British Journal of Environmental Sciences

Vol.3, No.2, pp.1-11, August 2015

Published by European Centre for Research Training and Development UK (www.eajournals.org)

POLYMORPHISM OF LIPOAMID-DEHYDROGENASE (DIAPHORASE) IN NATURAL POPULATIONS OF *HELIX POMATIA* LINNAEUS, 1758 (GASTROPODA, STYLOMMATOPHORA) AND *CALLIPTAMUS ITALICUS* (LINNAEUS, 1758) (INSECTA, ORTHOPTERA) UNDER CHRONIC EXPOSURE TO LOW DOSES OF RADIATION

Doichinov Al., D. Bachvarova, Ts. Ignatova-Ivanova*

Konstantin Preslavsky University of Shumen, 115, Universitetska Str., 9712 Shumen, Bulgaria.

ABSTRACT: *The effect of chronic exposure to low doses of radiation on the isoenzyme profile of diaphorase in individuals of natural populations of Helix pomatia (L.) and Calliptamus italicus (L.), inhabiting a territory with increased levels of radiation caused by continuous contamination with technogenic radionuclides has been studied. In individuals of the same species from regions with normal natural radioactive background there have been registered three isoforms of the gene-enzyme system DIA1, DIA2 and DIA3 distinguished by their electrophoretic mobility. This isoenzyme profile was retained in the various tissues and organs of the organism and was not dependent on the sex or the age of the individuals, which shows the high degree of conservatism of the enzyme system. Lack of DIA3 in the isoenzyme spectrum of diaphorase has been registered in representatives of the populations of H. pomatia (L.) and C. italicus (L.) exposed to chronic effective doses of radiation within the range of 0,84 - 3,36 mSv. In a single whole body exposure to radiation in the laboratory and receipt of equivalent dose of γ-rays of the order of 3,5 mSv there was a total loss of the activity of DIA3 which, however, did not change the activity of the other two fractions of the system. When the absorbed dose was increased to 6,23 mSv after irradiation with fast neutrons, the activity of all fractions of DIA2 was eliminated which, however, did not influence the activity of DIA1. These changes in the ratio between the isoforms were dependent on the dose of radiation. The change in the isoenzyme spectrum of diaphorase at the time of exposure to low doses of radiation has most probably an adaptive character associated with the provision of energy to the body in terms of acidosis and increasing its resistance to the chronic effects of radiation.*

KEYWORDS: Diaphorase, γ-rays, Radio-resistance, Chronic radiation.

INTRODUCTION

The continuous expansion of the applications of ionizing radiation in industry and medicine and the contamination of the biosphere with technogenic radionuclides imposes the necessity of a realistic assessment of the consequences from the increase of the natural radioactive background and the influence of small doses of radiation on large groups of people and natural communities in vast regions of the planet.

Contemporary science has sound knowledge of the effects of acute high doses radiation exposure on the bio-molecules, cells and organs in the body, but it does not have sufficient data or theoretic ideas on the effects of increased technogenic radiation as well as on the influence of prolonged exposure to low doses of radiation on the natural communities of organisms [16, 27]. The patterns associated with the clinically exhibited symptoms and

irregularities occurring at the time of acute radiation sickness are both inadequate and insufficient to account for the damage caused by low doses of radiation, therefore, principally new approaches and mechanisms should be offered [29]. The consequences of chronic radiation exposure include radically different ways of radiation effects on the single organism as well as on the populations of species; they also include new mechanisms of change in the cell metabolism and in the functioning of biotic communities. The majority of lesions in the biological systems caused by low doses irradiation are not induced directly by radiation itself, but are the indirect consequence of violation in the regulation systems, the mechanisms of repair and restoration, changes in the immune and antioxidant status of the organism or increased sensitivity to the other factors of environment [4, 6, 20]. It has turned out that the damage done to chromosomes and the malignant cell transformation caused by exposure to low doses of radiation are about an order of magnitude more potent than could be expected from the extrapolation of the effects of acute irradiation with high levels of radiation [15, 28]. This necessitates a more serious study of the mechanisms of influence of this new ecological factor on the biota at all levels of biological organization of the systems.

The effect of prolonged radioactive exposure to low doses of radiation does not cause a clinically definite picture which makes the changes in the organism difficult to register [14]. To this goal, during the last few years the heterogeneity of proteins and more specifically the polymorphism of gene-enzyme systems have been used very frequently as they have turned out to be a very sensitive marker for induced change at the genetic level.

The aim of this paper was to study the effect of chronic exposure to low doses of radiation on the polymorphism of lipoamide dehydrogenase (diaphorase) in individuals of natural populations of *H. pomatia* (L.) and *C. italicus* (L.) permanently inhabiting a region with increased technogenic radioactive background.

The isoforms of diaphorase have been studied in some species of the genus Drosophila, representatives of *H. pomatia*, some mammals, as well as in humans [8, 13, 19, 22)]. Diaphorase (lipoamide dehydrogenase) is an enzyme of the group of oxidoreductases – 1.6.4.3., which catalyze the reaction of recovery of lipoic acid in amide-dehydro-lipoic acid. It actively participates in the processes of exchange of energy and respiration of the cell by balancing anabolic and catabolic processes at cellular and organism level by regulating the speed of reversible oxidization of NAD.N. and/or NADPH.N. The enzyme is comprised of one polypeptide chain and has three isomorphic forms which makes it a suitable object of research on the influence and changes at genetic level.

MATERIAL AND METHODS

Region of research: The study covers a territory of 206 dka in total, situated 10 km south of Burgas, Southern Bulgaria, with geographic coordinates: E - 42° 45′ 071″; N - 27° 60′ 226″. The area is a sandy beach located in Vromos bay nearby cape Atia in the syncline of Burgas (Fig. 1).

Fig. 1. Map and location of the region of study

The region is characterized with a strong influence of Mediterranean climate which governs dry and hot summers and mild and wet winters. There is no permanent snow cover and the cumulative rainfall is about 700 mm/m^2 per year.

The biota is highly influenced by the Mediterranean climate and is comprised mostly of thermophilic individuals which determine its sub-Mediterranean character. The flora of the studied region is very poor represented by few species with low population numbers – *Eryngium campestre* Linnaeus, 1753, Angiosperms, Apiales; *Hordeum sp*., Angiosperms, Poales; *Raphanus raphanistrum* Linnaeus, 1753, Angiosperms, Brassicales; *Ammophila arenaria* (L.) Link Angiosperms. The low floristic diversity is crucial to the distribution, species composition, and the number of populations of animals, which include various representatives of the Pontic and Mediterranean elements – *Lumbricus terrestris* Linnaeus, 1758, Oligochaeta, Haplotaxida; *Helix pomatia* Linnaeus, 1758, Gastropoda, Stylommatophora; *Balanus balanus* (Linnaeus, 1758), Maxillopoda, Sessilia; *Tettigonia caudata* (Charpentier, 1842), Insecta, Orthoptera; *Calliptamus italicus* (Linnaeus, 1758), Insecta, Orthoptera; *Gryllus campestris* Linnaeus, 1758, Insecta, Orthoptera; *Rana ridibunda* Pallas, 1771, Amphibia, Anura, etc.

For decades as a result of copper mining and disposal of tailings from the copper mine Rosen, Vromos bay has been radioactively contaminated with various α-, β- and γ-emitting radionuclides in such quantities that they have caused an increase in the effective dose from 0,84 to 3,36 mSv. A territory identical in bio-geographic aspect, located in the aquatory of the town of Tsarevo (Nestinarka beach) was used as a marker of reference. This territory is located 70 km south of the town of Burgas. It has not been subjected to similar anthropogenic impact and is characterized with effective dose of radiation within the range of 0,22 to 0,24 mSv.

The animals used for a single exposure to γ-rays and neutron in the laboratory were individuals from natural populations of *H. pomatia* (L.), inhabiting the region of the town of Shumen. This area differs from the territory of research in soil-climatic, geo-morphological, as well as biotic respect but it is characterized by a radioactive background which is average for the country with effective dose of radiation from 0,14 to 0,18 mSv.

Methods of research: The counter for low α- and β-activity NA 6201, "Tesla" has been used to measure low background α- and β-activity in samples of the sand in both the researched and the referent territories. The total γ-activity in various parts of the biotope has been defined on the spot by an X-ray-gamma dosimeter of the type VAJ 27 040. The type of radio-nuclides and the content of chemical elements in the sand were established through an X-ray fluorescence elemental analysis [30]. ^{60}Co with activity of 5 mCi and two main lines – the first one with energy of γ-quant of 1137,23 keV and the second one with energy of 1332,51 keV were used for the irradiation of the animals with γ-rays. In order to obtain a higher dose for the same period of exposure the animals are irradiated with quick neutrons. They cause the same biological effects, however, their quality factor is ten times higher than that of γ-rays. Pu-Be source of the type IBN 8-5 with activity of radionuclide ^{238}Pu not more than 2,4.10^{11} Bq and a stream of fast neutrons at an angle 4 $\pi\frac{sr}{r}$ - 1,07.10^{7} n/s was used to irradiate the animals under research with fast neutrons.

The isoenzymes of the diaphorase were divided electro-phoretically in 13% starch gel at 10 V/sm for 4 hours at 4°C in gel buffer - 0,028 M Tris - 0,003 M citric acid, pH 8,6 and electrode buffer - 0,3 M H_3BO_3 - NaOH, pH 7,6. The isoforms were visualized after the method of Hope and Vincent [7] (with some fluctuations) for 2 hours at 37^{0}C in 0,05 M phosphate buffer, pH 7,4, including: NADN, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium Bromide (MTT) and 2,6-dichlorophenolindophenol (2,6-DPP). Enzyme activity was measured spectrophotometrically at 600 nm through reduction registration of 2,6-DPP in 0,2 M phosphate buffer, pH 7,4, including: NADN, 2,6-DPP and enzyme extract.

RESULTS

During the conducted radiological research it was found out that as a result of technogenic contamination the area of research is characterized with increased levels of the effective dose of radioactive rays, at the rate of 3,5 up to 14 times more than the normal amount for the southern part of the Black Sea in Bulgaria. In the researched area there were registered increased levels of radio-nuclides among which the content of ^{238}U, ^{232}Th, ^{214}Bi, ^{214}Pb, ^{137}Cs and other elements exceeded by two orders the one of the referent territory. (Fig. 2).

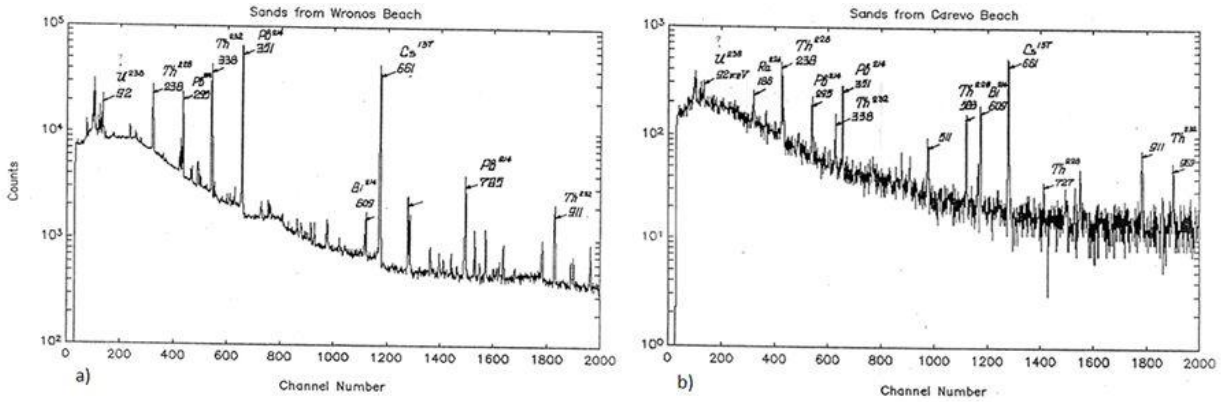

Fig. 2. Signature X-ray-fluorescence analysis of sand from
a) Vromos beach and b) Nestinarka beach, the town of Tsarevo

Therefore in the formation of the higher levels of radioactivity the main role is attributed to the daughter products – ^{214}Bi and ^{214}Pb acquired at the division of the radioactive families of ^{238}U and ^{232}Th which together with Cs, K, Sr, As, Mo, Zn and Se are the main reason for the considerably high γ-activity on Vromos beach compared to the one on Nestinarka beach. The patch-like mosaic character of the radioactive contamination is due to the specific horizontal distribution of radionuclides, their vertical stratification in the layer of sand as well as to the inner irradiation at the expense of radionuclides discharged by the seabed through the waves which enter the human body through the air, food, and water.

Research on the isoforms of the diaphorase locus in members of natural populations of *H. pomatia* (L.) and *C. italicus* (L.), inhabiting the controlled territory has distinguished three main (DIA1, DIA2, and DIA3) and in the case of DIA2 and DIA3 some additional fractions whose distribution in the various tissues and organs of the organism proved to be similar (Fig. 3). The three main bands of the electropherogram were most probably the result of genes coding the main multiple molecular forms of the enzyme, while the minor fractions are the result of epigenetic (post-transcriptional and post translational) modifications.

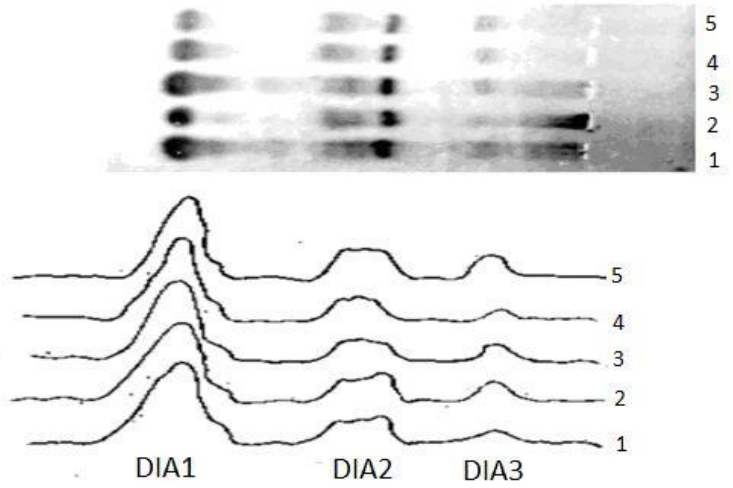

Fig. 3. Isoenzyme spectrum of diaphorase of *Helix pomatia* (L.) and *Calliptamus italicus* (L.) from the controlled territory (Nestinarka beach, the town of Tsarevo)

Starts: 1 - diaphorase of *C. itallicus* (♂); 2 - diaphorase of *C. itallicus* (♀); 3- diaphorase of *C. itallicus* (subad); 4 - diaphorase of hepatopancreas from *H. pomatia*; 5 - diaphorase of heart from *H. pomatia*

Diaphorase 1 in all species subject to this research was the isoenzyme with highest activity represented through an intensively staining band and in all probabilities it was the main and predominant form in the organism. Diaphorase 2 was represented through one main well staining fraction along with some additional fractions with lower activity. Diaphorase 3 was the isoenzyme with lowest anode mobility and relatively lowest activity. In the examined species it was exhibited as a thin band whose intensity of staining varies widely.

In respect to its composition DIA1 was the most homogeneous isoform of the enzyme, while DIA2 and DIA3 showed some structural heterogeneity. It is namely the latter that could

explain the small differences in the total relative diaphorase activity of the separate organs in the studied individuals (Table 1).

Table 1. Relative diaphorase activity of an extract from various organs of *H. pomatia*

Source	µM 2,6-DPP min/mg protein
Leg	12,80 ± 0,03
Hepatopancreas	11,05 ± 0,012
Kidney	10,95 ± 0,01
Lungs	8,02 ± 0,02
Heart	10,36 ± 0,01

The research on the isoenzyme spectrum of diaphorase of populations of *C. itallicus* and *H. Pomatia* permanently inhabiting Vromos beach showed differences which distinguished it significantly from the isoenzyme spectrum of the same species from the controlled territory. (Fig. 4).

Fig. 4. Polymorphism of diaphorase of *C. itallicus* and *H. Pomatia* inhabiting Vromos beach
1 – reference; 2 – *C. itallicus* from Vromos beach; 3 – *H. pomatia* Vromos beach

Only two of the multiple molecular forms of the gene-enzyme system – DIA1 and DIA2 were registered in the isoenzyme spectrum of diaphorase, while the third isoform – DIA3 was not indicated at all. The changes in the composition of the isoforms of the diaphorase locus at prolonged chronic exposure to low doses of radioactive radiation were identical for the two types studied (Fig. 4).

Individuals from the population of *H. Pomatia* from the region of Shumen were exposed to radiation with γ-rays and fast neutrons in order to clarify the genesis of the changes in the isoenzyme spectrum of diaphorase. As a result, identical changes in the complex of the diaphorase gene-enzyme system were established (Fig. 5). The absorption of an equivalent dose of the order of 3,5 mSv caused a total loss of the activity of DIA3 which had not affected the activity of the other two fractions of the system. Elimination of the activity of all fractions of DIA2 was observed after an increase of the absorbed dose to 6,23 mSv after radiation with fast neutrons; the activity of DIA1 was not affected (Fig. 5).

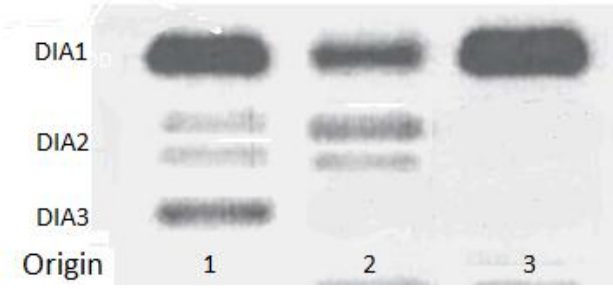

Fig. 5. Isoenzyme spectrum of diaphorase of *H. Pomatia* irradiated with different doses of γ-rays and fast neutrons

1 – after irradiation with a dose of 1,75 mSv; 2 – after irradiation with a dose of 3,5 mSv; 3 – after irradiation with a dose of 6,23 mSv

The obtained results showed that the molecule of the diaphorase is a monomorphic protein which consists of one subunit. Successive discreet elimination of the isoenzymes of the system confirmed that each of the three isoforms of the enzyme was coded by a single gene, each with its own system of regulation. Discontinuance of the synthesis of some of the multiple forms of the enzyme and the retention of others in case of outer influences confirmed their different significance for the organism. Functions were predominantly carried out by diaphorase 1 which was the main and most important isoform of the gene-enzyme system, while the elimination of diaphorase 3 and diaphorase 2 in all probability did not lead to considerable loss of functions and disruption of metabolism. It can be assumed that in evolution terms the multiple molecular forms of diaphorase did not appear simultaneously and that the first one was the gene of DIA1 which was the ancestor of the system and in the course of evolution had initiated the genes of DIA2 and DIA3.

DISCUSSION

The results obtained within the course of this research showed that chronic irradiation with low doses of radioactive radiation caused changes in the activity of the genes coding the multiple molecular forms of diaphorase. This lead to the elimination of some allele fractions and to a change in the isoenzyme profile of the gene-enzyme system. A loss of the activity of DIA3 was registered in individuals from the natural populations of *H. pomatia* (L.) and *C. italicus* (L.), permanently inhabiting the region of research; this, however, did not affect the quality and activity of the other two isoforms of the enzyme.

It is possible that the observed changes in the isoenzyme profile of diaphorase were connected with the adaptation of organisms through broadening of the range of their individual rate of response to prolonged radiation exposure and with the increase in the radio-resistance of the population. Or, it could also be an impairment causing imbalance in the metabolic pathways and distortion of functions leading to negative consequences. If the discontinuation of the DIA3 isoform synthesis of the enzyme is an adaptive change it is expected that it would be secured and maintained in the population from various forms of the team and that that will increase its radio-resistance and its successful adaptation to the specific conditions of the environment. In both cases, however, this genetic polymorphism of

the diaphorase, governed by the multiple alleles of its genes predetermines the potential diversity of the morphological and physiological properties of the organisms within the population and its heterogeneity [1].

During a single whole-body irradiation in the laboratory of *H. pomatia* (L.) individuals from populations inhabiting a region with normal natural radioactive background (the town of Shumen) it has been determined that the degree of alterations in the isoenzyme profile of diaphorase depended on the dose of radioactive radiation. Irradiation of organisms with a dose of 3,5 mSv caused loss of the activity of DIA3 and an increase in the dose to 6,23 mSv caused loss in the activity of DIA2 as well. The functions of the organism in these conditions were ensured only by DIA1 which is NAD.N. and NADPH.N. dependent and was the isoenzyme form of the diaphorase system which was dominant in quantity and functional activity. These changes in the structure of the isoenzymes of diaphorase were the result of the blocking of the activity of Dia-2 and Dia-3 genes which synthesize the polypeptide chains of the two fractions – DIA2 and DIA3. Suspension in the expression of both genes leads to changes in the ratio of anabolic and catabolic processes in the cell. Diaphorase 2 and 3 are NADPH dependent and their main function is to provide energy for some synthetic processes through transfer of reducing equivalents to NADPH. The elimination of their activity will transfer energy to the reduction of NAD and the production of NAD.N. which is the main substrate in the respiratory chain of mitochondria.

It is known that the first reaction of the organism to the effect of chronic exposure to low doses of radiation which exceed by tens the natural radioactive background is reduction in the interchange of gases as well as in oxygen consumption [10, 12, 17, 21]. This "oxygen effect" alleviates the negative consequences from the radioactive radiation by restricting the formation of active oxygen radicals and other reactive oxygen types whose interaction with the cellular structure can lead to various alterations and damages in the structure, as well as to the death of the cell [5, 18]. Reduction in the quantity of oxygen and its derivatives in the cells leads to reduction of the damage caused by ionizing radiation. There is an increase in the dose (from 954 R to 1153 R) causing $LD_{50/30}$ to rats and in 33 days of adaptation to the conditions of low partial pressure of oxygen the semi-lethal dose is reached at even bigger irradiation (1317 R). In addition, there is an increase in life expectancy, reduction in the mortality rate, improvement in the blood and overall state of the body and the results remain constant regardless of the type of hypoxia [24].

In case of deficiency of oxygen in the tissues, however, the main source of energy is the glycolysis whose low effectiveness could hardly suffice for the energy needs of the body. In these conditions the body tries to redirect the main part of the reducing equivalents to the respiratory chain of mitochondria in order to increase the synthesis of ATP [26]. This could happen through change in the ratio and activity of the multiple molecular forms of the enzymes which participate in the bio-energetic functions of the cell, which is actually possible through the isoforms of the diaphorase.

SELINA et al. [23] have proven that when the NAD.N. dehydrogenase of the respiratory chain in mitochondria is blocked as a result of oxidative stress the synthesis of DIA1 increases which in itself shunts the transfer of electrons in the affected spot by transferring the reducing equivalents directly to cytochrome-c reductase restoring the oxidative phosphorylation and the synthesis of ATP.

Therefore, most probably, the changes in the composition of the multiple molecular forms of diaphorase registered in natural populations exposed to chronic radiation is an adaptive reaction aimed at an increase in the intensity of the oxidative phosphorylation and energy synthesis which is a prerequisite for the successful adaptation of the body when in a state of radioactive contamination of its environment.

Apart from energy provision to the organism in cases of exposure to low doses of radiation, diaphorase 1 fulfils other functions as well. It is considered a part of the enzymes which are responsible for the struggle of the cell with oxidative stress. That enzyme group possesses a wide range of activities responsible for the utilization of xenobiotics as well as for the stabilization of some pro-apoptotic factors [2, 3]. It has been established that the expression of the NADN dependent diaphorase (DIA1) increases substantially in response to oxidative stress and the introduction of xenobiotics in the environment [23]. Therefore, the elimination of two of the isoforms in the isoenzyme spectrum of diaphorase and the preservation of diaphorase 1 is an adaptive reaction having to do not only with energy provision to the organism but also with the successful struggle of the cell against the forming reactive oxygen compounds and increase in its radio-resistance in conditions of chronic exposure to low doses of radiation [11].

Said dynamics of the diaphorase enzyme system is very fast and has no latency period, it is not dependent on the individual peculiarities of the organisms but occurs simultaneously in all individuals of the population and depends on the dose of radioactive emission.

Diaphorase is not the only gene-enzyme system which reacts to changes in its isoenzyme profile as a result of chronic exposure to low doses of radiation. Studies conducted by the authors [9] on natural populations of *C. italicus* inhabiting Vromos beach and exposed to the influence of the same levels of radioactivity as described above, have shown inversion in the ratio and quantity of the isoenzymes of lactatdehydrogenase. In conditions of prolonged exposure to doses of 0,84 to 3,36 mSv/h there was an increase in the relative share of isoforms of M-type (HM_3 and M_4) which are predominant in tissues with prominent anaerobic exchange and which function in state of hypoxia, while in the representatives from populations inhabiting the controlled territory – Nestinarka beach, the isoenzyme spectrum is with clearly defined aerobic type with predominance of H_3M and H_4 fractions which operate in bigger content of oxygen.

The changes registered in this study in animals from the radioactive beach Vromos is confirmed by an array of other research on the state of genetic indices in rodents from the area of Chernobyl nuclear power plant disaster [25].

In this sense radio-sensitivity or radio-resistance of biological systems is above all a cellular problem, as the effects of radioactive emissions are mostly connected with impairment in some structures and functions of the cell. In all probability impairment of the integrated bio-chemical reactions can alter the life-functions of whole tissues, organs and systems and thereby determine the overall reaction of the organism towards radiation.

Therefore, prolonged radioactive exposure to low doses of radiation causes changes in the gene activity of the genotype of the separate individuals and readjustment of the bio-chemical and bio-physical functions assuring the individuals' successful adaptation to the conditions of the environment. Clarification of the mechanisms of this adaptation requires an in-depth study of the changes in the rate of reaction of the separate organisms, the functioning of the reparative processes, change in the sensitivity to the other ecological factors which are of

non-radiation nature, as well as in the mechanisms of adaptation of the population in the conditions of chronic exposure to low doses of radiation.

References:

[1] Altuhov Y. P. (2003) Genetic processes in populations. M.: Academkniga 431.
[2] Asher G., Lotem J., Cohen B., Sachs L., Shaul Y. (2001) Regulation of p53 stability and p53- dependent apoptosis by NADH quinone oxidoreductase 1 // Proc. Natl. Acad. Sci. U S A—V 98 1188–1193.
[3] Asher G., Lotem J., Kama R., Sachs L., Shaul Y. (2002) NQO1 stabilizes p53 through a distinct pathway //Proc. Natl. Acad. Sci. U S A —V 993099–3104.
[4] Burkart W. (1999) Damage pattern as a function of radiation quality and other factors (Caractéristiques deslésions provoquées dans les structures cellulaires, en fonction de la qualité du rayonnement et des autres facteurs), Comptes Rendus de l'Académie des Sciences - Series III - Sciences de la Vie 322 (2-3) 89-101.
[5] Cao Z., Tsang M., Zhao H., Li Y. (2003) Induction of endogenous antioxidants and phase 2 enzymes by alpha-lipoic acid in rat cardiac H9C2 cells: protection against oxidative injury // Biochem. Biophys. Res. Commun 310 979–985.
[6] Daniels R. D. (2011) A meta-analysis of leukaemia risk from protracted exposure to low-dose gamma radiation [archive], Occupational and Environmental Medicine 68 457-464
[7] Hope V.T., Vinsent S.R. (1989) Hisochem. Cytochem 37 653–661.
[8] Doichinov A. S., Ralchev K. H., Ivanova N. (1987) Sub-cellular localization of isomorphs of diaphorase in *D. virilis*, Annual of HIP, book Biology 36-43
[9] Doychinov, A, D. Bachvarova, D. Kamenov. (2002) Contamination of radionuclides and their influence on the lactatdehydrogenetic gene-enzyme system in Caliptamus italicus. // 11th International Symposium "Ecology-2002",Bourgas, Bulgaria 138-143.
[10] GarcIa , J., Kimeldorf, D. J., Koeling, R. A. (1955) Science 122 (3160) 157-158.
[11] Kargin V. I., Motovilov K. A., Vuysokih M. Y., Yaguzhinskiy L. S. (2008) Interaction of positively charged analogue of ubiquinone (MitoQ10) with DT-diaphorase from liver mitochondria // Biological membranes 25 34-40.
[12] Kimeldorf, D. J., Garcia, J., Rubadeau, D. O., Radiat. Res. (1960) 12 (6) 710-718.
[13] Koistinaho J., Swanson R. A., De Vente J., Sagar S. M. (1993) NADPH diaphorase (nitric oxide synthase)-reactive amacrine cells of rabbit retina: putative target cells and stimulation by light. Neuroscience 57 587–597.
[14] Kudyasheva A. G., Shishkina L. N., Shevchenko O. G., Bashluykova L. A., Zagorskaya N. G. (2004) Biological effects of radioactive contamination in populations of rodents. Ekateringburg: UrO RAN 214.
[15] Mothersill Seymour. (2005) Radiation-induced bystander effects: Relevance for radiation protection of human and non-human biota Radioprotection 40 (3), 297 - 306.
[16] Oghiso Yoichi, Satoshi Tanaka, Ignacia B. Tanaka III, Fumiaki Sato. (2008) Experimental studies on the biological effects of low-dose-rate and low-dose radiation, International Journal of Low Radiation 5 (1).
[17] Overall, J. E., Brown, W. L., Logie, L. C., Brit. J. Radiol. (1959) 32 (378) 411-414.
[18] Purdom-Dickinson S. E., Lin Y., Dedek M., Morrissy S., Johnson J., Chen Q. M. (2007) Induction of antioxidant and detoxification response by oxidants in

cardiomyocytes: evidence from gene expression profiling and activation of Nrf2 transcription factor // J. Mol. Cell Cardiol 42 159–176.
[19] Ralchev K. H., Doichinov A. S., Dunkov B., Simeonovska M. (1987) Electrophoretic spectrum of diaphorase in D. virilis, Genetics and selection, annual, Sofia 20 (3) 196-202.
[20] Rannou A., Gourmelon P. (2009) Eléments de réflexion sur le risque sanitaire posé par le tritium, Livre blanc sur le tritium, Autorité de sûreté nucléaire.
[21] Robergs RA, Ghiasvand F, Parker D. (2004) "Biochemistry of exercise-induced metabolic acidosis.". Am J Physiol Regul Integr Comp Physiol 287 (3): R502–16. doi:10.1152/ajpregu.00114.2004. PMID 15308499
[22] Sandell J. H. (1985) NADPH diaphorase cells in the mammalian inner retina. J. Comp. Neurol 238 466–472.
[23] Selina L. V., K. A. Motovilov, L. S. Yaguzhinskiy. K. I. Agladze. (2013) Restoration of the contractile activity of the primary culture of rat cardiomyocytes, mitochondrial inhibitors suppressed NADH-dehydrogenase, under the influence of hydrophilic quinones, MIPT Studies 5 (1) 129-139
[24] Shishkina L. N., Kudyasheva A. G., Zagorskaya N. G., Shevchenko O. G. (2005) Indices of antioxidant status in tissues of offspring of Tundra voles (Microtus oeconomus Pall.) from regions with elevated natural radioactivity // Radiation biology. Radioecology 45 (4) 474-479.
[25] Shishkina L. N., Kudyasheva A. G., Zagorskaya N. G., Shevchenko O. G., Taskaev A. I. (2011) Participation of lipid peroxidation in the mechanism of adaptation of rodents to the radioactive contamination zone around the Chernobyl nuclear power plant // Radiation biology. Radioecology 51 (1) 185-200.
[26] Taegtmeyer H., Golfman L., Sharma S., Razeghi P., van Arsdall M. (2004) Linking gene expression to function: metabolic flexibility in the normal and diseased heart // Ann. N Y Acad. Sci.1015 202–213.
[27] Tanooka H. (2001) Threshold dose-response in radiation carcinogenesis: an approach from chronic beta-irradiation experiments and a review of non-tumor doses. Int J Radiat Biol 77 541-551
[28] Tubiana Maurice, André Aurengo. (2004) La relation dose-effet et l'estimation des effets cancérogènes des faibles doses de rayonnements ionisants, Rapport à l'Académie nationale de médecine.
[29] Wakeford R. T. , E. Janet. (2010) The meaning of low dose and low dose-rate (Editorial) [archive], Journal of Radiological Protection 30 (1)1-3.
[30] Yanminchev V. D., Uzunov N. M., Vasileva Zh. N. (1991) Practice in Nuclear Physics, Shumen, Helios