Modeling of Alpha-Particles' Epigenetic Effects in Short-Term Test on *Drosophila melanogaster*

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***Abstract*—**In recent years, interest in ecogenetic and biomedical problems related to the effects on the population of radon and its daughter decay products has increased significantly. Of particular interest is the assessment of the consequence of irradiation at hazardous radon areas, which includes the Almaty region due to the large number of tectonic faults that enhance radon emanation. In connection with the foregoing, the purpose of this work was to study the genetic effects of exposure to supernormal radon doses on the alpha-radiation model. Irradiation does not affect the growth of the cell, but rather its ability to differentiate. In addition, irradiation can lead to somatic mutations, morphoses and modifications. These damages most likely occur from changes in the composition of the substances of the cell. Such changes are epigenetic since they affect the regulatory processes of ontogenesis. Variability in the expression of regulatory genes refers to conditional mutations that modify the formation of signs of intraspecific similarity. Characteristic features of these conditional mutations are the dominant type of their manifestation, phenotypic asymmetry and their instability in the generations. Currently, the terms "morphosis" and "modification" are used to describe epigenetic variability, which are maintained in *Drosophila melanogaster* cultures using linkaged X- chromosomes, and the mutant X-chromosome is transmitted along the paternal line. In this paper, we investigated the epigenetic effects of alpha particles, whose source in nature is mainly radon and its daughter decay products. In the experiment, an isotope of plutonium-238 (Pu^{238}), generating radiation with an energy of about 5500 eV, was used as a source of alpha particles. In an experiment in the first generation (F_1), deformities or morphoses were found, which can be called "radiation syndromes" or mutations, the manifestation of which is similar to the pleiotropic action of genes. The proportion of morphoses in the experiment was 1.8%, and in control 0.4%. In this experiment, the morphoses in the flies of the first and second generation looked like black spots, or melanomas on different parts of the imago body; "generalized" melanomas; curled, curved wings; shortened wing; bubble on one wing; absence of one wing, deformation of thorax, interruption and violation of tergite patterns, disruption of distribution of ocular facets and bristles; absence of pigmentation of the second and third legs. Statistical analysis by the Chi-square method showed the reliability of the difference in experiment and control at $P \leq 0.01$. On the basis of this, it can be considered that alpha particles, which in the environment are mainly generated by radon and its isotopes, have a mutagenic effect that manifests itself, mainly in the formation of morphoses or deformities.

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I. Introduction

ALMATY is a city with the highest natural radiation in the Kazakhstan, is which rich in such natural resources as minerals, metal ores and natural gas and oil reserves. Kazakhstan has 12% of the world's uranium resources and may be exposed to a variety of hazardous materials including radon [18], a radioactive gas occurring naturally as an indirect decay product of uranium. Radon gets out of the earth surface through five tectonic faults crossing the Almaty city territory. Radon and its decay products are sources of α-radiation - a stream of heavy positively charged particles [1]. In nature, alpha particles occur as a result decay of heavy elements atoms, such as uranium, radium and thorium. Emanation (a release of radon into the air pores) happens when the radium decay took place near the soil surface and it is mainly carried out by recoil energy produced by a radon nucleus in the process of radium nucleus disintegration.

According to estimates of the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) the population receives more than 75% of the annual individual radiation dose from radon and its isotopes [2]. Radon and its decay products emissions are supposed to be dangerous for living organisms and can cause oncological diseases in humans. In human body, radon facilitates some processes also leading to lung cancer. The decay of radon nuclei and its daughter isotopes in the lung tissue causes a micro-burn, as the whole alpha particles' energy is absorbed at its decay point. Combination of radon and smoking is especially hazardous and increases the disease risk. According to the US Department of Health, radon had regarded to be the second factor (after smoking) that causes lung cancer, mostly, of bronchogenic (central) type. Lung cancer caused by radon irradiation is the sixth most frequent reason causing death from cancer [3]. Radon radionuclides cause more than a half radiation dose, which a human body receives from natural and technogenic environmental radionuclides [1], [4]. For this reason, the aim of present work is to study the mutagenic activity of α-radiation using the *Drosophila melanogaster* test-system based on method of linkaged X-chromosome.

II. Materials and Methods

The plutonium isotope – Pu^{238} isotope was used as source of α-radiation. Alpha-rays are one of the ionizing radiation types performing a stream of rapidly moving, positively charged particles (alpha-particles). The main source of this radiation is the radioactive isotopes and daughter products of a natural radon gas. One of the peculiarities of alpha-radiation is its low penetrating power. The penetrating power of alpha particles in a substance (the path along which ionization occurs) is very short (hundreds of millimeter in biological media, 2.5-8 cm in air). However, along a short path, alpha particles create a great number of ions. That provides a relative biological efficiency, 10 times greater than when exposing the X-ray and gamma radiation.

Testing of α-radiation genetic activity was carried out using the fruit fly *Drosophila melanogaster*. So, some tests based on incidence of different mutations types have been developed for drosophila. The processes occurring in the *Drosophila melanogaster* are extremely interesting for the community of researches engaged in developmental genetics [5]. This fly is chosen as an object in the variety genetic schemes, as it is one of highly researched and well characterized higher organism in genetics. Approximately 2/3 of genes that are responsible for a human disease are homologous to genes in *Drosophila melanogaster* genome. The main biochemical processes in *Drosophila melanogaster* and mammalian cells are identical. In addition, advantages of *Drosophila melanogaster* include the fact that it has a metabolism similar to human and similar enzyme systems involved in digestion. This makes it possible to find invisible mutagens, which acquire genotoxicity in metabolism process. Tests based on *Drosophila melanogaster* are recommended by WHO for studying the mutagenic and toxic activity of anthropogenic xenobiotics and pharmacological agents [6].

The method of linkaged X-chromosomes based on *Drosophila melanogaster* was used to determine the mutagenic effects of α-radiation. The inheritance of concatenated, or linkaged, X-chromosomes was first described by Möller in the study of the mutagenic effects of X-rays (γ-radiation) [19]. The test system of linkaged X-chromosomes can be used to detect visible recessive, sex-linked mutations in the first generation in males, or to detect mutations with non-autonomous manifestation, called conditional. The method is based on the fact that the linkaged X-chromosomes are always transmitted together, since they are connected by a centromere. In females with coupled X chromosomes, the Y chromosome is also present in the genotype, which they receive from the male. When such females ($\widehat{XX}Y$) are crossed with normal males (XY) in the next generation, females ($\widehat{XX}Y$) and males (XY) receiving one X chromosome from the father, and the Y chromosome from the maternal are born [7].

Somatic recombination is the exchange of genetic material between the homologous chromosomes of somatic cells in mitosis, which leads to the formation of mosaic individuals [8]. The purpose of this method is the complex detection of mutations induced in somatic cells of drosophila.

If the mutagen-treated males are crossed with females, then all mutations that occur in the X-chromosome of the males are transmitted to the descendant males, therefore, all recessive mutations occur in the first generation of hemizygous males. Consequently, the mutation rate is defined as the ratio of males' number that has mutations to the total number of males studied in the first generation.

In the experiment, two *Drosophila melanogaster* test stocks were used: PE-2 and Oregon. The PE-2 stock was created at the Institute of Molecular and Cellular Biology of the Siberian Branch of the Russian Academy of Sciences (IMCB SB RAS) and was kindly provided to us. Phenotypic markers of the PE-2 stock are *y – yellow* body, *v – vermillion* eyes in females and *y – yellow* body, v^+ - red (normal) eyes in males. The Oregon is wild-type *Drosophila melanogaster* stock. The PE-2 stock has the «gene position effect», it means a change in gene activity depending on its position in the genome, the probability of which increases with the presence of linked sex chromosomes in the genome. A. Sturtevant discovered this phenomenon in 1925 [20], and in 1935, Dubinin and Sidorov noticed that with the effect of the position the gene is not lost, but only its state changes [9]. The so-called conditional mutations (morphoses) are also associated with the gene position effect, because such mutations more often damage not the structural, but the regulatory elements of the genome responsible for transcription, replication, compaction and other important processes of regulation and implementation of genetic information [10].

The cultures of the genetic lines and all crosses were kept and propagated on a standard medium [11].

III. Results and Their Discussion

The method of linkaged X-chromosomes and XY-chromosomes was used to assess the mutagenic activity of radon and its daughter decay products in *Drosophila melanogaster*. In the experiment, we used the PE-2 stock females with chromosomal rearrangements crossed with males of Oregon stock irradiated by isotope Pu^{238}.

Cultivation of flies, selection and crossing were carried out at a temperature of approximately 22 °C. Females and males older than 5 days were not used in the experiment, since old individuals accumulate spontaneous mutations in the genetic material in the process of vital activity, which can distort the obtained data. One irradiated male and two virgin females were placed in each tube. Irradiated males were crossed with virgin females of the PE-2 stock according to Fig. 1.

Every culture of first generation (F_1) is analyzed visually for revealing morphological mutations after flying-out. The formation of morphoses is one of the properties of conditional mutations that are not associated with the primary structure of DNA and occur in regulatory genes responsible for the formation of signs of intraspecific similarity [12]. Morphoses are non-hereditary morphological disorders (deformities), which are formed as a result of exposure to stressful environmental factors on the body. In our case, α-radiation was a stress factor.

In the first generation (F_1), morphoses of the wings, eyes, body, thorax, antennas, as well as the formation of melanomas in different parts of the body were detected (Fig. 2). These morphological changes indicate mutagenic and carcinogenic activity of radon and its radioactive daughter products.

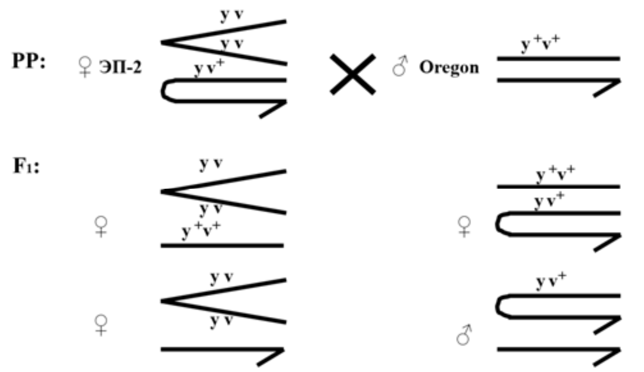

Fig. 1 The scheme of conducting a short-term genotoxicity test using the *Drosophila melanogaster* PE-2 stock containing linkaged X-chromosomes and XY-chromosomes and Oregon-R stock

Fig. 2 Morphoses detected in first generation *Drosophila melanogaster* of the EP-2 test-system: a) morphoses combination - extreme mutant wing phenotype (without a wing), deformation of the head, thorax and abdomen; b) black plaques on a body (melanoma); c) group of small spots (melanomas); d) melanoma and abnormal tergite pattern; e) moderate mutant wing phenotype (improperly outspreaded wing); f) melanoma

Analysis of the imago with morphoses showed that they have practically no effect on the flies' life activity: they do not interfere with their existence, mating, and even giving birth. In the descendants of the irradiated ones in the next generations (F_2 and F_3), various deformities can often be observed, and all that morphoses astonish with their diversity and depth of disorders that do not affect the viability and breeding of the flies. Under the usual conditions for the cultivation of fruit flies in the absence of stress factors, experimenters may also encounter morphoses, but this happens very rarely [13].

As can be seen in Fig. 2, morphoses can form on any part of the body, or at the same time on different parts. There are two major classes of morphoses: "tissue +" and "tissue –" [14], [15]. "Tissue +" is a variety of tissue neoplasms: dark spots (or melanomas) resembling necrotic spots, blisters on the wings and on the abdomen. "Tissue –" is the absence of a part of tissue or organs: absence of a wing, facets of the eye or the whole eye, absence of a leg. As noted by researchers and observed in our experiments, asymmetry is a common feature of morphoses [15]. The formation of morphoses in the first generation (F_1) did not depend on the sex of the flies, which is explained by the disruption of the work of the regulatory genes of autosomal chromosomes as a result of α-irradiation.

The frequency of occurrence of morphoses was estimated to evaluate the possible genotoxic effect of α-radiation on individual development of flies (Table I).

TABLE I
FREQUENCY OF MORPHOSES IN FLIES IRRADIATED WITH α-PARTICLES AND UNIRRADIATED FLIES

Index / Sample	Number of flies analyzed (absolute)	Absolute frequency of morphoses	Relative frequency of morphoses, %
With α-radiation (expirement)	1873	33	1.8
Without α-radiation (control)	1792	7	0.39

Visual analysis was accompanied by counting the number of flies without mutations - *a*, *c*, and with conditional mutations (morphoses) - *b*, *d*. Comparison of the results in the experiment and control was performed by the Chi-square method with the amendment of Yates [16]. The calculations were performed according to (1):

$$\chi^2 = \frac{\left(|ad-bc|\frac{N}{2}\right)^2 N}{(a+b)(c+d)(a+c)(b+d)} \tag{1}$$

where *a, c* – flies without mutations in the experiment; *b, d* – flies with mutations in the experiment; *N* – the total number of flies.

The summarized results and results of the statistical analysis are presented in Table II.

TABLE II
EXPERIMENT AND CONTROL RESULTS IN THE 2X2 TABLE

Experiment	*a* (the number of flies without morphoses)	*b* (the number of flies with morphoses)	∑
	1840	33	1873
Control	*c* (the number of flies without morphoses)	*d* (the number of flies with morphoses)	∑
	1785	7	1792
∑	3625	40	3665

A statistical experimental data processing in the method of linkaged X-chromosomes and XY-chromosomes showed that $\chi^2_{exp} = 14.7$, a $\chi^2_{table} = 6.6$ at $k = 1$ and $P \leq 0.01$. Therefore at $P \leq 0.01$ $\chi^2_{exp} > \chi^2_{table}$. For this reason, we can affirm that alpha-radiation possesses a mutagenic effect. Therefore, we reject the null hypothesis that the induction of morphoses does not depend on α-radiation [17].

IV. CONCLUSION

For determination the genotoxic effect of radon and its daughter decay products, we used test-system of *Drosophila melanogaster* with linkaged X-chromosomes and XY-chromosomes. The genotoxicity of α-radiation manifested itself in the formation of conditional mutations that relate to mutations with the gene position effect, that is, their manifestation depends on a change on gene position in the genome. They affect precisely the regulatory elements of the genome, which are responsible for important regulatory processes, the implementation of genetic information and for the formation of signs of intraspecific similarity.

According to the obtained results, a statistically significant difference in the frequency of morphoses induced in the X-chromosome of male *Drosophila melanogaster* of Oregon stock upon alpha irradiation was revealed. The non-parametric Chi-square test demonstrated that the distribution of frequencies in experiment and control is statistically different at a 99% probability level. Thus, when Drosophila is irradiated with alpha rays, their genotoxic activity is manifested.

In this study, the genotoxic effect of α-radiation of radon and its decay products on the development of the test-system with linkaged X-chromosomes linkaged XY-chromosomes of *Drosophila melanogaster* was studied. Morphological manifestations of α-radiation genotoxicity were revealed: morphoses or deformity of the wings, eyes, abdomen, chest, antennas and melanomas or pigmented body parts. These results indicate mutagenic and carcinogenic activity of radon and its daughter decay products.

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