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DNA-Repair, Chromosome Alterations and Chromatin Structure under Environmental Pollutions

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DNA-REPAIR, CHROMOSOME ALTERATIONS AND CHROMATIN STRUCTURE UNDER ENVIRONMENTAL POLLUTIONS

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International Symposium

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SELECTIVE DNA REPAIR IN ACTIVE GENES

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My colleagues and I have discovered intragenomic heterogenicity in the processing of damaged DNA in mammalian cells. The efficiency of removal of pyrimidine dimers has been mapped in and around the active dihydrofolate reductase (DHFR) gene in Chinese hamster ovary (CHO) cells. Repair within the DHFR gene was shown to be much more efficient than that in silent upstream sequences or in the genome overall. In mouse cells we have shown that sequences in the active c-abl protooncogene are repaired much more efficiently than are sequences containing the inactive c-mos protooncogene. Tissue specific and cell specific differences in the coordinate regulation of protooncogene expression and DNA repair may account for corresponding differences in the carcinogenic response. Preferential repair of active genes may account for the fact that rodent cells are as UV resistant as human cells in spite of lower overall repair efficiencies. We also find a dramatic difference in the efficiency of repair in the transcribed versus the nontranscribed DNA strand of the DHFR gene in both CHO and human cells.
It is likely that unrepaired transcription-blocking lesions rather than the blockage of replication forks are responsible for cell lethality. In fact the efficient replicative bypass of certain bulky adducts has been documented. Persisting damge in unexpressed regions and silent genes may result in higher levels of mutation and/or chromosomal alterations in those regions of the genome.

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IONIZING-RADIATION-INDUCED GENETIC DAMAGE TO NATURAL POPULATIONS AND PROCESSES OF ADAPTATION

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The criteria for evaluating the genetic effects of ionizing radiation on flora and fauna have been analysed. It has been found that the damaging effects of radiation are estimated most adequately by the genetic damage criterium, which intergrates the products of the frequencies of induced mutations of all kinds and their relative viability. Methodological problems of evaluating the genetic damage caused by ionizing radiation have been studied taking as examples the natural populations of Arabidopsis thaliana, Androsace septentrionalis and others. It has been shown that on the basis of this criterion the genetic effects of the environmental pollution can be forcasted and the ecological shifts occurring in natural populations can be evaluated.

The damaging effect of ionizing radiation evokes the processes of genetic adaptation to various influencing factors in the exposed natural populations of microorganisms, plants, and animals; these processes may involve different mechanisms and develop in various directions. One of the commonest ways of radioadaptation is the selection of radioresistant forms which are less severely damaged than the initial ones. The increased radioresistance of the populations of herbaceous plants and unicellular algae, exposed to chronic beta-radiation, was shown to be the result of an increased efficiency of their radiation damage repair systems (single-strand breaks repair and an intensity of the nonscheduled synthesis of the DNA). When comparing the radiorsistance of the populations of plants which grow under conditions of chronic beta-, gamma-, and alpha-irradiation, it was found that the selection of radioresistant forms occurs most effectively in the populations, which are chronically exposed to rare-ionizing types of radiation. No forms of plants were found to have an increased resistance to chronic alpha-radiation. The rate of repair processes in such populations is reduced as compared with the control group. From this we can conclude that there are various evolutionary ways in the natural populations exposed to rare- and heavy-ionizing radiation.

INFLUENCE OF GENOTOXIC ENVIRONMENT ON DNA-REPAIR OF HUMAN LYMPHOCYTES AND A CORRELATION BETWEEN CHROMOSOME ABERRATION AND DNA-REPAIR

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In our previous work it was found that in vivo administration of different genotoxic agents increased the DNA-repair in murine lymphocytes. To study the effect of different genotoxic environmental factors on the DNA-repair of human lymphocytes, three populations were examined using a standardized method. An increased DNA-repair was found in 20-30% of persons occupationally exposed to genotoxic environment, in contrast to 9% in the control group. The proportion of this subpopulation was not related to the level of exposition. A decreased repair capacity of lymphocytes was detected in 9% of the control persons while in 10-11% of the population exposed to genotoxic environment depending on the level of exposure. Both phenomena, mentioned above, were independent of the type of genotoxic environment studies in this work. In a population occupationally exposed to Radon-222 a significant but not very close correlation was found between the capacity of DNA-repair and the ratio of the chromosome aberrations and the level of exposure.

CONTINOUS INDUCTION OF UNSCHEDULED DNA SYNTHESIS BY GAMMA IRRADIATION

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The induction of DNA-synthesis in non-S-phase cells is a very sensitive measure of a preceding damage of the DNA. Usually, in an in vivo - in vitro test (treatment of an animal, incorporation of H3-thymidine in a cell suspension) the damaging of DNA takes place hours to days before the evaluation. In this case, the time course of the UDS-induction after a single dose of 1 Gy gamma irradiation should be observed for a long time (21 months). C57 black mice served as test animals. In an age of about 80 days they were irradiated and the induction of unscheduled DNA synthesis was measured at ten points of time during the whole life-span of the animals. Although the repair in this gamma radiation damge in DNA is a very quick process - with centrifugation in alkaline sucrose you find a half time of some minutes - an induction of unscheduled DNA synthesis coudl be seen at the irradiated animals until the end of their life (640 days). The reason for the could be permanent disorders in cellular regulation caused by the gamma irradiation.

RADIATION-INDUCED GENE AMPLIFICATION IN MAMMALIAN CELLS

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Amplification of selective genes occurs physiologically during development and differentiation and can be induced by a wide variety of DNA damaging agents in vivo and in vitro. Amplified DNA sequences give rise to characteristic chromosomal aberrations, the so-called double minute chromosomes (DM`s) and homogeneously staining regions (HSR`s), both frequently found in human tumours.
We are interested in the effect of radiation on oncogene activation in normal and repair deficient cells. According to current ideas amplification of oncogenes themselves or the rearrangement of sequences subsequent to amplification can change the expression of critical genes and lead to the formation of a cancer cell.
We can show that ionizing radiation of various LET (^{60}Co-γ, ^{241}Am-α or the decay of ^{125}I incorporated in the DNA) as well as UV light induce amplification of integrated viral DNA sequences (SV40) and of two oncogenes (Ki-ras and Ha-ras) in SV40-transformed Chinese hamster embryo cells. The induction of amplification is dose and time-dependent and the results suggest that all types of DNA damage (single- and double-strand breaks or base damage) whether they are repairable or not can trigger the amplification process. With respect to human cells repair-deficient skin fibroblasts have been found to be more competent for gene amplification than normal cells under all conditions tested.
At present the mechanism of gene amplification in mammalian cells is not understood. The fact that specific gene amplification can be achieved at low doses of alpha particles and even in unirradiated cells after fusion with irradiated cells indicates a trans-acting mechanism, possibly through a diffusible protein.

CYTOLOGICAL ANALYSIS OF RADIATION-INDUCED GENE AMPLIFICATION IN C57BL/6 MICE

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Inbred mouse strain C57BL/6 pre-treated with or without the drug DADH (N,N`-Diacetyl-1,6-Diaminohexan) by daily feeding for five consecutive days to a total dose of 1 mMol of the drug in aqueous solution, were exposed to an acute dose of 1 Gy gamma radiation. Appropriate controls without radiation were maintained both for water and DADH-fed groups of animals. At 10 and 36 hours post-irradiation the animals from all the four groups (designated as A to D with each group having six animals) were sacrificed and their bone marrows harvested and cultured for chromosomal analysis. Special emphasis was placed on the estimation of the frequency of occurrence of extra chromosomal elements, such as minutes, single or double (DM`s) as a possible assay for radiation-induced gene amplification (presumably representing the proliferation of specified genes causing initiation of a very early tumori-genesis event). Gamma irradiation alone yielded the highest frequency of such minute chromosomal structures concerned (DM`s), which however was significantly reduced by DADH pre-treatment. The data are discussed in the light of current information in the field of gene amplification pertaining to carcinogenic induction.

INDUCIBLE PROCESSES IN DNA REPLICATION AND REPAIR AFTER γ-, UV-IRRADIATION AND ACTION OF SOME CHEMICALS ON MAMMALIAN CELLS

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A restoration of DNA synthesis in mammalian cells after γ-, UV-irradiation and action of some chemicals damaging of DNA structure and/or disturbing of replication can also be an inducible process as many other phenomena. It was suggested that involving of inducible factors in restored mode of DNA synthesis endows the replication with essential characteristics. For checking this suggestions an action of 5-fluordeoxyurdine (FUDR, 10^{-6}M) hydroxyurea (HU, 10^{-3}M), γ- and cycloheximide (CH, 1 mg/ml) (inhibitor of protein synthesis) on DNA replication and repair in HeLa cells was studied.

The agents observed induce in HeLa cells a mode of DNA synthesis which is resistent to γ-, UV-irradiations or heating. This correlates with changes in chromatine structure and perhaps depends on the modification of the latter.

For studying possible inducible characteristics of restored process of DNA synthesis the irradiated cells were incubated with CA (10 mg/ml) or (actinomicyn D (AcD) - 0.05 mg/ml). It was shown that CH and AcD have no influence on irradiation inhibition of DNA synthesis. A rate of ^{3}H-thymidine incorporation reaches quickly an initial level in the cells without CH or AcD, (at 4-5 hours after irradiation). In the presence of CH or AcD restoration of DNA synthesis does not occur. The first γ-irradiation of cells with a dose of 5 Gy stimulates in 4 hours of restoration of DNA synthesis after the second dose of 10 Gy.

An postreplicative DNA repair in UV-irradiated HeLa cells occurs with the high rate after the previous action of FUDR, HU or UV-irradiation. Under this conditions daughter DNA strands have few gaps. In cells of confluent culture action of HU, CH, γ- or UV-rays stimulates DNA replication.

Possible molecular mechanisms of DNA replication and repair and their role in radiation reaction of mammalian cells are discussed.

ENVIRONMENTAL INFLUENCES ON THE SENSITIZATION BY DRUGS MEASURED IN PERIPHERAL BLOOD LYMPHOCYTE CHROMATIN

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We worked out an "allergokinetic test method" by which the transformation within T-lymphocyte subsets due to drugs to which the person is allergic can be measured after a short time of incubation (Balo-Banga and Pfeiffer). This test is based on quantitative microscopic analysis of neutral red-chromatin birefringence in lymphocyte nuclei (Balo-Bang et al., 1980; Racz, Balo-Banga, Vedrova, Kubanova, 1984). The rate of transformation showed a positive correlation in over 500 cases with the extent and severeity of skin manifestations of drug allergies. The sensitizations could be characterized, therefore, by a number (called "score") expressing transformation related to critical drug concentrations. These "scores" were also used to distinguish between drugs that could be made responsible for symptoms and those that could not. In comparison of those values between the capital city, Budapest, of high environmental pollution with Esztergom, a small town near Budapest of low environmental pollution in the period of 1st April 1983 to 1st May 1986 we observed significant differences. There were generally higher "scores" in Budapest with higher frequency of the more severe bullous and generalized manifestations. The comparison of the results (338 tests in Budapest and 185 tests in Esztergom) within the above time interval with those between the 1st May 1986, and the 1st June 1987 has resulted in a relative increase of sensitization as measured by the "scores" in both centres, without any alteration in the rates positive to negative. With no detectable increase in "classical" air-pollutants, these results stress the importance of elevated radioactivity in both centres.

HIERACHY OF GENOME RESISTANCE SYSTEMS TO RADIATION DAMAGE

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Formation of the species-specific radioresistance (RR) is determined by the size and peculiarities of structural organization of genome, as well as by DNA reparation efficiency. Through objective computer classification of RR for 79 unicellular organisms distributed by A. Sparrow into 8 radiotaxa and independent selection consisting of 183 organisms the hierarchical system of cluster resolution was shown to exist as 2 (viruses and pro(eu)caryotes); 3 (viruses, pro- and eucaryotes); and 4 (s-DNA (RNA) viruses, 2s-DNA viruses, haplonts and diplonts) taxa, totally meeting the internal and external criteria of classification quality. Hierachy of radiotaxa directly reflects the structural genome transformations during progressive evolution and is dependent on the basically different possibilities for radiation damage reparation. Radiotaxonomy based on the cell nucleus physical parameters remains effective within the caryotaxon IY as well. Existence of three discrete resolutions in conformity with the plant phylogenesis from the Coniferae-Gymnospermae (radiotaxon III) to Angiosperae. Monocotyledoneae (radiotaxon II) and Dicoetyledoneae (radiotaxon I) has been shown by computer analysis of the higher plants` RR in vegetation (65 species) and as resting seeds (99 species). The evolutionally advanced species are characterized by marked radiopolymorphism. Essentially complete coincidence of the results of radiotaxonomy and significant correlation between the seed and plant RR ($r = 0.91 \pm 0.08$) prove basical similarity of evolutionally developed genetical mechanisms taking part in the RR formation at different stages of ontogenesis. To test the hypothesis of the small taxonomic groups determination by fine structural organization on genetical systems, the factor analysis of electrophoresis spectra of prolamines and isoenzymes of hexaploid wheat cultivars contrast by RR has been carried out, this allowing to select genetically related cultivar groups with similar RR levels, avoiding reference to any radiobiological information.
The results obtained allow to formulate the evolutionary-genetical conception of RR and to take this as a basis for an explanation, from united positions, the inter- and intraspecies organisms differentiation by their resistance to damaging effect of radiation.

REPAIR STUDIES ON METHYLATED DNA OF CHO CELLS

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Modifications of nucleic acids by chemical alkylations modify these macromolecules in that way that their cell functions are influenced or even changed. If not repaired alkylated DNA can induce formation of cancer. The preferred DNA targets for alkylating chemicals are the positions N-7 and O-6 of guanine. Influence of N-Methyl-N-nitrosourea (MNU) causes a relative high degree of O-6-Methylguanine, a product which is in close correlation with formation of cancer.
Formation and repair of different methylated DNA products were investigated in ^{14}C-MNU treated CHO cells. After moderate acidic DNA hydrolysis released purine bases were separated by High Performance Liquid Chromatography (HPLC) and formed N-3-, N-7-, O-6-Methylguanine and N-3-Methyladenine determined by Liquid Scintillation Counting.
Within an incubation period of 2 hours at 37°C in vivo with CHO cells no release of methylated products from DNA was found. Cell pretreatment with 7 rad γ-radiation caused repair stimulation of N-methylated products, especially of N-7-Methylguanine, but no removal of O-6-Methylguanine.

DNA REPAIR AND HUMAN DISEASES

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A survey of lymphocytes taken from the patients with some hereditary diseases (e.g. Marfan`s syndrome, homocystinuria and others), as well as with some diseases which are characterized by a hereditary predisposition (e.g. schizophrenia and gout), has revealed the anomalies of DNA repair under the exposure to 4-NQO, UV-, and gamma radiation. Besides, a survey of lymphocytes among the patients with Xeroderma pigmentosum and healthy donors was carried out. The inhibition of DNA repair was established by the following three methods: 1. Vaccinia virus and induced mutagenesis; 2. determination of breaks in DNA and their resynthesis; 3. unscheduled synthesis of DNA. These cells were also shown to have an increased level of sister chromatid exchanges (SCE). As an additional test, we carried out a study of the ability of human interferons to protect human lymphocytes from the action of mutagens. It had been established earlier that the pretreatment of human lymphocytes with interferon reduces the number of induced SCE, since interferon catalyses the unscheduled synthesis of DNA, as well as the process of the resynthesis of breaks in DNA.

In the lymphocytes taken from the patients affected by some diseases, interferon fails to protect the cells from the action of certain mutagens. This is accounted for the damage to the genes which are responsible for the protective function of inerferon.

Thus, anomalies of DNA were found in patients with some diseases, characterized by a hereditary predisposition. The anomalies of DNA repair conceivably underlie the molecular-genetic mechanism which triggers the development of a number of human dieseases.

PHENOTYPIC COMPLEMENTATION OF XERODERMA PIGMENTOSUM GROUP-A AND GROUP-F CELLS BY MICROCELL-MEDIATED TRANSFER OF DIFFERENT SINGLE HUMAN CHROMOSOMES

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Fibroblasts from patients afflicted with the cancer prone syndrome xeroderma pigmentosum (XP) exhibit sensitivity to UV radiation and a reduced capacity to repair DNA damage. Although the marked UV sensitivity of XP-A and XP-C cells would appear to offer amenability to a gene cloning strategy by phenotypic complementation, transfection experiments utilizing total genomic human DNA from normal cells have failed to reproducibly yield phenotypic complementation of these cells.
We have pursued an alternative strategy for examining the complementation of XP cells, based on microcell-mediated transfer of human chromosomes tagged with a selectable marker. Human chromosomes tagged with the neo gene and maintained in human/mouse hybrids were transferred to XP-A or XP-F cells and recipients were selected for G418 resistance. Transfer of most chromosomes had no effect on the UV sensitivity of XP-A cells, but increased the UV sensitivity of XP-F cells.
Independent human/mouse hybrids have been identified which contain single human chromosomes that complement either XP-A or XP-F cells, but not both. The complementation is observed at high frequency following microcell chromosome transfer, occurs in the absence of detectable rodent DNA transfer, and cosegregates from complemented XP clones with the selectable chromosome (neo)marker. Levels of complementation of UV sensitivity for both XP-A and XP-F cells are substantial, but not fully restored to that of wild-type cells. Quantitative measurements of DNA repair reveal that complemented XP-A clones consist of a mixture of fully complemented and uncomplemented cells, whereas in complemented XP-F clones, all cells demonstrate equivalent, partial levels of complementation. We are presently characterizing the complementing chromosomes in the hybrids to permit chromosome assignment, subchromosomal mapping, and cloning of genes responsible for the phenotypic complementation of XP-A and XP-F cells.

GENOME UNSTABILITY AS A CONSEQUENCE OF DNA-REPARATIVE AND IMMUNE SYSTEM ABNORMALITIES

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The analysis of cell level with cytogenetic abnormalities of 720 normals and individuals with different deviations in organism immunoreactivity has been done in this study. 2.5% normals have an increased level of cells with cytogenetic abnormalities. Further thrice - repeated examination of these patients let "eliminate" sporadic cases of genome unstability from the given group. The remain, 30% cases, had reliable decrease in excisionic reparation, and the rest marked significant changes in immunoreactivity organism state. Only two individuals had simultaneous disturbance of both systems. A great number of examined having ataxia teleangiectasia, Down`s syndrome, schizophrenia, myopathy and other diseases showed the increased level of cytogenetically aberrant cells, simultaneously had decrease of immune and DNA-reparative system activity. Side by side with this, there has been registered the decrease of endogeneous interferon activity produced by T-lymphocytes of patients with schizophrenia.

The data given, in our opinion, indicate of the fact that there is a pre-clinical period preceding the state of "frue" genome unstability characterized by decrease of DNA-reparative system activity or immunosuppression. The disease with a marked state of genome unstability, as a rule, is accompanied by simultaneous disturbance of both immune and DNA-reparative system. The connection between them, perhaps, is realized with the help of interferon as a mediator and interferongenesis disturbance may lead to the development of the marked genome unstability state.

ABILITY OF ADDUCTS FORMED IN A SHUTTLE VECTOR BY REACTIVE METABOLITES OF 1-NITROPYRENE AND BENZO(a)PYRENE TO INDUCE MUTATIONS WHEN THE PLASMID REPLICATES IN HUMAN CELLS

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A shuttle vector, pZ189, containing a gene for ampicillin resistance and the supF gene coding for tyrosine suppressor tRNA, as well as the SV40 origin of replication and T antigen, was reacted with various concentrations (2 µM to 60 µM) of tritium-labeled 1-nitrosopyrene (1-NOP) in the presence of ascorbic acid, or (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,1 tetrahydrobenzo(a)pyrene (BPDE) and allowed to replicate in human cells. With either carcinogen, there was a linear relationship between the concentration of carcinogen used and the number of adducts formed, and a linear decrease in transforming ability as a function of the number of adducts, with 8 adducts per plasmid lowering the transformation efficiency to 37% of the control. There was a linear increase in the frequency of supF gene mutants in the progeny of the carcinogen-treated plasmids following replication in human cells. However, the BPDE-induced adducts, which are formed principally at the N^2 position of guanine, were 4 times more effective at inducing supF mutants than were 1-NOP-induced adducts, which are formed at the C-8 position of guanine. Agarose gel electrophoresis and DNA sequencing analysis showed that the majority of the carcinogen-induced mutations consisted of single base substitutions, GC -> TA. Each agent induced its specific spectrum of "hot spots", but there were two in common. Studies with two other structurally-related polycyclic aromatic carcinogens are in progress. (NIH/NCI CA21253 and Contract 87-2 from Health Effects Institute).

POLY(ADP-RIBOSE)-SYNTHESIS AND EXCISION REPAIR IN LIGHT SENSITIVE SKIN DISORDERS

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Several data suggest a relationship of poly(ADP-ribose) (PAR) - synthesis to DNA repair. Further on it is known that some trace elements have an influence on the semiconservative and unscheduled DNA synthesis (UDS). Previously we found certain alterations in the UV-light induced UDS as well as in the contents of trace elements in the lymphocytes of patients with light sensitive skin disorders. In the recent study PAR synthesis was investigated in correlation to the UDS after UV-irradiation in the lymphocytes derived from patients with polymorphic light eruption, cutaneous porphyrias and xeroderma pigmentosum variant. Parallel with it zinc, copper and manganese contents in the chromatin of the cells were measured by neutron activation analysis. NAD incorporation was generally lower in the cells of light sensitive patients than in those of the controls. Some correlation occurred between the contents of trace elements studied and UDS as well. It seems that PAR investigations throw new light upon our understanding of the pathomechanism of photodermatoses.

THE MODIFICATION OF CHEMICAL MUTAGENESIS IN THE GROUPS OF GENETIC RISK

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The recent investigations of the cytogenetic effects of the new classes of protective substances are conducted on different test-systems, especially with due regard for repair processes. In the lymphocyte cultures from the groups of genetic risk (patients with bronchial asthma, familial mediterranean fever, xeroderma pigmentosum and in healthy donors the protective effect of interferons (IF) and thiol compound WR2721 are investi-gated.

For the first time we use our test-system with the increased interval (46 hrs) between the time of the addition of mutagens, alkylating agents, thiophosphamide and fotrin, and protectors. The considerable protective effect of natural and recombinant leucocitar IF on the chromosomal aberrations is obtained. That effect has cellular nature and is independent from the inter-action of investigated substances. The natural IF did not change the level of SCE but the recombinant IF increased it. In the groups of patients with bronchial asthma and healthy donors there was no significant difference in their action.

In the cultures from xeroderma pigmentosum patients with the defect of postreplicative repair system ("variant") WR2721 has no protective effect, that was registered in the control group. The investigated dosis of mutagens induced the significant increase of one-strand DNA level in cell lysate chromatography (L. Abramian, 1988). The role of repair processes in the protective cytogenetic effect is discussed.

TRICHOTHIODYSTROPHY - A UV-SENSITIVE DISORDER

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Trichothiodystrophy (TTD) is an autosomal recessive disorder characterised by brittle hair with reduced sulphur content, ichthyosis, peculiar face and mental and physical retardation. Some patients are photosensitive. A previous study by Stefanini et al. (Human Genet. 74 (1986) 107-112) showed that cells from 4 patients with TTD had a molecular defect in DNA repair, which was in the same complementation group as xeroderma pigmentosum, group D. We have found a variety of different responses in a detailed molecular and cellular study of the effects of UV light on cells cultured from 4 further unrelated TTD patients. Cells from patient 1 were normal in cell survival, excision repair, DNA and RNA synthesis following UV irradiation, whereas in cells from patient 2 all these responses were similar to those of excision-defective XP cells. In cells from patient 3 cell survival was normal following UV-irradiation, even though repair synthesis was only 50% of normal, and RNA synthesis was severely reduced. In patient 4 excision repair was normal but RNA synthesis was reduced. Our results show that the molecular defect in TTD patients is very heterogeneous. They pose a number of questions about the relationship between the molecular defect in DNA repair and the clinical symptoms of XP and TTD.

THE CHERNOBYL FALLOUT IN SALZBURG/AUSTRIA AND ITS EFFECT ON PERIPHERAL BLOOD CHROMOSOMES

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The province of Salzburg is situated in a region with relatively high deposition of Chernobyl fallout, caused by heavy rain during the critical days. Great many measurements of the environmental radioactivity and the Cs137 and Cs134 content of all kinds of food have been carried out. Furthermore the Cs-burden of several 100 persons has been measured with a whole body-counter. Due to the incorporation of Cs a group of persons received effective whole body doses up to 1.5 mSv.
Inhabitants of a region with elevated natural radioactivity, whose individual annual alpha and gamma radiation burden was assessed, showed a significant rise of chromosome aberration frequencies with dose, at doses beneath 2 mSv. Therefore also the additional radiation burden, given above, due to the incorporated Cs might induce higher chromosome aberrations. Furthermore we have of some persons aberration values of several 1000 mitoses from the time before the accident, which could be taken as comparison.
To get reliable results the radioactive environments of the testpersons will have to be chequed and a great many mitoses must be analysed. This work is going on and the results will be published at the symposium.

THE GENERAL MECHANISM OF CHROMOSOME MUTAGENESIS: THEORETICAL AND APPLIED ASPECTS

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Mutation, perhaps, is one of the most widely used genetic terms uniting all changes in genetic apparatus and phenotype changes inherited stablely. In number of cases the nature and mechanisms of mutation formation is well known. On this base the new terminology, such terms as recombination, transposition, arised for those phenomena belonged to general mutation category before. In connection to consider chromosome aberrations (CA) is interesting in as much as CA is very important quantitative index of eukariotic cell genetic apparaturs disturabance. In literature term "chromsome aberrations" has a wide spread synonym - structural chromosome mutation. By using the method of reproduction we pointed out a considerable part of chromosome breaks, which before belonged to true deletions (double breaks of DNA) by mistake, can reflect processes of chromatin reparation. These "pseudobreaks" being registered in first K-mitosis after influence over cell by different types of mutagens (ionizing radiation, 5-fluorodeoxyuridine, nitrosoethilurea) are able to repair completely in next nuclear cycle and follow that they cannot be identical to true structural mutations. Providing there is no changes of primary DNA structure in the process of chromatin reparation the pseudobreaks have no mutation significance in general. Our results show exchange aberrations to predominate in spectrum of CA after pseudobreaks reparation. Only part of exchanges - symmetrical reciprocal translocations - are able to pass meiosis and in this way it has mutation significance. On this account the problem of formation of exchanges becames an important question of chromosomal mutagenesis theory. Experiment on CA reproduction in C.Capillaris cells answered this questions synonymously: the central stage of exchange formation without reference of mutagen type is mechanism identical to homological recombination on principle. The experimental data proving CA formation takes place as the result of damage of small parts of eukaryotic cell genome are adduced.

SISTER CHROMATID EXCHANGES IN LYMPHOCYTES OF PERSONS EXPOSED TO LOW LEVELS OF IONIZING RADIATION

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In previous studies we observed decreased levels of Mitomycin C inducible SCEs (= sister chromatid exchanges) in lymphocytes of persons occupationally exposed to low levels of radiation. After the Chernobyl accident we had the possibility to investigate SCE frequencies of persons working in Shlobin (USSR) during and after April 1986. Both, spontaneously occurring and Mitomycin C induced SCEs were studied, and the rates in exposed persons compared with those of controls staying in Austria during the respective time. Low radiation exposure caused an significant increase in spontaneous SCEs, and a statistically highly significant decrease in inducibility of SCEs by the alkylating agent. Results will be discussed with respect to an induction of DNA repair by low dose radiation.

SISTER CHROMATID EXCHANGES AND INHIBITION OF DNA SYNTHESIS IN IRRADIATED HUMAN CELLS

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X-ray irradiation inhibits DNA synthesis and enhances frequency of sister chromatid exchanges (SCE) in normal human lymphocytes. On the contrary, cells from patients with Down`s syndrome, Xeroderma pigmentosum (form II) and progeria, characterized by radioresistant DNA synthesis, do not show such increase in SCE frequency. We suggest that radiation-induced increase in SCE frequency is rather a result of inhibition of DNA replication, than a result of direct damage of chromosomes by ionizing radiation. It is in agreement with Painter`s (1980) hypothesis according to that SCE are formed due to asynchronous completion of replication in contiguous replicon clusters. So, probability of SCE formation is more the lower rate of replication. Thus, the extent of radiation damage cannot be measured directly by the SCE frequency.

SCEs AND CHROMOSOME ABERRATIONS IN HUMAN LYMPHOCYTES IN VITRO TREATED WITH QUERCETIN

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Quercetin is one of the most toxic representatives of flavonoid group that is widely distributed in edible plants. The mutagenities of various flavonoids have been extensively studied, but there have been few reports on their genotoxic effects on mammalian cells. Quercetin has mutagenic activities in microbial mutation assay systems (Sugimura et al. 1977). A weak but not relevant increase in the SCE rate was induced by quercetin in V79 Chinese hamster cells (van der Hoeven et al. 1984). Since humans are exposed to quercetin daily, it is important to have more information on its genotoxic effects on mammalian cells. In the present study the effect of quercetin on human lymphocytes in vitro was investigated. Full blood cultures were treated with several concentrations of quercetin (0.5, 2.5, 5, 10, 15 and 20µg/ml). Time of treatment was 3, 24 and 48 hours. This exposure resulted in the increase of the SSE frequency in treated cells. Chromosome aberrations are also being analysed.

COMPARISON OF CYTOGENETIC TESTS FOR MONITORING OVEREXPOSURES FROM IONIZING RADIATIONS

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The search for proper biological indicators of radiation injuries did not result in technique fulfilling all the requirements as yet (1). So far, the dicentric chromosome aberration analysis can be used as the most reliable assay in radiation accidents (2). A more simple cytogenetic technique has come into the limelight of research, i.e. the detection of micronuclei in lymphocytes (3). The consequent dose-effect relationship makes the technique promising (4).
The yield equations of chromosome aberrations, micronuclei in lymphocytes without and with blocking their cytokinesis will be compared with special emphasis on the practical uses of these indicators in cases of accidental overexposures.

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COMPREHENSIVE EVALUATION OF ENVIRONMENTAL AGENTS` MUTAGENICITY BY DNA REPAIR AND CYTOGENETIC DAMAGE

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Any method developed for assessment of genotoxic effects of environmental agents has some limitations and shortcomings. Some years ago the chromosome aberrations were the most widely used. Now the test panel is often supplemented by the estimation of sister chromatid exchanges and DNA repair synthesis. To estimate the mutagenicity of occupational health hazards it is necessary to consider the results of more than one test. The best way is to use the methods for evaluation of mutagen activity, both in vivo and in vitro. Sometimes the results of these 3 tests are dicrepant. That may be connected with either the assessing different sides of the interaction between mutagenic factors with the cell or the existence of significant interin-dividual variability and some peculiarities of dose curves of genetic damage induction. The results of testing several occuptional groups and comparative assessment of mutagen action on human cell culture have been used to substantiate the conclusion that considered methods should not be referred to as interchangeable, but as the complement of each other.

MUTAGENIC ACTIVITY OF SMOKING AND ALCOHOL CONSUMPTION IN MEN

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The abstract did not arrive in time.

DEFECT IN DNA TOPOISOMERASE II IN ATAXIA-TELANGIECTASIA CELLS

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Ataxia-telangiectasia is a heterogeneous genetic disorder characterized by a variety of clinical, cellular and molecular abnormalities. A number of features that include chromosome instability, abnormal DNA rearrangements, defective DNA repair, radioresistant DNA synthesis and cell cycle anomalies can be explained by what has been termed a defect in DNA processing. To date it has not been possible to identify a common biochemical defect in A-T or indeed to understand how a defect in a single gene could explain the "DNA abnormalities" and the other characteristics of this syndrome. Over the last several years a variety of approaches have been employed to determine the biochemical defect. These have included investigations of chromatin structure in A-T cells, transfection of high molecular weight DNA into these cells in an attempt to correct the defect, and more recently the study of factors recognizing chromatin structure. It seems likely that the DNA topoisomerases, enzymes capable of modifying chromatin structure, play important roles in such processes as DNA replication and DNA repair in eukaryotic cells. Accordingly we have determined the activities of DNA topoisomerase type I and II in A-T cells.
DNA topoisomerase type I and II activities were determined by serial dilution in nuclear extracts from control and ataxia-telangiectasia lymphoblastoid cells. Topoisomerase I activity, assayed by relaxation of supercoiled plasmid DNA, was found to be approximately the same in both cell types. In order to remove interference from topoisomerase I, the activity of topoisomerase II was measured by the unknotting of knotted P4 phage DNA in presence of ATP. The activity of topoisomerase II was markedly reduced in two ataxia-telangiectasia cell lines, AT2ABR and AT8ABR, compared to controls. This reduction in activity was detected with increasing concentration of protein and in time course experiments at a single protein concentration. A third cell line, AT33ABR, did not have a detectably lower activity of topoisomerase II when assayed under these conditions. The difference in topoisomerase II activity in the ataxia-telangiectasia cell lines examined may reflect to some extent the heterogeneity observed in this syndrome.

EFFECT OF IONIZING RADIATION ON UNSCHEDULED DNA SYNTHESIS (UDS) IN HUMAN AND MURINE LYMPHOCYTES

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It was found in 1982 in an area of high natural radioactivity in South China that the inhabitants continuously exposed to low level radiation about 3 times as high as usual background showed an increase in UDS in the peripheral blood lymphocytes. Experiments have since then been performed to study the effect of different doses of x- and γ-rays on the level of UDS in mouse splenocytes. Whole body irradiation (WBI) with single doses between 0.5 and 6.0 Gy (dose rate 0.26 Gy per minute) caused marked inhibition of UDS in the splenocytes in a dose-dependent manner with the regression equation of $E=0.89\lambda-0.098D$, $r=-0.954$. Within the same dose range the reduction in number of nucleated cells in the spleen was more marked. WBI with single doses within 250 mGy caused an entirely differnt picture where stimulation of UDS of the splenocytes occurred after 50 mGy. Continuous γ-irradaition at a dose rate of 5.4 mGy/6h/d also caused stimulation with a cumulative dose of 130 mGy. Following WBI with doses between 0.5 and 8.0 Gy the DNA polyermase activity of the splenocytes was inhibited with a very steep slope within 2 Gy above which the doseresponse curve became flattened, suggesting that the decrease of UDS was not in parallel with the inhibition of the total DNA polymerase activity in the cells. Data on relative changes of DNA polymerase α and β following different doses of ionizing radiation will be presented.

MALIGNANT TRANSFORMATION OF HUMAN FIBROBLASTS BY ONCOGENE TRANSFER

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There is no reproducible method for the malignant transformation of human fibroblasts, or in fact, any normal human cells with carcinogens. As a possible model for such transformation process, we have transfected human fibroblasts with various oncogenes. Normal cells transfected with mutant H-ras or N-ras oncogenes became morphologically transformed, produced foci, and exhibited anchorage independence. However, the cells did not form tumors in athymic mice and did not acquire unlimited lifespan. Transfection of H-ras, N-ras or K-ras oncogenes into a near-diploid immortal fibroblast cell line caused similar changes. However, these cells produced malignant tumors in athymic mice. Studies on these transformed cells, as well as human fibrosarcoma-derived cell lines, showed that they grow in serum-free medium containing 0.1 mM calcium without addition of EGF or PDGF. Normal fibroblasts require EGF or PDGF for growth in this medium. These data suggest that for human fibroblasts to become malignant cells, they must acquire some measure of growth factor independence. Immortality is probably also required.
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DNA REPAIR IN CELLS OF C_{57}bl MICE AFTER RADIATION AND IN VIVO TREATMENT WITH A DNA SITE MODULATOR OF POLY(ADP-RIBOSE)-POLYMERASE

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Poly(ADP-ribose) is one of the most important cellular regulatory molecules which can change the chromatinstructure and function. Its synthesis can be modulated by interaction of substances with the DNA binding site of the poly(ADP-ribose)-polymerase. This mechanism is connected with the antitransforming ability of these drugs. In long term experiments with C_{57}bl mice some changes in DNA repair of cells, determined by autoradiographic studies and in the structure of supercoiled DNA measured by nucleoid sedimentation could be found after treatment with a poly(ADP-ribose)-modulator and radiation. Compared to the γ-irradiated group of mice the combined treatment group (modulator and γ-irradiation) showed a lower incidence of lymphomas.

MOLECULAR PROPERTIES OF ADENOSINE DIPHOSPHORIBOSE TRANSFERASE (ADPRT), A DNA-BINDING PROTEIN AND ITS PARTICIPATION IN SIGNAL TRANSDUCTION FROM ENVIRONMENT TO GENE REGULATION

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ADPRT (Mr 116-120,000) is a specific nuclear protein that upon binding to certain DNA structures is converted to an enzyme synthesizing covalently protein bound helical homopolymers of ADPR, with NAD as substrate. The NAD/NADH ratio reflects the environmental metabolic regulation of this enzyme, since NADH is not a substrate. We have identified the polypeptide structure of ADPRT, located DNA and NAD binding domains and specified the DNA structures to which ADPRT binds by foot-printing and gel-electrophoretic methods, that recognize specific structural features. These specific features are: AT rich regions, coinciding with bent, cruciform, and anti Z antibody-binding structures that are also sensitive to S1 nuclease. Specific octadeoxyribonucleotides were synthesized, replacing long DNA sequences for maximal catalytic and binding reactions of ADPRT that occur cooperatively with histones. We propose that the specific structural DNA domains, identified by ADPRT binding, may be either gene initiator or replicative structures, predicting gene regulation by ADPRT under physiologic conditions and anomalies in DNA function following environmental damage.

DNA REPAIR WITHIN THE HIGHLY-ORGANIZED DNA-PROTEIN COMPLEXES

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DNA within the eucaryotic nucleus is packed in association with proteins forming higher order chromatin structures. The super-coiled replicon domains are tightly bound to a proteinaceous skeletal structure of nucleus named nuclear matrix (NM). This intranuclear organization of DNA appears to participate not only in the replication and transcription but also in the repair of damaged DNA. Due to multilevel DNA organization the uniformly distributed DNA damages are repaired with a different efficiency in the divers nucleotide sequences. The higher rate of DNA repair occurs at the DNA fragments tightly bound to the NM as compared with the bulk DNA.

The high level of unscheduled DNA synthesis at the sites of DNA attachment to the NM seems to be due to location of the DNA-repair enzymes at the NM and due to formation of the spatial compartments on it with the optimal condition for DNA-repair. The DNA tightly bound to the NM is enriched in the actively-transcribed and replicated sequences which are repaired preferentially with a higher rate as compared with untranscribed DNA sequences.

There is a reason to propose the participation of specific proteinases in the control of DNA-repair within eucaryotic nuclei. DNA-dependent and specific to H_1-histone proteinase firmly associated with the NM may be involved in such kind of regulation. This proteinase is active only in the presence of denaturated DNA or DNA containing single strand breakes produced by ionizing radiation and DNAse 1. It specifically cleaves DNA-bound proteins and makes possible the accessibility of DNA damage for the repair enzymes.

POLY(ADP-RIBOSE) AND CHROMATIN ORGANIZATION IN DNA EXCISION REPAIR

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De novo poly ADP-ribosylation of chromatin proteins, particularly of the enzyme poly(ADP-ribose)polymerase, is a stereotype response of higher eukaryotes to DNA damage. We have found that DNA damage also induces an up to 680 fold stimulation of poly-(ADP-ribose) catabolism in mammalian chromatin. Using a reconstituted in vitro system with the pure enzyme poly(ADP-ribose)-polymerase, histone H2B, and 5`-end labeled 146 bp core DNA fragments, we obtained evidence that the automodification reaction of the polymerase may operate as a protein shuttle mechanism on DNA templates by providing alternative binding sites for DNA binding proteins. Incubation of nucleosomal core particles with poly(ADP-ribose)polymerase and NAD caused the release of core DNA at reduced electrophoretic separating forces as compared to non ADP-ribosylated controls. Poly(ADP-ribose)-depleted mammalian cells exhibit a deficiency in the formation of free, non-nucleosomally organized domains in the course of DNA excision repair and fail to excise bulky DNA adducts. - Based on these results we propose that the poly ADP-ribosylation system of higher eukaryotes operates as a protein shuttle mechanism on damaged templates which is involved in the local and transient disruption of chromatin organization in DNA excision repair.

DNA REPLICATION AND POLY(ADP-RIBOSYL) ACTION OF CHROMATIN

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The abstract did not arrive in time.

DNA REPAIR DURING NEURINAL DIFFERENTIATION OF A MOUSE TERATOCARCINOMA CELL LINE

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Mouse teratocarcinoma cells (PCC7S) were induced to differentiate to nerve soecific direction by retinoic acid and dibutyryl cyclic AMP. The DNA repair capacity of differentiated and undifferentiated cells were compared after ultraviolet light (UV) and methylnitrosourea (MNU) treatment. It was found that the incision step of excision repair and the removal of 7-methyl guanine is largely diminished in differentiated nerve cells.

INCREASED ADP-RIBOSYLATION OF HISTONES DURING THE PROLIFERATION OF THYMUS CELLS, INDUCED BY TRANSIENT INHIBITION OF PROTEIN SYNTHESIS BY EMETINE IN VIVO

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Intraperitoneal injection of emetine (33 mg/kg) induces in thymus a cell-migration linked oscillation of cell population (Antoni et l.: Int.J.Immunopharmacol. 9, 333-340, 1987). Coincidental with a transient decrease and subsequent rise of cells both DNA and to a lesser extent RNA synthesis exhibit oscillation and especially DNA synthesis overshoots beginning 3 days after emetine administration. The apparent poly ADP-ribose polymerase activity in permeabilized thymus cells parallells the kinetics of oscillation - DNA synthesis, suggesting alterations in chromatin conformation that regulates the availability of ADPR-acceptor sites. The increase in protein ADP ribosylation does not appear to coincide with an increase in poly ADP-ribose polymerase protein contents since gel electrophoresis reveals only marginal changes of ADP-ribosylation of the ADPRT protein (116 kDa). However significant increase in the ADP-ribosylation of histones take place. Since ADP-ribosylation of histones dissociates these DNA binding proteins from DNA sites, process tantamount to "de-repression", our observation indicates a histone ADP-ribosylation induced gene activation in proliferating thymus cells. The gene repressor role of histones has been previously proposed (Weintraub: Cell 38, 17-27, 1984).

ADP-RIBOSYLATION AND HYPERTHERMIA

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ADP-ribosylation represents a kind of posttranslational modification of proteins directly involved in: DNA-repair, organization of chromatin structure, differentiation and transformation of cells.
Metabolism of ADP-ribose in mammalian cells in vitro is rapidly altered following DNA damage. In order to see how hyperthermia (specific environmental stress that alter various intracellular events) affect intracellular level of (ADP-ribose)n, we exposed mammalian cells to different hyperthermic treatment. Results show that hyperthermia change ADP-ribose metabolism in a way partly described in literature. Additionally we found that hyperthermia may induce either relaxation or condensation of the nucleoid structure. Since, the activation of ADPR-transferase demands the generation of DNA strand breaks, we concluded that observed relaxation of the structure may directly be connected to simulataneous stimulation of ADP-ribosylation.

DIFFERENTIATION CONTROL IN CELL CYCLE AND DEVELOPMENT BASED ON THE ALTERATIONS OF HIGHER ORDER CHROMATIN STRUCTURE. THEORY AND APPLICATION TO RADIATION AND CHEMICAL ACTIONS

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The proposed theory based on the interdependence of differentiation state and the state of supermolecular chromatin structure in the cell cycle and development (maturation). The predictions of the theory give an opportunity to account for some experimental facts concerning the alterations of chromatin domains during differentiation and in the cell cycle. Coordinated genes switching is explained by discrete chromatin transitions between free energy levels, specifically that should lead to oscillations of chromatin condensation degree in the nucleus.

One of the predictions deals with the nature of an initial event of malignisation - the aquirement of the ability for unlimited proliferation. The decrease of the mean loop size and of the duration of quiescent state and also the block of differentiation on the initial stages of transformation are forecasted.

In connection with the problem of radiation and chemicals action a number of predictions concerning the dependence of transformation probability on the state of chromatin structure follows from the theory, as well as the dependence of differentiation state, probability of cell death and initiation of transformation on quantity and "quality" of chromatin damages caused by external factors.

USE AND MISUSE OF METABOLIC INHIBITORS IN ANALYSING EUKARYOTIC DNA REPAIR PATHWAYS

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One of the classic ways of studying a biochemical pathway is to apply a specific inhibitor and look for the accumulation of intermediates. This approach has been used with DNA repair; but since no inhibitors specific to repair are known, great care must be taken in interpreting results.

The clearest effect is the accumulation of incomplete repair sites that occurs when cells treated with, for instance, ultraviolet light are incubated with DNA synthesis inhibitors - hydroxyurea (HU) and cytosine arabinoside (araC) or aphidicolin (aph). DNA resynthesis is blocked but incision continues at further sites of damage. AraC and aph inhibit DNA polymerase α by competing with dCTP at its binding state. What does HU do in this system? As an inhibitor of ribonucleotide reductase, it tends to reduce dNTP concentrations. Generally, few breaks accumulate with HU alone after UV, so it seems that resynthesis can take place with a limited supply of dNTPs. HU potentiates the inhibitory effect of araC or aph, perhaps by depleting the dCTP pool; but in hamster cells, with a very high level of dCTP that is not affected by HU, this drug still enhances the effect of the polymerase inhibitor.

On removing inhibitors, artificially accumulated DNA breaks are readily ligated, at a rate many times faster than the initial incision. This ligation is not inhibited by 3-aminobenzamide, so poly ADP-ribose synthesis is not required to activate the ligase in this pathway, even though it is implicated in other kinds of repair.

The DNA synthesis inhibitor novobiocin blocks topoisomerase II. It prevents incision of UV-damaged DNA, abolishing the accumulation of DNA breaks induced by HU and araC. A role for topoisomerase in repair, modulating DNA topology and making damage sites accessible, is attractive. But novobiocin also inhibits ATP metabolism, and its effect on incision may be explained without implicating topoisomerase.

A valuable use of inhibitors is in discriminating between different repair modes employed by the cell. Inhibitor-based assays can contribute to the identification of novel chemicals as potential carcinogens; and they have a role in characterising repair-defective mutant cell lines. Examples of these applications will be described.

SUPERVISION OF CELLULAR RESPONSE TO RADIATION BY HUMAN INTERFERON

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Cells of a human cell strains, with high sensitivity to radiation (X-ray and UV)-killing and low capacity for DNA-repair, when pretreated with human interferon (HuIFN) preparations before irradiation, acquired an enhancement of radiation-induced DNA-repair replication synthesis in association with recovery from inhibition of total cellular DNA-synthesis and radiation-survival. The enhancement and induction effects of HuIFN were observed, irrespective of the kind of HuIFN preparation used (α, β or γ, and natural or recombinant). Furthermore, frequencies of ouabain- or 6-thioguanine-resistant mutation induced by UV decreased. The findings suggest that HuIFN supervises radiation-induced DNA-repair and its-related functions and might confer an error-free state on the radiation-sensitive human cells examined here.

HUMAN INTERFERONS MODIFY DNA REPAIR

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We have found a new biological function of human interferons, namely, their capacity to protect human cells from the action of some physical and chemical mutagens. The following criteria were applied for evaluating the protective effect of interferons: formation of sister chromatid exchanges (SCE), chromosomal aberrations (CA) and breaks in DNA, as well as viability of cells and intensity of DNA repair synthesis.

The pretreatment of cells with a natural interferon decreased the number of SCE and CA, which had been induced by the mutagens of various origin. This is accounted for the ability of interferon to enhance certain phases of DNA repair and, probably, "to stabilize" the structure of DNA.

In the case of photomutagenic action of 8-MOP on human lymphocytes, when only monoadducts, or both monoadducts and interstrand cross-links are formed, the antimutagenic effect of interferon is shown only with respect to interstrand cross-links.

Unlike the natural interferon, the recombinant α_2-interferon failed to have any effect (SCE) on the lymphocytes of clinically healthy donors exposed to gamma-radiation. In repair-deficient cells (Marfan`s syndrome) the protective effect of the natural interferon with respect to 4-NQO and gamma-radiation was found to be significantly reduced, and that of α_2-interferon was not manifested at all.

Thus, the capacity of interferon to alter the repair of DNA conceivably depends both on the type of interferon and on the genome of the cells under study.

EFFECT OF EPIDERMAL GROWTH FACTOR AND INSULIN ON THE KINETICS OF RADIATION-INDUCED DNA LESIONS REPAIR

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Epidermal growth factor (EGF) in the combination with insulin have earlier been shown to increase the rate of γ-ray indused DNA single-strand breaks (SSB) repair in Swiss 3T3 cells in the presence of 10% serum (V.N. Bildin, et al., DAN SSSR, 1987, 292, 220-223). To study the effect of both EGF and insulin in terms of possible regulation of the repair process in cells, the time-course dependence of SSB and DSB repair have been investigated either in the presence of EGF (10 ng/ml) and insulin (1 µg/ml) or without these factor in the medium either complemented with 10% serum or without serum on Swiss 3T6 cell exposed to ionizing radiation at a dose of 5 - 10 Gy using methods of neutral and alkaline elution as well as centrifugation on alkaline sucrose gradients. The absence of serum in the incubation medium during 18 to 24 hours before irradiation results in the sharp decrease in the rate of the repair of both SSB and DSB. When the cells were exposed to EGF and insulin immediately before irradiation the processes were restored to a significant extent. Thus, within 45 min after irradiation at a dose of 200 Gy the proportion on unrepaired SSB in cells incubated in the medium supplemented with 10% serum makes up 12% while that of in the medium without serum makes up 70%, that of exposed to both EGF and insulin does not exceed 30%.

The data suggest that EGF together with insulin, in the absence of other serum components, are involved in the regulation of the repair of radiation-induced DNA lesions.

CYTOGENETIC AND MODIFYING EFFECTS OF CELL METABOLIC INHIBITORS

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It was taken for granted that production of mutations by external factors was always connected with the local damages in DNA. In 1978 N.V. Luchnik coined an idea that the increase of spontaneous level of structural mutations produced by some factors was due to the damage of repair systems rather than to local DNA lesions. In such a case the observed mutations are formed from unrepaired spontaneous lesions of DNA.

The term pseudomutagenesis was proposed to distinguish this phenomenon from the production of mutations by nucleotropic agents. The following pecularities of mutation production by pseudomutagens may be predicted.

1. The frequency of aberrations produced by different pseudomutagens in the same organism is similar. The frequency of aberrations produced by the same pseudomutagen in different organisms may be different.
2. The mutation frequency is the same in broad range of concentrations or doses.
3. A combined treatment with two pseudomutagens produce the same effect as each of them used separately (the lack of additivity).
4. The relative frequency of different types of structural mutations ("aberration spectrum") differs from that produced by "true" mutagens and is sometimes similar to that among "spontaneous" mutations.
5. Pseudomutagens increase the effect of "true" mutagens showing a synergism.

Investigations conducted by the authors using both animal and plant cells showed that all the cell metabolic inhibitors studied were in fact pseudomutagens.

MODIFICATION OF TRANSFORMATION OF C3H 10T1/2 CELLS BY A DIFFERENTIATION INDUCING SUBSTANCE

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Transformation of C3H 10T1/2 cells was induced by 3-methylcholantrene. Treatment with hexamethylene bisacetamide (HMBA), a differentiation inducing and poly(ADP-ribose)-synthesis modifying substance, influences expression of multilayered foci in a treatment schedule dependent manner. Inhibition of transformation occurred only if HMBA was present after the genotoxic damage. After HMBA treatment most of transformed cells showed an end-differentiation like form.

MUTAGENIC DAMAGE FROM RADIATION AND CERTAIN CHEMICAL VIA CLASTOGENIC FACTORS. PROTECTION BY SUPERPEROXIDE DISMUTASE.

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The survival of aerobic organisms in an oxygen environment involves a complicated interplay between the physiological generation of very reactive chemical species called "free radicals", and the ability of the organisms to control their level in the tissues. Disease states, xenobiotics and other environmental stress can overwhelm defense mechanisms and cause cytotoxicity.
Oxygen-derived free radicals such as O_2^- and OH·, induce DNA damage and may therefore play a role in mutagenesis and carcinogenesis. However the action of these free radicals on DNA cannot always be "direct", in particular when they are generated extracellularly. Because of their high reactivity, they cannot reach the target nucleus, and we have to assure the formation of secondary chemical species that can. DNA-damaging material described as chromosome breakage or clastogenic factors (CF) may represent such intermediates. CF are low molecularweight substances (under 10000 DA) and have been observed as an indirect effect of irradiation, in chronic inflammatory diseases and in the hereditary disorders Ataxia telangiectasia and Bloom's syndrome, known for their high risk of cancer and leukemia. They may be generated in vitro by exposure of cells to a source of O_2^-. This can be prevented by superoxide dismutase. Certain chemicals seem to exert their DNA damaging effect also via the formation of CF. The tumor promoter tetradecanoyl phorbolmyristate acetate (TPA) and asbestos fibers are examples herefore. The TPA-induced CF is mutagenic in V79 cells at the HGPRT locus. The formation of mutants can be prevented by pretreatment of the cells with superoxide dismutase.
CF formed in presence of O_2^- and acting via formation of O_2^- may be important intermediates in carcinogenesis and mutagenesis, a process which can be influenced by superoxide dismutase.

CYCLOPHOSPHAMIDE - INDUCED CHROMOSOME ABERRATIONS IN GOLDEN HAMSTERS DECREASED BY Phs LECTIN

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The anticlastogenic activity of mitogen-active susbtance, Phs lectin was studied in vivo on 39 golden hamsters distributed into 6 treatment groups. One of them served as a positive control, and the animals were injected intraperitoneally (i.p.) with 100 mg/kg cyclophosphamide (CP). Three of the experimental groups were treated i.p. with Phs, and the remaining two via stomach tube, 24 h before CP application or simulatenously. Three Phs doses were used - 110, 220 and 440 mcg/100 g. Numerical and structural chromosome aberrations (SCA) in bone-marrow cells were scored 24 h following the EP injection. Distinct antimutagenic effect of i.p. applied Phs in dose 220 mcg 24 h before CP administration was registered. The mean value of the cells with SCA decrease from 15.4% in the control group to 5%, and the total percentage of SCA is diminished from 32.7% to 8.7%. A similar, but not so expressed effect was observed in the groups with oral Phs application.
Possible mechanism of antimutagenic effect of Phs via immune system activation are discussed.

MODULATION OF THE MUTAGENESIS BY TOKOPHEROLS

U. ALEKPEROV

The abstract did not arrive in time.

THE MODERATE REPEAT DNA (MOBILE ELEMENTS) AND HOT SPOTS FOR CHROMOSOME MUTAGENESIS IN DROSOPHILA EUCHROMATIN

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The distribution patterns of the 10 different types of transposable elements and of the about 200 sites of chromosome breakages-rejoinings underlying the heritable autosomal rearrangements (inversions, deletions, translocations, etc.) induced by ionizing radiation throughout the spermiogenesis are being analyzed by in situ hybridisation of ^{3}H-DNA probes and a cytogenetic mapping on giant salivary chromosomes. Thus far, the single and "hot" spots for chromosome breakage-rejoinings have been shown to be found, as a rule, in the interband regions ("anchor" DNA) flanked by mobile elements of strictly definite nature. It is important that the locations of some spots are quite the same for different cell types. It follows that in the eukaryotic genome there exist qualitatively different "anchor-middle repeat DNA" complexes as the specific attachment structures for looped domains which have the topological function in common, but not quite uniform locations. The dynamic component of these complexes have been postulated to determine the unique pattern of chromatin in the nucleus under study, whereas the static one ensures the identical topological arrangement of interphase chromonema in the different cell types of Drosophila.

INFLUENCE OF CHROMATINE STRUCTURAL ORGANIZATION ON NEUTRON AND γ-IRRADIATION CYTOGENETIC EFFECTS

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The change in structural and functional (stationary) state of the chromatine occurring during the cell cycle must influence differently on the formation of primary lesions (PL), induced by various types of ionisation radiations, and responsible for structural chromosomal aberrations and lethal lesions formation. Neutrons, characterized by significant locality of energy release, may cause clusterization of PL, i.e. the great number of single-strand breaks and double-strand breaks of DNA per unit of volume. In rare ionizing radiation clusterization of PL is absent or decreased greatly.

We investigated the chromosome aberrations yield in culture of human lymphocytes in different stages cell cycle after irradiation. The human lymphocytes were irradiated by gamma-rays ^{60}-Co and fast neutrons (mean energy 0.85 MeV) in low and middle-dose range. Linear dependence of cytogenetic effect on the dose in all cycle stages was found to be characteral for neutrons. The character of dose dependences for aberration frequency in gamma-radiation depends on the cell cylce at the moment of irradiation. For example, in irradiation of cells in G_0-stage in the dose range 0.05 - 0.5 Gy plato was observed, in radiation at the beginning of G_1-phase (10 hours after the FGA stimulation) it was significantly smaller - 0.05 to 0.2 Gy, and 27h after FGA stimulation the plato on dose curve was absent.

The experimental data received are discussed from the point of view of differencies repair system activation in cell after change of structural and functional (stationary) state of chromatin, occurring during the cell cycle.

ACTIVITY OF DNA REPAIR SYNTHESIS IN CELLS OF HIGH ORGANISMS WITH ACUTE AND PERSISTENT VIRAL INFECTION

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The investigation results of influence of number infectious viruses - influenza, measles, mumps, herpes on repair capacity of human cells and animals in different types of interaction of virus and cell: acute infection and persistant infection are present.
The experiments were made on interweaving lines of HeLa, L-41, cultures of lymphocytes of peripheral human blood, and also in mice of CBA and C57BI/6 lines.
It was shown that with acute type of infection active reproduction of investigating viruses accompained temporal inhibition of DNA repair synthesis in cells. We found in cellular systems with low reproduction of viruses the similar of genotoxic action of biological agents and mutants of physical or chemical nature. It was observed stable inhibition of repair capacity of cells at prolonged persistence of influenza viruses and herpes in mice organism.

A COMPARATIVE CYTOGENETIC ANALYSIS OF CELL CULTURES WITH IMPERFECT AND ACTIVE REPARATION SYSTEMS CONTAMINATED WITH POLIOVIRUS OF TRIPLE VACCINE BY SABIN

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The analysis of chromosome set and the pathology of the division of lymphocyte in healthy donors and patients with various forms of muscular dystrophies having a decreased activity of excising system of reparation as well as the cultures of hen embrions with imperfect (IFEH) and normal (NFEH) systems of reparation after their contamination with living vaccine for poliomyelitis (LVS) has been carried out. The comparison of the consequences of IFEH and NFEH contamination shows a sharp increse in the number of cells with C-mitosis in the cultures NFEN, with the level almost tenfold as much as the controlled one after the contamination. A simultaneous increase in the anaphase and telophase has also been found. No increase in such pathologies of the division in the contaminated LVS IFEH has been registered. At the same time it should be mentioned that in the intact IFEH and the cultures of T-lymphocyte of the patients with muscular dystrophy we observed a higher level of cytogenetic changes than in the cells with the normal system of reparation. Among the cytogenetic changes the more frequently observed were the cell with chromatid breaks as well as premature divergence of chromatids in the centromere.
We are inclined to explain the distinctions in the level of cytogenetic changes in the cultures with imperfect and normal systems of reparation by the presence of imperfect virus particles in poliovaccine which are unable to induce cytogenetic changes with the normal system of reparation and thus make their contribution to the mutagenictiy potential of the vaccine.

SISTER CHROMATID EXCHANGE DYNAMICS AS A MIRROR OF REPAIR

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Sister chromatid exchange (SCE) formation reflects the appearance of primary DNA damage. Examination of SCE formation may give some information about repair of DNA damage. Such an examination may be realized by several ways.

1. Investigation of the lifetime of DNA damage realising into SCE in subsequent cell generations. The essence of such investigation is analysis of SCE formed in the 1st, 2nd and 3rd subsequent cell cycles after pulse treatment with genotoxic compound. This may be achieved by using a number of methods:
   (I) SCE analysis in the endoreduplicated cells;
   (II) employment of different schemes of cell treatment and fixation times;
   (III) analysis of SCE in three-way differential stained M3 metaphases;
   (VI) analysis of reciprocal and nonreciprocal SCE in M3.
   The most preferable methods are (III) and (IV) because they make it possible to discerne DNA damage induced in the 1st cell cycle by genotoxic compound and DNA damage induced in the subsequent cell cycles by its metabolites.
2. Investigation of dynamics of SCE appearance and cancelation at different fixation times, and the analysis of liquid holding recovery effects on the DNA damage.
3. Investigation of the elevation or reduction of genome stability to the damaging action of genotoxic factors after treatment with various modulators of cell metabolism.

All above mentioned principles are substantiated by the experimental material obtained in the laboratory of Ecological Genetics.

MODIFICATION IN G_2-PHASE OF THE REALIZATION OF CHROMOSOMAL ABERRATIONS OF HUMAN LYMPHOCYTES INDUCED BY IRRADIATION IN G_0-PHASE

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The prupose was to study the ability of 5-fluorodesoxyuridine (5-FUDR) and adenosine diphosphate (ADP) to modify the radiation lesions of chromosomes. Human lymphocytes were irradiated (1 Gy) in G_0-phase of the cell cycle and after 48h cultivation with FGA they were treated for 2 hours with 5-FUDR (10^{-7}M) or ADP (10^{-4}M). Then the number and type of chromosomal aberrations were determined. It was shown that the treatment of non-irradiated lymphocytes in G_2-phase with 5-FUDR or ADP induced only chromatide type of aberrations. With 5-FUDR the same treatment in G_2-phase of lymphocytes that had been irradiated in G_0-phase did not influence chromosomal types of aberrations, but with ADP the same treatment reduced the number of chromosomal types of aberrations. The data obtained indicate the presence of long-living potential damages in the genome of irradiated cells. The probability of their realization into chromosomal aberrations can be reduced by ADP action on G_2-phase of the cell cycle.

CORRELATION BETWEEN RADIORESISTANCE OF CHRONICALLY IRRADIATED POPULATIONS OF HIGHER AND LOWER PLANTS AND DNA REPAIR

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The selection of radioresistant forms with more efficient repair system of single-strand breaks in DNA was found to occur in chronically irradiated populations of Chlorella vulgaris. There is a correlation between efficiency of repair and radioresitance in algae strains.

It has been shown that populations of Vicia cracca, which have been growing for a long time under exposure to chronic alpha- and beta-radiation, differ in their radioresistance from the control group. In the case of beta-irradiation the increased radioresistance correlates with more intensive DNA repair synthesis, but it does not depend on the effectiveness of the formation and repair of single-strand breaks in DNA. The gamma-induced repair synthesis of DNA was found to be normally implemented by β-like DNA polymerase, whilst in radioresistant population it also involves DNA polymerase α. Unlike the control group, this synthesis is partially inducible. The intensity of the repair synthesis is directly proportional to the dose rate of chronic beta-irradiation.

The populations of V.cracca growing under conditions of chronic alpha-irradiation habe become more radioresistant than the control group. The effectiveness of the repair of gamma-induced breaks in DNA does not differ from that in the control, and the experimental populations. The intensity of DNA repair synthesis in radiosensitive populations decreases after exposure to high doses of additional alpha-radiation. The observed effects are already plainly seen after exposures to relatively low dose rates of chronic alpha-radiation. This, evidently, proves the increased genetic risk of chronic alpha-irradiation.

Thus, various microevolutionary events occurring in chronically alpha- and beta-irradiated populations of plants are, conceivably, connected various combinations of primary damages of DNA, having different repair capacities. The adaptation of plant populations to chronical irradiation is manifested only rare-ionising irradiation. This depends on a more efficient functioning of their repair systems of radiation-induced DNA damage.

DISTRIBUTION OF POSTREPLICATION INDUCED BREAKS OF DNA IN DIFFERENT GROUPS OF CHROMOSOMES

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During prolonged action of small doses of some agents which are polluting the environment, chromosomal breaks can be induced by: 1. interaction of above mentioned agents with and 2. as we suggest by evolutionarity acquisited reaction of a cell, which has exhausted its adaptational and compensational potential, "searching" for new genetic variants, which are adopted to the changed environment. The activation of this programme is achieved by chromatine transition in a new stationar state. This hypothesis is proved by preposition known from general pathology: small doses (as it was shown for mutagens) cause "preserval" effect to the following more strong action of pathogen factors. The question appears if the influence of chemical agents upon interphase cells in different groups of chromosomes causes an equal quantitiy of potential DNA breaks. The human lymphocytes and Djungarian hamster fibroblasts were treated by N-methyl-N-nitrosourea. Then the cells were brought to metaphase, then metaphase chromosomes were extracted, which were fractionated according to their sizes. The quantitiy of DNA single breaks was determined. In both cell types the larger quantity of postreplication single strand breaks was found in the DNA of small chromosomes. But larger quantity of reparation radioactive label (^{3}H-thymidine) for DNA mass unite was found in the chromosomes with bigger size. This fact indicates that larger quantity of single DNA breaks in small chromosomes is conditioned by difficult reparation of their DNA. It may be so because of bigger specific gravity of heterochromatine sites in small chromosomes. Acquired data are discussed in the sence of analysis of genetic consequence of cell adaptation to different unfavourable conditions of environment.

REPAIR OF DNA, CONTAINING URACIL, IN MAMMALIAN CELLS

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Uracil might appear in DNA either via spontaneous (heat-induced) deamination of cytosine residues or via incorporation of dUMP during replication. In the absence of repair deamination of cytosine results in GC->AT transitions. The enzyme uracil-DNA glycosylase was found which is able to release uracil residues from DNA and decrease the rate of spontaneous mutagenesis. Uracil-DNA glycosylase also recognizes uracil, incorporated during replication, although this process is not mutagenic. It is known that stimulation of dUMP incorporation or decreased efficiency of DNA-uracil repair may increase mutagenesis, recombination and chromosome aberrations, but biological role of "replicative" uracil and its repair in normal cells remain unclear. In this report the data will be discussed, which suggest that incorporation and excision of uracil residues serves as a mechanism of polarization of mismatch correction.

LYMPHOCYTES OF PEOPLE OCCUPATIONALLY EXPOSED TO HEAVY METALS POSESS REDUCED REPAIR ACTIVITY

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A survey of 19 workers occupationally exposed to Co and Mo for not less than 10 years was made both for the criterion of formation of breaks in DNA, induced by a corresponding metal and by reference-mutagen, 4-NQO, and for the indices of reactivation and mutagenesis of Vaccinia virus.
Most of the workers exposed to Co and Mo were shown to have a relative resistance of lymphocytes to the formation of 4-NQO-induced breaks in DNA, as well as a reduced ability for their resynthesis. These data correlate with the indices of the virus reactivation, which also appeared to be increased under the exposure to 4-NQO, as compared with the lymphocytes of healthy donors. In addition, the lymphocytes of the workers have an increased level of spontaneous mutagenesis of the virus, which may be accounted for the impairments of the excision repair. Thus, the data have been obtained on the inhibition of the excision repair in lymphocytes of the workers occupationally exposed to metals, although their cells appeared to have a relative resistance to mutagens. It can be inferred that the ways of repair of DNA damage caused by 4-NQO and the metals under study are generally similar.

STUDIES OF THE RADIATION-INDUCED CHROMOSOMAL ABNORMALITIES IN SISTER CELLS: A NEW REGULARITIES OF REALIZATION OF CHROMOSOME STRUCTURAL ALTERATIONS

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Cytogenetic analysis of sister cells from populations affected with gamma- and UV-rays allowed to discover some new regularities of realization of sister chromatid exchanges (SCEs) and chromo-some aberrations.

Chinese hamster cells synchronized at G_0-stage and labelled with 5-bromodeoxyuridine for three cycles were affected with UV-irradiation (254 nm) or ^{60}Co gamma-rays two cycles before fixation. The sister cells were revealed in the slides fixed in situ.

A new type of abnormality related to persistent chromosomal alterations (PCAs) was revealed in sister cells as pair of abnormal SCEs. PCAs being identified as abnormal SCEs were found to occur spontaneously at relatively high rate and PCA frequency was increased three fold in average after UV-irradiation (10 J/m^2). The ordinary method of PCA estimation based on comparison of spontaneous and induced SCE levels did not detect any induction of PCA.

Cytogenetic analysis of sister cells resulted in a detection of a new mechanism of aberration formation. It was found that except aberrations capable to reproduce their structure after replication (dicentrics, rings and fragments) the aberrations with another type of reproduction were present. After replication the fragments with cross-linked complimentary chains gave rise to the structures of symmetric (palindromic) architecture. The frequency of these fragments is directly proportional to the dose of UV- and gamma-radiation.

It is suggested to investigate the yield of abnormally replicating structures after irradiation of cells at different stages of the cycle.

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