *Boletaceae*) corresponds to these requirements. The main reason influencing the choice *Leccinum* has become detection of abnormal peculiarities of a structure of rDNA site, playing a key role in albumen biosynthesis. Data received to the present time permit one to assume that *Leccinum* species can have plastic genome capable to change in various ecological conditions. This zero hypothesis requires further experimental check.

Materials and Methods

As material for allocation DNA, the ectomycorrhizal terminations of Picea abies roots selected on plot 20 x 20 m^2 in the old forest situated in the Priozersky district of the Leningrad region have served. Soil samples D 25 mm, H from 30 to 120 mm, defined by presence of stones, were taken in squares 1x1 m - 9 samples in square located 2,8 m far from the south-western corner, 6 samples on diagonal of a square in the centre of the plot and 9 samples in square placed 2,8 m far from the north-east corner.

The ectomycoirhizas were described in terms of morphological, anatomical and molecular features using the ITS/PCR/RFLP method. We have successfully amplified 77 samples of individual ectomycoirhizas and divided them into 23 different ITS-types in tree squares 1x1 m. The results of sequence have shown that some ITS-types from different squares are identical and the total number of different sequences was 11. The nucleotide sequences of ectomycoirhizas were identified by comparing them with the International Nucleotide Sequence

Database (INSD) using BLAST and Phylip 3.5 for determination of phylogenetic placement in case the conformity is lower than 95 %. The accession numbers of these sequences were in INSD with AY237171 on AY2371811, submission in May 2003.

Results

From ectomycoirhizas three species were identified (*Rozites caperata*, *Suillus variegatus*, *Cortinarius traganus*) and relationship with seven species or species groups is determined (*Paxillus involutus; Amanita fulva; Clavulina cinerea; Russula postiana* and *Russula integra; Russula foetens* and *Russula integra; Lactarius mitissimus*, *Lactarius fulvissimus* and *Arcangeliella borziana*). Received data have allowed analysing distribution pattern of studied ectomycorrhizal fungi in the soil (Ivanov, 2005a).

But one sequence AY237178 stays unidentified. More over, it possessed abnormal length of ITS1-5,8S-ITS2 by the size 1600 bp. In April 2004 it was identified as *Leccinum versipelle* (Fr.) Snell. (= *L. testaceoscabrum*) after the ITS1-5,8S-ITS2 rDNA for species from *Leccinum* was sequencing and brought into INSD by Den Bakker et al (2004a, b).

The reason for abnormal length phenomenon of that ITS1 is organized by repeating minisatellites sequences named also VNTR (Variable Number of Tandem Repeats). Sequence GAAAAGTA was observed 12 times and CTAATAGA – 11 (Ivanov, 2005b). Probably, this is the first discovery of minisatellite-like repetitive sequences in ITS1 among basidiomycetes whose rDNA were investigated. But VNTR are usual at plants, animals and human DNA. They are characterized by high variability and are used for population analysis.

Discussion

It is necessary to note that until now such abnormal size of ITS1 site at representatives of other *Boletales* genus is found out. So, for *Suillus variegatus* identified from ectomycoirhizas the size of ITS1-ITS2 site was 860 bp (Ivanov, 2005a). But it is early to make final conclusions because for many *Suillus*, *Xerocomus*, *Tylopilus* species sequences 28S are determined only. At the same time, at *Boletus edulis* ITS1 sequence of the small size 204 bp the site CCCCTTTCT was observed twice.

The sequences containing minisatellites have very high intra-species variability. The experience of their application as markers in genetic examination has shown high efficiency for control of reliability of an origin of domestic animals breeds and differentiation of cultural plants sorts. Such markers give large information for lines with close origin and permit one to analyze distinctions at population level. But such high variability does not permit one to work on species or higher level. Therefore to investigate phylogenetic relationships between *Leccinum* species on

the base of ITS1 rDNA sequences is impossible. For these purposes it is necessary to look for other target sequences and concentrate attention on sites coding ferments.

For *Leccinum*, beside the found genetic differences there were the difficulties in the systematic plan. "Crux et scandalum agaricologicorum" - so it was underlined by B.P. Vassilkov (1956a).

Species of *Leccinum* is widely distributed in taiga forests; it plays an important ectomycorrhizal role and has interesting phylogenetic relationships. Except for that, almost all species from *Leccinum* belong to delicious products of food and very popular among many people. Any amateur to gather mushrooms pays attention on huge variety in *Leccinum* species.

Latest papers about *Leccinum* in Russia were published in 1956 by mycologist B.P. Vassilkov. Inside *Leccinum scabrum* 12 ecological forms were allocated and in *Leccinum aurantiacum* - 7 forms (Vassilkov, 1956a, b). These papers were a preparation for the guide on *Boletaceae*. It is a pity that this book has not been issued.

According to R. Watling (1970) system key to *Leccinum* genus totals thirteen species. On the system offered in the monograph by Lannoy and Estrades (1995) in this genus about seventy species are allocated (quoted on Kirk et al, 2001)

May be, the *Leccinum* species are useful as biomonitors of radioactive and chemical pollution in wood communities? To answer this question it is necessary to investigate species' genetic structure (for example by RAPD) and understand what these species are: (i) the organisms with high variability depending on ecological conditions or (ii) species complexes in the state of a divergence or (iii) independent species.

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COMPLEX ASSESSMENT OF ENVIRONMENTAL RADIOACTIVITY IN YEREVAN

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Today, the issue of radioactive pollution of biosphere is acquiring a special topicality worldwide due to specificity of ionizing radiation. Rapid development of nuclear technologies and energy, nuclear weapon testing, accidents in nuclear power plants have brought to the occurrence of short- and long-living man-made radionuclides - environmental pollutants. Such pollutants are especially harmful for any living organisms, their habitats and biosphere as a whole as they may easily join the natural process of element turnover. All this dictates a necessity of complex radioecological environmental monitoring i.e. understanding of past and present observations, studying radionuclides transfer in the environment and assessing their impact upon the ecosystem [1].

This paper highlights the results of first ever complex radioecological research in system atmospheric precipitation-soil-plant performed in Yerevan - the capital of Armenia - a densely populated city located some 30 km far from the Armenian NPP.

Material and Methods

The study material – atmospheric precipitation, soil, plant and moss samples were collected in Yerevan. To comprehensively assess radioecological situation in the city, the author processed and analyzed data obtained in period 1969-1997 [2] alongside with sampling, field and lab sample treatment and analyses performed by her own in period 1998-2003. Collating all the data obtained for the entire study period, compiling relevant database, producing β-radioactivity distribution maps are important constituents of this independent research aimed to getting an exhaustive picture of Yerevan environment radioactive pollution. Atmospheric precipitation samples were collected through the sedimentation method [2], soil, plant and moss samples - by the method developed at the V.V. Dokuchaev Soil Institute [3]. All the samples were processed and prepared for further analysis. ^{40}K, ^{226}Ra, ^{232}Th, ^{210}Pb, ^{137}Cs concentration was determined through γ-spectrometric analysis on NaI(Tl) semi-conductor and HpGe detectors (Canberra, USA) using Genie-2000 computer software, ^{90}Sr concentration – by β-spectrometric analysis on NaI(Tl) detector (Progress, Russia). Gross β-radioactivity measured on β-radiometer RKB4-1eM (Russia). KCl was used as a calibration standard. Totally, processed, collated and interpreted were data on 408 atmospheric precipitation, 847 soil, and 22 plant and moss sample analyses.

Based on the results, the Yerevan soil β-radioactivity distribution maps (1:10000) were produced by the author using GIS Arc View 3.2 and Surfer 6.04 Programs.

Results and Discussion

The *basic* outcomes of this radioecological research in atmospheric precipitation-soil-plant system in period 1969-2003 in Yerevan are as follows:

1. **Atmospheric precipitation: A)** Radionuclide composition is mainly represented by ^{40}K, ^{226}Ra, ^{232}Th, ^{137}Cs, ^{90}Sr. **B)** Gross β-radioactivity level variations are timed to nuclear weapon testing in different regions of the world and global pollution. In period 1969-1977 (1st stage) β-radioactivity level significantly varied: max value in 1971 (1857,4 Bq/m^2/y), min. - in 1973 (111,0 Bq/m^2/y), high indices were observed in 1974 (1073,8 Bq/m^2/y) and 1977 (743,8 Bq/m^2/y). In 1989-2001 (2nd stage) an inclination to β-radioactivity level decrease and stabilization was established, mean value making 304 Bq/m^2/y *vs.* 672 Bq/m^2/y in 1969-1983 (Fig. 1).

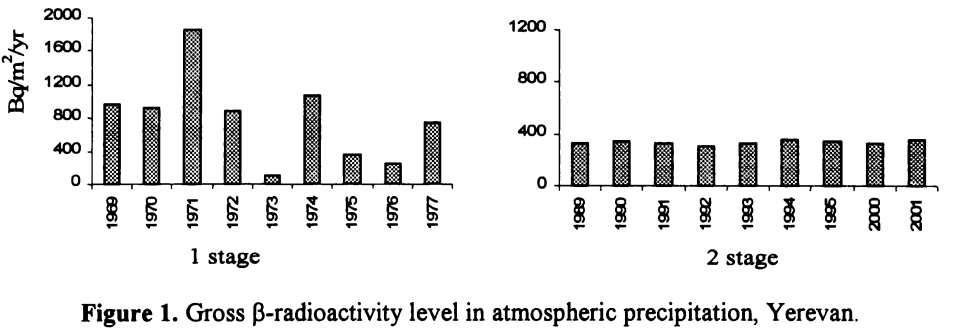

Figure 1. Gross β-radioactivity level in atmospheric precipitation, Yerevan.

The same is true for global radionuclides ^{90}Sr and ^{137}Cs which contents decreased by 36,6 and 45,5% at the 2nd stage, respectively.

2. **Soils. A)** As of 2000, Yerevan soils radionuclide composition is mainly represented by ^{40}K, ^{226}Ra, ^{232}Th, ^{137}Cs, ^{90}Sr. In 2000 ^{137}Cs concentration decreased by some 20% *vs.* 1990. Wholly, the research results on soil radioactivity correlate to those on atmospheric precipitation.

B) In 1990, Yerevan soils gross β-radioactivity was distributed unevenly throughout the city: max. value 919 Bq/kg, min.– 538 Bq/kg (Fig.2). The analysis of soil gross β-radioactivity in 2002 *vs.* 1990 testified to decrease of Yerevan radioactive pollution level and increase in areas characterized by natural background (500-600 Bq/kg). The produced Yerevan soil gross β-radioactivity distribution map is included into General Urban Development Plan.

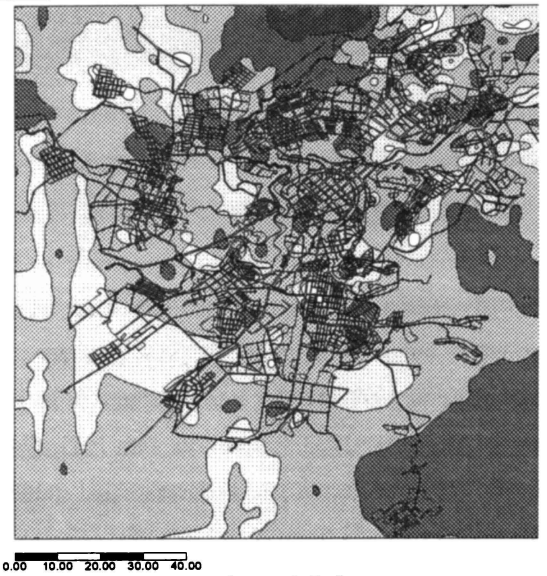

Figure 2. Gross β-radioactivity of soils in Yerevan as of 1990.

3. **Plants. A)** In period 1989-2001, $^{137}Cs/^{90}Sr$ ratios in plants averaged 5,2 *vs.* 1,9 in atmospheric precipitation, i.e. plants accumulate ^{137}Cs which concentration is 5-9 times higher than that of ^{90}Sr. **B)** As found out, relatively high gross β-radioactivity level in plants depends on local man-made pollution, as well. In 2003, gross β-radioactivity level insignificantly decreased *vs.* 2000: 1455 and 1556 Bq/kg respectively. γ-spectrometric measurements of **moss** samples (2000-2003) show that mosses accumulate larger amounts of radionuclides and particularly ^{137}Cs *vs.* plants and thus may serve radioactive pollution indicators.

Conclusion

1. The first long-term radioecological monitoring shows that large urban centers like Yerevan are exposed to both global and local radioactive pollution. 2. The obtained data and compiled databases may underpin further relevant investigations. 3. The gained experience and developed research methods may be applied in long-term complex radioecological monitoring of urban sites.

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USING THE RADIOCAPACITY PARAMETER FOR THE ESTIMATION OF RADIATION AND TOXIC INFLUENCES ON PLANT SYSTEM

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There is a necessity to find a parameter and a measure of ecosystem's state which would outstrip the reaction of the biological parameters under the stress influence and would allow estimating operatively the state of biota. Our research of behavior of the radionuclide tracer ^{137}Cs and the radiocapacity parameters by this tracer have allowed offering estimations of state of biota in ecosystem on the behavior and changes of the radiocapacity parameters [1].

The idea of the radiocapacity theory and of the radiocapacity factor for the first time was entered by Agre and Korogodin (1960) [3] and has been actively developed by us lately [2, 4].

The radiocapacity parameter relates with the fundamental characteristics of the nutrient interchange processes in the ecosystem. The present two-chamber ecosystem's model included the environment (water) and the biota with the containing of radionuclides *Y (x)* and *Z (x)* (*x* – the time), the radiocapacity factors for water and biota:

$$F_b \cong \frac{a_{12}}{a_{21}+a_{12}}, \quad F_w \cong \frac{a_{21}}{a_{12}+a_{21}}, \tag{1}$$

where a_{12} - speed of absorption of radionuclides tracer and a_{21} - speed of their outflow to the environment (in water).

And its ratio is proportional to the ratio of speeds of absorption and of outflow:

$$\frac{a_{12}}{a_{21}} = \frac{F_b}{F_w} = \frac{1-F_w}{F_w} = Z \tag{2}$$

It means that the above biomass and factor of accumulation of tracer by biota is better, the biota's state and the ratio of speeds of tracer absorption and outflow is higher. Thus, nutritious elements from water to biota are also better. And it makes possible estimating the reactions and changes of biota's state under influence of the various environmental factors using the radiocapacity parameters by tracer.

Also the parameter for the estimation of the synergism degree of the different factors action – the koefficient of synergism *P* was entered:

$$P = \frac{Z_{Cd+\gamma}}{Z_{Cd} \cdot Z_{o\delta u}} \cdot Z_0, \tag{3}$$

where Z_0 – is the biota radiocapacity relation for control; $Z_{Cd+\gamma}$ – the relation in case of radiation and toxic metal joint action; Z_{Cd} and Z_γ – the relation for the separate influence of each factor.

If $p=1$ - no synergism is present. If $p<1$, it can testify to the essential contribution of synergism that is strengthening two factors action in comparison with the action of each separate factor. If $p>1$, we deal with an antisinergism, when the first factor reduces negative action of the second one or on the contrary.

Materials and Methods

The research was carried out on the simplified models of plant ecosystem – water cultures of maize plants. The three-day sprouts were irradiated on the cobalt γ-device "Researcher" in dose 15 Gy and then landed on 0,5-litre jar with water. To the water of all experimental variants was added the tracer - ^{137}Cs (initial activity on each 0,5-litres jar was 3000 Bq). To the certain jars was added the $CdCl_2$ solution in concentration of 50 mkM/l. During the experiment (within 14 days) sampling water was carried out regularly for definition of residual activity by the tracer contents in it. The effect of synergism on the two factors interaction was appreciated through the radiocapacity parameters of maize plants influenced to joint and separated acute γ- irradiation on dose 20 Gy and fractionated salt $CdCl_2$ adding in concentration 50 mkM/l using. The time between the fractions (two fractions in 25 mkM/l each) was 6, 10 and 24 hours. The preparation and realisation of the experiment was done by the scheme analogous to previous.

Results and Discussion

The radiocapacity factor dynamics for water as a component of offered simplified system and the synergism index P time dependence is given in Fig. 1. and Fig. 2. It is shown that to the end of the experiment plants of control variant absorb almost all added to the water radionuclide-tracer quantity. The plants of experimental variants absorb to 50 % of activity added to water. The P values for combined action of the acute γ-irradiation and fractionated adding of $CdCl_2$ with the time of fraction 6 hours do not exceed 1. So synergism of interaction is present here.

Conclusion

The possibility of estimation of ecosystem's state by the radiocapacity parameters under the influence of γ-irradiation and toxic metal salt adding was proved.

Radiation and toxic factors acting to the plant ecological system interact with each other. The character of interaction on all of irradiation and salt $CdCl_2$ adding combinations is nonadditive. Maximal synergism is observed on case of combined influence of irradiation of plants in dose 20 Gy and fractionated $CdCl_2$ adding with the time of fraction 6 h (Fig 2).

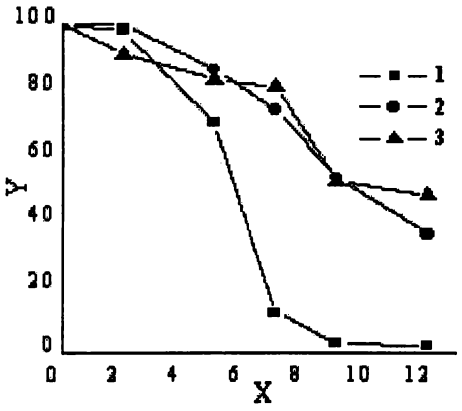

Figure. 1. The dynamics of change of the tracer contents in water. X – days of measuring; Y – content of tracer, %; *1* – adding of 50 mkM/l $CdCl_2$; *2* – acute γ-irradiation in a doze 15 Gy; 3 – control

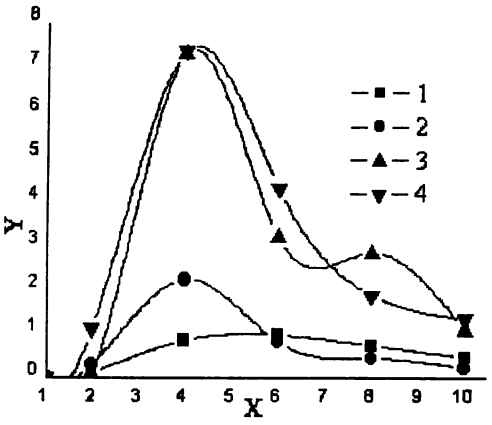

Figure 2. X – days of measuring; Y – *P* values. *1* – irradiation (20 Gy) with $CdCl_2$ adding with the time between fractions 6 h; *2* – irradiation with $CdCl_2$ adding with the fraction 10 h; *3* – irradiation with $CdCl_2$ adding with the fraction 24 h; *4* – irradiation with $CdCl_2$ adding without fractionating

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EVOLUTIONARY THEORY IN NEW MILLENNIUM

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The research aims to generalise the results of the development of evolutionary theory.

Materials and Methods

1. Analysis of the data obtained from the genetic and taxonomic researches of the group with clear evolutionary pathways – white fishes, fam. Coregonidae, (they were often called «quintessence of evolution», since they demonstrate abundance of morphs, ecological and geographic races, intraspecific hybrids as well as the fanciful patterns geographic distribution)
2. Analysis of evolutionary interpretations of the enigmatic case – the origin of bats.
3. Revealing the non-Darwinian evolutionary concepts containing valuable material.
4. Analysis of the formation and development of the modern "evolutionary synthesis".
5. Study of the attempts to base biology on the principles, on which other natural sciences are based – laws and periodical systems.
6. Study of the concepts aiming to extend evolutionary approach outside of biology.
7. Analysis of ecological history as a reflection of the evolution of mankind and biosphere.

Results and Discussion

1. The data on white fishes give grounds for the doubts in the geographic speciation model or, at least, in its universality. Modifications could be considered in some cases as evolutionary events even if they were not replaced for correspondent mutations. The predisposition to vary slowly or rapidly in definite directions should be considered as the significant factor of evolution. The evolution of variety could be explained in some cases just by age changes. The appearance of new species as a result of hybridisation is possible. It takes place through merging of two species into one in specific conditions, while occasional intraspecific hybrids originating in addition to parent species cannot give birth to new species.

2. All interpretations of "the enigmatic case" rest on the analysis of the existence of tendencies to vary in certain directions, which could be realised in specific conditions of environment.

The gliding animals represent a model to study the origin of flight. These animals are abundant in Asian tropical forests, rare in Africa, and absent in America. Such pattern of distribution was often considered as a chance in biogeography. However it corresponds to the differences of the arboreal tier structure of tropical forests in various continents. The tendencies to produce

"anomalous" structures suitable for flight could be fully realised only in the Asian forests since they have a rarefied highest arboreal tier. This case provokes an idea that any statement on randomness in evolution is just a declaration of the lack of knowledge.

3. The most promising concepts existing in the shadow of Darwinism are the concepts of directed evolution (more then 20 of them are known): organisms have a predisposition to vary in certain direction and this very predisposition determines evolution. To substantiate this idea the adherents of this idea very often appealed to the general laws of a nature or to principles, on which other natural sciences were based.

4. It is possible to construct in biology the systems reminding the periodical system and homological rows of chemistry. Two approaches could be used: the composing of the table of possible combinations of the characters of organisms and the construction of the parallel rows of variations. This possibility is based mainly on the existence of the similarities among groups of organisms, which are not related directly. Moreover, the other feature of the variety of organisms reminds periodical system. The Mendeleev's table is asymmetric: in the lower part of it the periodicity is noted not among elements but among the groups of elements, which tend to increase. The systems of biology are asymmetric too, despite the efforts of taxonomists to put them in Procrustean bed of convenient systems. The systems of every taxon become more and more asymmetric as the research of them is progressing: minor part of genera become large, they become separated into "sections", "superspecies", "subgenera" while major part of genera remains small. The same situation takes place among species: few species become large splitting into subspecies, morphs, ecotypes, etc. while the majority of them remains homogenous tending to be genera. The changes of age cause periodicity among groups of organisms like the changes of atomic weight cause it among chemical elements.

5. Besides evident successes Darwinian concepts contain a significant gap – the analysis of the constraints on variation. Both Darwin and the authors of modern version of natural selection theory tried to hide or discredit any data on this subject.

Such a situation could be traced in the case of the analysis of the variation of one bird species (*Pachycephala pectoralis*) by Th. Dobzhansky (1937) and E. Mayr (1942). To characterise it a table demonstrating the combinations of 5 their alternative characters was composed. 8 combinations were known. Mayr cited this data to demonstrate abundance of variability noting that almost all-possible combinations were found out. However it is very easy to calculate, that the number of possible combinations is 32. Nobody noticed that 8 is not "almost 32", because the belief in unlimited variation was firmly established. Such a situation is typical for the analysis of the material supplied by nature to selection. It is considered abundant, but it is only "8 of 32".

The contradiction of selection and constraints seem to be less sharp in recent Darwinian interpretations. The constraints are being examined, and in some special cases are considered as the effective factor of evolution. However such "compromises" concern just isolated instances of empirical studies. In general even the "modernistic" Darwinism follow the traditional view on the evolution wandering through vast space of adaptation instead a moving on rails created by constraints on variation.

6. The concepts on the development of biosphere and/or mankind usually do not descend to the levels of variation and speciation. At the same time the extrapolations of the data on the evolution of species to the upper levels also usually remain unfinished. Moreover they tend to be negatively influenced by anthropocentrism. They often consider man as a special phenomenon, which could control not only its own evolution, but also the evolution of the whole biosphere.

7. The data on ecological history indicates on the spontaneous transformation of nature irrespectively of human desire. Despite the great power of intellect mankind cannot control some global processes. For example, the main tendency in fisheries is overfishing. However even the developed countries cannot do anything to restrict it and to wait a little for the restoration of fish populations. Man exterminates them and then produces fish artificially despite the fact that fish breeding is much more expensive and causes ecological problems.

The expression of such processes is the evolution of technology. Mankind has transformed into the measure for the reproduction and perfection of it.

Conclusion

Evolution takes place spontaneously because of the incapability of organisms to reproduce exact copies of them. The real number of potential variations of organisms is lesser than expected one due to physical and chemical constraints. This means that organisms have a predisposition to vary in certain directions and this very predisposition determines evolution. Specific conditions of environment can hinder some of these directions. Concerning evolution of man and mankind only the directions contributing to the progress of technology are allowed.

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I would like to express my gratitude to Konrad Lorenz Institute for Evolution and Cognition Research, since it provided me ideal conditions for the work at the final stage of this research, to E. Kovalenko for comments and to V. Korogodina who convinced me to submit this paper.

ECOTOXIC EFFECTS IN *PINUS SYLVESTRIS* L.POPULATIONS GROWING IN THE VICINITY OF A RADIOACTIVE WASTE STORAGE FACILITY AND IN THE 30-KM ZONE OF THE CHERNOBYL NPP

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The influence was studied of the regional enterprise for processing and temporary storage of radioactive wastes, "Radon" LWPE (Leningrad region), on plant components of natural ecosystems.

Materials and Methods

Cones and young shoots were collected (in autumn, 1997–2002 and in May, 1998–2002, respectively) from trees of three Scotch pine populations located at the LWPE territory, in the centre of the Sosnovy Bor town, and at 30-km distance from the town in an ecologically safe site chosen considering the wind rose. A similar experiment was carried out earlier in the 30-km Chernobyl NPP zone, where populations of Scots pine within the asphalt-concrete plant (ACP, "Red wood") site and the Cherevach village (rather safe site within the 30-km ChNPP zone) were examined. The population within an ecologically safe area of the Kaluga region was used as a reference. The collected cones were allowed to ripen up to opening. Seeds were germinated in thermostat at 24^0 C in Petri dishes on a filter paper. The seedling roots at the cell stage of first mitosis (10-15 (в опубликованных статьях указано 8-14 мм – несоответствия не должно быть!) mm in length) were fixed in ethanol-acetic acid (3:1). Squashed temporary preparations of seedling root apical meristem and intercalar meristem of young needles were stained with acetic orcein. All anaphase and telophase cells were analyzed in each preparation and aberrations were scored; the aberration spectrum analysis was carried out with separation of chromatid (single) and chromosome (double) bridges and fragments, multipolar mitoses, and also chromosome laggings (genome mutations).

Results and Discussion

Cytogenetic analysis revealed an increased damage level in meristematic cells of reproductive (seeds) (Figure 1) and vegetative (needles) organs of Scotch pine (Pinus sylvestris L.) sampled in the Sosnovy Bor town and at the LWPE territory [1, 2]. The frequencies of cytogenetic disturbances at the 'Radon' LWPE site were, however, well below those in the Scots pine populations from the sub-lethal zone (the ACP site) of the 30-km Chernobyl NPP zone (Figure

1). It should be noted that, while the incidence of cytogenetic damage in the samples from the 30-km Chernobyl NPP zone increased with radiation exposure, the cytogenetic damage found in the seed and needle samples from the 'Radon' LWPE site could not be attributed to the radiation exposure alone.

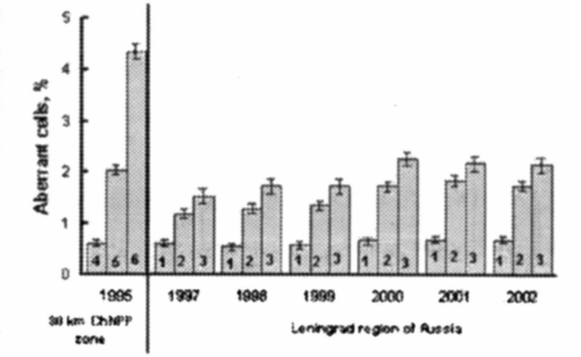

Figure 1.: Aberrant cell frequency in root meristem of *Pinus sylvestris* L. seedlings from the 30 km Chernobyl NPP zone (1995) and Leningrad region of Russia (1997-2002).
1 – Bolshaya Izhora (reference population)
2 - Sosnovy Bor town
3 - 'Radon' LWPE
4 – Obninsk (reference population)
5 – Cherevach
6 - ACP

Additional information on the possible factors affecting the trees may be obtained from an analysis of the mutation spectrum. Mitotic anomalies occurrences in the Scots pine populations of Sosnovy Bor and 'Radon' LWPE sites exceeded corresponding reference levels [2]. At these two sites, cytogenetic damages of rare type, so-called three-polar mitosis, were revealed, while there were no multipolar mitoses as in the reference population (Figure 2) as in the earlier Chernobyl-related study [1]. It allows a conclusion about the presence of significant components of chemical pollution in the Sosnovy Bor town (why?).

The regression analysis indicates that the frequencies of cytogenetic disturbances increase with time in all the tree populations of the Leningrad Region.

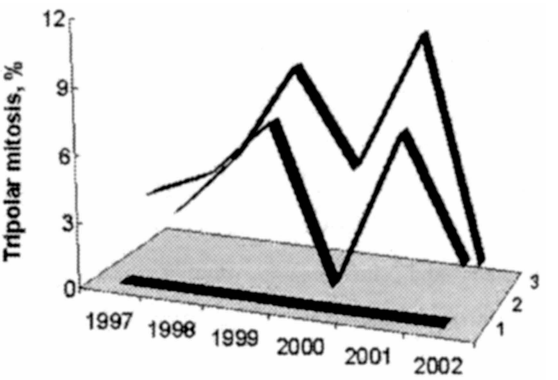

Figure 2. Frequency of tripolar mitoses in seedling root meristem of Scots pine trees growing in Lenigrad region (1997-2002) (per cent of the whole aberrations number).
1 – Bolshaya Izhora (reference population), 2 - Sosnovy Bor town, 3 – 'Radon' LWPE

The seeds sampled in the Leningrad Region were treated with acute γ-irradiation at a dose of 15 Gy. The seeds from the populations of the LWPE site and Sosnovy Bor town were found to be more

radioresistant in comparison to the reference ones (Figure 3). It gives evidence on the presence of a selection directed on increasing in an efficiency of the repair system. Such increased repair efficiency reflects an appearance of processes of genetic structure changes caused by long-term anthropogenic influence.

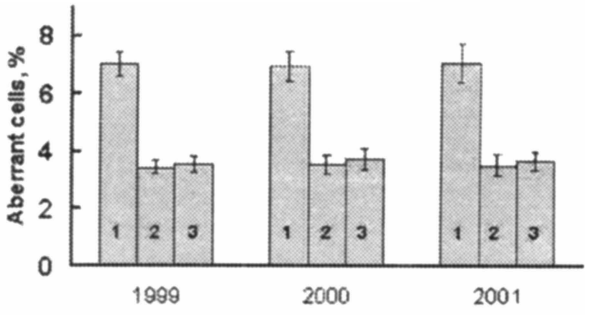

Figure 3. Aberrant cell frequency in root meristem of Scots pine seedlings grown from seeds exposed to an acute γ-ray dose of 15 Gy. 1 – Bolshaya Izhora (reference population), 2 – Sosnovy Bor town, 3 – 'Radon' LWPE

Conclusions

As a whole, our findings demonstrate that an analysis of the frequency and spectrum of cytogenetic disturbances in both reproductive (seeds) and vegetative (needles) structures of Scots pine may be used to quantify the differences in pollution-induced stress not only in areas with prominent damage to the conifers, but also in forests with slight or no visible symptoms of a pollution impact.

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ECOLOGY OF ECTOMYCORRHIZAL ASSOCIATIONS – RESEARCH IN NATURAL CONDITIONS

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Obligate mycotrophysm (fungi-depended nutrition) of the tree-plants from boreal zone is important characteristic its biology and ecology. Actually, in nature, it is not exists really and adapts for environment single plant, but symbiotic systems "plant – ectomycorrhizal fungi". Accedence in a symbiosis amplifies adaptive ability both partners and permits them to play dominate role in forest ecosystems. Mechanism of the interrelation of trees with ectomycorrhizal fungi and different effects this interrelation is intensive studied, but now it is not enough information about way for achievement of symbiotic effect in natural condition.

In this short paper we try to answer next question: which features structures of ectomycorrhiza dynamics, in nature (not in the experiment), can illustrate process of symbiotic system adaptability to changes environment. At list two way of this adaptability is postulated. The first is selection of mycorrhizal fungi species most optimal adaptable for this or another ecotope. The second is directivity and intensively changes in physiological interaction of symbiont (without exchange of fungi species).

Materials and Methods

We have studied reaction ectomycorrhizas on environmental changes with several ecological gradients – range adjoin ecotope (table 1). We studied next parameters of ectomycorrhiza structure. 1. Variety of the mycorrhizal mantles (according T. Dominic's classification in paraphrase of I.A. Selivanov [1]). 2. Abundance ratio of mycorrhizas with mantles of a plectenchimatous, pseudoparenchimatous and unstructured construction. 3. Cross size of ectomycorrhizal tips and size of the individually symbiont in this – root diameter and thickness of fungal mantle.

Two first groups of parameters might be connect with diversity of ectomycorrhizal fungi. Symbiont size changes, in our opinion, might be descry as exterior conspicuity of physiological alternation (changes in volume of resource expended on forming of absorbable organs, tensity of "work" effected this organs).

Table 1. Characterization of the observing ecological gradients

Name of the gradients	Geographical location	Main ecological factor	The investigated arts of trees	Quantity of the trial areas	The investigated period, years
1T	Middle Ural	toxicity of soil (Cu, Zn, Pb, Cd)	*Abies sibirica, Picea obovata, Pinus sylvestris*	4-14	1994-1998
2T	Middle Ural	pollution of atmosphere (SO_2, NO_x)	*Pinus sylvestris*	12	2001
3T	South Subural [a]	pollution of atmosphere (NO_x, SO_2, Cl_2)	*Pinus sylvestris*	3	2002
4N	North Ural	altitude	*Picea obovata*	8	2004
5N	North Ural	changes of soil fertility	*Picea obovata*	6	2004

[a] material is capacitated by G.A. Zaitsev (IB USC RAS).

Results and Discussion

Analyze of our data allows to draw inference about existence of species peculiarity absolute (in micrometer) sizes of ectomycorrhizal tips and each symbiont sizes. The biggest tips is character for *Abies sibirica*, tiny for *Pinus sylvestris*.

Estimated limits on all variety sampling of root-fungi ratio in ectomycorrhizal tips of three tree species is equal. In the one ecotope, middle part of fungal mantle, in the cutting area of ectomycorrhizal tips (or its volume) is not consist less than 16 and more than 30%. Absence of species variety over featured of the comparatively symbiont's input in the common volume of the simbiorgans is interpreted by high ecological (conditionally of concrete biotope) changes of its structures.

Both diversity and sizes ectomycorrhizal tips is changes in the observing ecological gradients (table 2). Situation is predominant: when declined condition of tree existence (simbiosystems) is decreased set diversity of fungi mantles. Interrelation of micorrhizas with different mantles structures in the prenominate gradients change not one-valued. Average thickness of the mantles under "disadvantageous condition" comparison with "advantageous" is decrease in one case and increase in another. Nevertheless, if ecotopes to rank only with feature of its "advantageous" or "disadvantageous" edaphic situation (not considering changes in overground environment of the biotope) we acquire that fungal symbiont always developed better at low soil fertility.

Conclusion

In nature (in the environmental gradients), reaction is observed which attest to avail of both theoretical waiting manners of symbiosystem "fungi – tree" adaptation for changes environment. "Fine tuning" of tree absorbing apparat on actual ecological condition is executed by two modus. First modus: changes in ratio of ectomycorrhizal tips with fungal mantles of different structure. Second modus: changes of symbiont dimension and vitality. Herein, intensification of fungi component significance adjoin with increase of the charge on absorb organs.

Table 2. Parameters of diversity and sizes of the ectomycorrhizal tips in the ecological gradients

Name of the gradients	Conditions		
	favourable	transitive	adverse
	Shanon's index		
1T	1,18-1,98	1,13-1,94	0,99-1,71
2T	1,45-1,97	1,39-1,59	1,15-1,35
3T	1,44	not data	1,08-1,26
4N	1,23-1,61	not data	1,55-1,76
5N	1,70-1,79	1,69-1,79	1,55-1,58
Part of mycorrhyzas with pseudoparenchimatous mantles, %			
1T	17,1-42,7	12,2-50,0	0,0-15,1
2T	47,7	48,6	21,4
3T	47,0	not data	35,0
4N	28,1	not data	43,8
5N	31,6	48,4	56,4
	Average thickness of mantle, μ		
1T [a]	19-33	25-36	27-51
1T [b]	18	18	19
2T	24	24	19
3T	18	not data	16
4N	15	not data	17
5N	17	19	25

[a] *Picea obovata* and *Abies sibirica*;
[a] *Pinus sylvestris*.

Acknowledgements

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