COMPARATIVE CHARACTERISTICS OF LIPID PEROXIDATION IN SMALL INTESTINE AT PROGENY IRRADIATED RATS

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It has been determined that the influence of low–dose ionizing radiation manifests itself in 10–month progeny of radiation–exposed rats in the form of a significant increase in concentrations of primary and secondary metabolites in the examined small intestinal lymph nodes, whereas the 2– and 5–month progeny of radiation–exposed rats' level of the examined products does not change significantly. An analysis showed that the lipid peroxidation (LPO) in structural membranes of the small intestinal lymph nodes in 2– and 5–month generations of radiation–exposed animals does not change for a valid value.
Keywords: *low–dose radiation, rats progeny, small intestine, lipid peroxidation*

Introduction.
One of the important biological effects of low-dose ionizing radiation is adaptive responses and genetic instability, the phenomenon of low-dose hyper-radiosensitivity [1]. A range of low-dose radiation effects on living cells are possible key factors in evaluating «risk of low-dose radiation», but there remains little of the coherence required among robust data that can be used with confidence in risk assessments [2]. It is known that serious accidents at massive nuclear power plants lead to low-dose irradiation of a large number of victims [3, 4]. Radiation–induced oxidative stress leads to mutations at target DNA loci, chromosomal rearrangements, epigenetic alterations and defective double strand breaks repair, contributing to the perpetuation of a genomic instability phenotype of the affected cells [5]. The progeny of irradiated cells display changes, such as aneuploidy, micronucleus formation, sister chromatid exchanges, gene mutations, amplifications and chromosomal destabilization, resulting in the generation of an aberrant clonal population of cells [6].
One of the most prone to radiation induced damage is gastrointestinal system [7]. Chronic intestinal radiation injury causes a change in bowel pattern, diarrhea, fecal incontinence, pain, and intestinal blood loss [8]. Particular interest of modern medical science is a comparative characteristic of physiological changes in the immunocompetency organs in progeny exposed to gamma– and neutron radiation [9]. A more detailed study of metabolic changes taking place in the peripheral organs of the immune system of the offspring of people exposed to ionizing radiation can complement to the full disclosure of pathogenesis, development of new methods of diagnostics, prediction, and treatment of immune dysfunction in the progeny. The existing level of scientific knowledge and practical experience allows making several principal conclusions, according to which in case of low–dose exposure no deterministic effects were noticed among the generation of persons exposed to radiation, and the risk of stochastic effects is hypothetical only.
The research purpose: determination of level of LPO products – diene conjugates (DC) and malondialdehyde (MDA) in the small intestinal lymph nodes in the various age of the generations of white rats exposed to low–dose gamma radiation.
Materials and methods.
To fulfill the objectives, we provided eight series of experiments with 190 white outbred reproductive rats (228 ± 5 g) of both sexes. The first series included control rats (n=30); the second series included radiation–exposed rats (n=70); the third, fourth, fifth, and sixth series included the offspring of rats exposed to gamma radiation (0.2 Gy), 15 rats in each group (n=15). Rats were given water ad libitum, were fed with standard mice pellets and were adapted for one week prior to drug administration in the following atmosphere: 22±1°C, 50±5% relative humidity, ventilation at 15 air renewal cycles/h and 24–h light–dark cycle.
We obtained permission for performing the research protocols and all animal experiments, and followed the guidelines of the Local Ethics Committee of the Semey State Medical University (Minutes № 3 dated 16.04.2014 of the Meeting of the Ethical Committee).
Before the exposure, topometry and dosimetry of the rats were performed. The images of irradiated animals after displaying were directly input in the planning system using network connection with the computer by electronic tablet. Rats were placed in well–ventilated special boxes and treated with whole body 0.2 Gy. The source of radiation was ^{60}Co–γ and was provided by Czech–made radiotherapeutic instrument «Teragam» in Regional Oncological Center (Semey, Kazakhstan).
Rats small intestinal lymph nodes were converted into homogenate which was prepared according to the method described by Lossow (1964) with slight modifications. Rats were exsanguinated by withdrawal of blood from the heart under chloroform anesthesia. Small intestine was removed and washed with 30 ml of 0.9% NaCl (physiological solution) and placed in ice cold 0.9% NaCl. The small intestine was minced with a surgical knife and homogenized using Potter–Elvehjem type of homogenizer in 50 ml of 0.1 M potassium phosphate buffer of pH 6.8. After 30 min, the homogenates were centrifuged for 30 min at 10,000 rpm at 4°C. The level of LPO products was estimated by the content of DC through Z. Placer method (1970) and MDA through S. Konyukhova method (1989).
The obtained results of the study were processed by the commonly accepted SPSS Statistical program (version 17.0). Statistical comparison was made by a Student's t–test, and a P–value < 0.05 was considered significant.
Results.

It is know that low-dose γ-irradiation has positive effects against oxidative damage that are different from high-dose irradiation [10], induces various stimulus effects [11, 12], activates anti-oxidative functions in some organs [13]. The small intestine is among the most rapidly self-renewing tissues in adult mammals [14]. In our experiment, we studied the quantitative content of the LPO products in the small intestinal lymph nodes in various-age progeny of rats exposed to gamma radiation (0.2 Gy).

Figure 1 shows that the effect of low–dose gamma radiation on the animals determines the decrease in the DC levels to 0.91±0.06 cu in the small intestinal lymph nodes in 2–month rats progeny, while in the control group, this value is equal to 0.92±0.72 cu (p>0.05). When the quantitative content of DC in 5–month control animals were studied, the value equaled to 0.84±0.05, and in the experimental rats to 0.95±0.04 (p>0.05).

In the experimental 10–month rats progeny, valid changes in the levels of DC in small intestinal lymph nodes were detected, which is confirmed by the numeric data, according to which the control group rats' quantitative content of the LPO product was equal to 2.56±0.18 cu, while in the experimental group this value was equal to 3.21±0.23 cu, i.e. an increase by 25.39 % was determined (p<0.05).

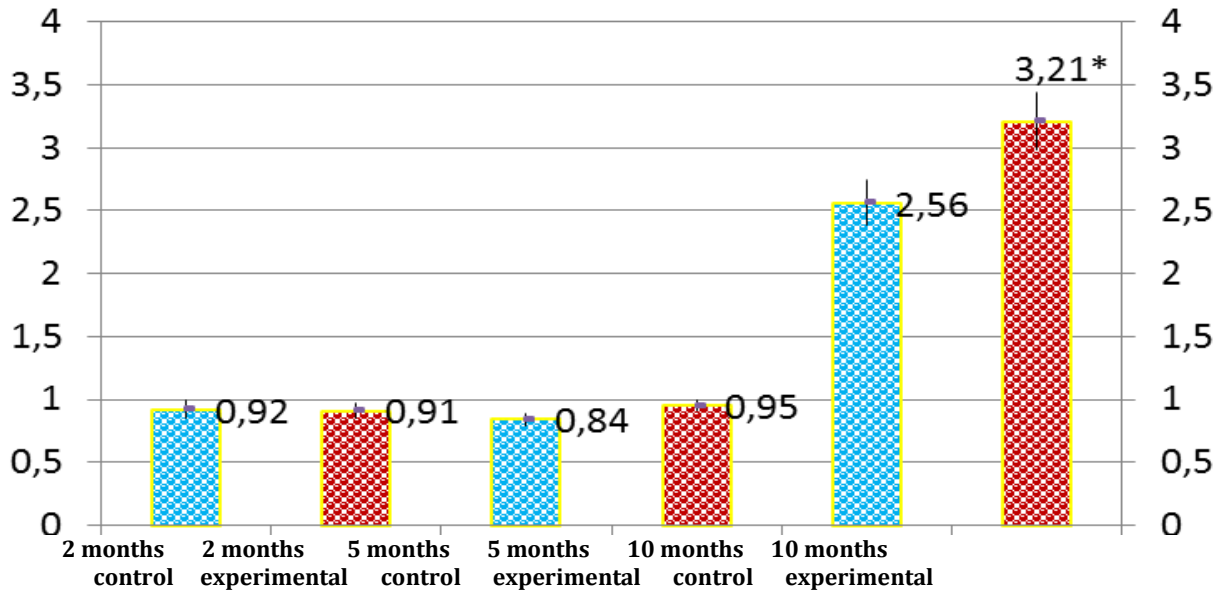

Figure 1 – The dynamics of changes in the quantitative content of DC (in cu) in the small intestinal lymph nodes in 2–, 5–, and 10–month progeny of irradiated rats

An analysis of data in publications showed that the decrease in the LPO in structural membranes of the small intestinal lymph nodes in 5–month progeny of radiation–exposed animals is confirmed by the stimulation of adaptive reactions as a consequence of the increased reactivity of the antioxidant system of cells.

According to the purpose of the research, the next objective of our work was to determine the quantitative content of the secondary product – MDA, which is produced during the oxidation decomposition of lipid hydroperoxides and phospholipids. Subsequent to the radiation–induced oxidative stress, intracellular LPO increases as a result of the oxidative transformation of multi–unsaturated lipid acids to MDA [15]. The measurement of this aldehyde provides a convenient index of LPO [16].

The numeric data show that the MDA level in the small intestinal lymph nodes in 2–month rats progeny exposed to low–dose ionizing radiation was equal to 0.25±0.02 nM/mg, while in control animals the value was equal to 0.28±0.01 nM/mg, p>0.05 (Fig. 2). When studying the quantitative MDA content in 5–month control and experimental animals, we detected no valid changes: the control group had 0.31±0.02 nM/mg, and the experimental group's rats had 0.33±0.02 nM/mg (p>0.05). When comparing the MDA levels in 10–month progeny of the animals of the control and experimental groups, we observed valid changes: in the control group, the value was equal to 0.42±0.02 nM/mg, while in the experimental groups, it was equal to 0.49±0.02 nM/mg, i.e. there was an increase in the MDA level by 16.66 %, p<0.05. It is known that hypoplasia of lymph nodes causes the disturbance of immune response, aggravation of autoimmune processes [17].

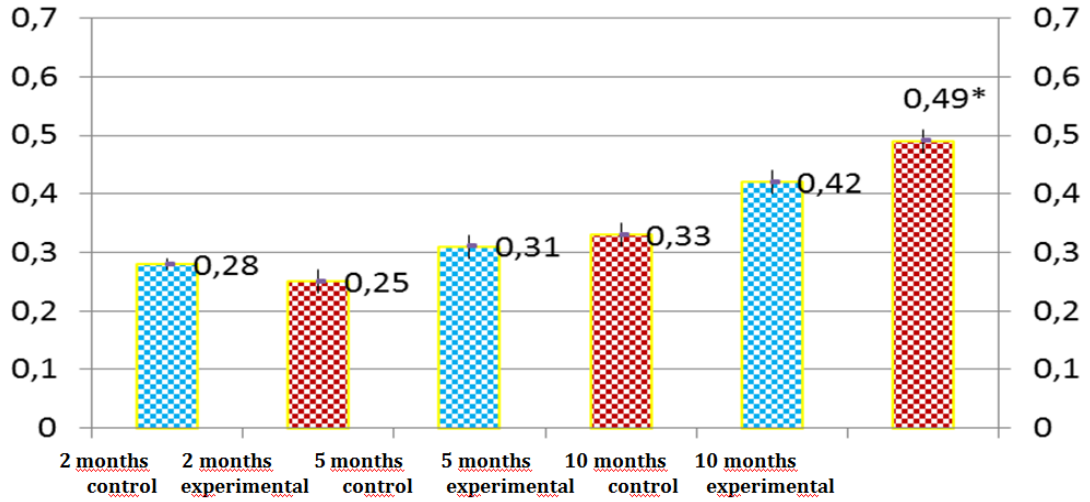

Figure 2 – The dynamics of changes in the quantitative content of MDA (nM/mg) in the small intestinal lymph nodes in 2–, 5–, and 10–month progeny irradiated rats

Analyzing the above said related to the radiation effect on the offspring, we can note that we could observe valid changes in the levels of DC and MDA in the studied small intestinal lymph nodes in 10–month progeny of experimental rats. An analysis of the data in publications showed that compared with control group, the MDA levels in the ileal tissues significantly increased [18]. Excessive accumulation of various LPO products in the organism, which have a destructive influence on the biological membranes, changes the activity of a large number of enzymes along with the dysfunction of the most important biochemical processes in the radiation–exposed organism [19]. Indirect effects of LPO are less apparent but no less detrimental. The aldehydes that are produced as a consequence of LPO are biologically active [20].

Our research also pays attention to the comparison of the levels of LPO products in the immunocompetency organ in various age rats progeny exposed to gamma radiation, which were determined by dividing experimental rats into three groups. We determined the LPO products, DC and MDA, in the small intestinal lymph nodes in 2–, 5– and 10–month control rats.

Table 1 shows that the quantitative content of DC in 2–month offspring of intact rats equaled to 0.92±0.07 cu, in 5–month rats—0.84±0.05 cu, and in 10–month ones—2.56±0.18 cu. Comparing the obtained data, we can note a valid increase in the level of DC in the rats of the third group by 178.26 % ($p<0.001$) compared to the first group, and by 204.76% ($p<0.001$) compared to the second group.

Table 1 – Quantitative content of DC (cu) and MDA (nM/mg) (M±m) in examined the small intestinal lymph nodes in 2–, 5– and 10–month control rats

LPO products	2–month control rats	5–month control rats	10–month control rats
	Group 1	Group 2	Group 3
DC	0.92±0.07	0.84±0.05	2.56±0.18 *** 000
MDA	0.28±0.01	0.31±0.02	0.42±0.02 *** 0
Remark: 1 – valid for the first group: * – $p<0.05$, ** – $p<0.01$, *** – $p<0.001$, 2 – valid for the second group: o – $p<0.05$, oo – $p<0.01$, ooo – $p<0.001$.			

One of the objectives of our experimental research was to compare the quantitative content of MDA in the small intestinal lymph nodes in the control groups of generations of radiation–exposed rats. Table 1 demonstrates that the MDA level in 2–month control animals equaled to 0.28±0.01 nM/mg, in 5–month — 0.31±0.02 nM/mg, and in 10–month rats — 0.42±0.02 nM/mg. The LPO products' level in the third group turned out to be higher by 50.0% ($p<0.001$) compared to the first group, and by 35.48% ($p<0.05$) compared to the second group. The obtained results show that the increase in MDA content is associated with the decrease in the antioxidant protection of the adult organism.

Discussion.

Ionizing radiation decreases the total antioxidant capacity of the organism and results in an imbalance between pro–oxidants and antioxidants [21]. The excessive reactive oxygen species (ROS) produced during irradiation exposure can cause tissue damage through LPO [16]. When cells or tissues are exposed to ionizing radiation, the water molecules undergo radiolysis and produce free radicals [22]. Proteins, nucleic acids and lipids are the major targets for ROS, generating DNA strand breakage, DNA–protein cross linking, and lipid peroxide production [23]. These toxic products initiate a cascade of events on the molecule level [24]. With aging of cells, their enzymes, antioxidants and smaller antioxidant molecules become progressively inactivated due to the failure of the antioxidant systems to overcome the constant influx of ROS. The accumulation of free radical–induced carbonylated proteins accelerates, indicating the age or state when cells in the individual become increasingly more susceptible to ROS–mediated damage [25]. Low–dose radiation affecting membranes changes the functional activity of cells, their sensitivity to neurohumoral factors, initiates the processes of LPO and causes hydrolysis, which entails disturbance of the structure and functionality of biological membranes. The disruption of biological membranes and the products of the cell structure degradation largely contribute to the development of genetic damages.

Thus, low–dose–rate irradiation causes genetic damage and passes it down to the next generation [26]. The incidence of genetic disorders in the descendent generation is result from genome instabilities in the parent's generation [27]. The absence of dependence of the aberrant cells and chromosome aberrations frequencies on the year of birth of children after the accident at the Chernobyl Nuclear Power Plant suggests a nonspecific character of the action of low dose ionizing radiation on sex cells of the parents, zygotes and somatic cells of the children. It was demonstrated the individual expression of genomic instability induced by low–dose irradiation and its transgenerational phenomenon in the children' organs born to parents irradiated as a result of the nuclear accident [28].

Conclusion.

In conclusion, the obtained experimental data indicate that in the studied lymph nodes of the small intestine of 2– and 5–month intact rats, the quantitative content of DC and MDA does not change considerably, while there is a valid increase in 10–month rats progeny.

The level of DC and MDA in 2– and 5–month rats progeny exposed to gamma radiation (0.2 Gy) does not change for a valid value. The effect of low–dose ionizing radiation manifests itself in 10–month offspring of radiation–exposed rats in the form of a valid increase in concentrations of DC and MDA in the examined small intestinal lymph nodes.

REFERENCES

1 Kadhim M.A., Moore S.R., Goodwin E.H. Interrelationships amongst radiation-induced genomic instability, bystander effects, and the adaptive response // Mutat. Res. – 2004. – Vol. 568. – P. 21–32.
2 Blyth B.J., Sykes P.J. Radiation-induced bystander effects: what are they, and how relevant are they to human radiation exposures? // Radiat. Res. – 2011. – Vol. 176. – P. 139–157.
3 Ishihara H., Tanaka I., Yakumaru H., Tanaka M., Yokochi K. et al. Quantification of damage due to low-dose radiation exposure in mice: construction and application of a biodosimetric model using mRNA indicators in circulating white blood cells // J. Radiat. Res. – 2016. – Vol. 57, № 1. – P. 25–34.
4 Riecke A., Rufa C.G., Cordes M. et al. Gene expression comparisons performed for biodosimetry purposes on in vitro peripheral blood cellular subsets and irradiated individuals // Radiat. Res. – 2012. – Vol. 178. – P. 234–243.
5 Fachin A.L., Mello S.S., Sandrin–Garcia P. et al. Gene expression profiles in radiation workers occupationally exposed to ionizing radiation // J. Radiat. Res. – 2009. – Vol. 50. – P. 61–71.
6 Huang L., Snyder A.R., Morgan W.F. Radiation–induced genomic instability and its implications for radiation carcinogenesis // Oncogene. – 2003. – Vol. 22. – P. 5848–5854.
7 Kiang J.G., Garrison B.R., Gorbunov N.V. Radiation combined injury: DNA damage, apoptosis, and autophagy // Adapt Med. – 2010. – Vol. 2. – P. 1–10.
8 Berbée M., Hauer–Jensen M. Novel drugs to ameliorate gastrointestinal normal tissue radiation toxicity in clinical practice: what is emerging from the laboratory? // Curr Opin Support Palliat. Care. – 2012. – Vol. 6. – P. 54–59.
9 Узбеков Д.Е. Кайрханова Ы.О., Hoshi M., Чайжунусова Н.Ж., Шабдарбаева Д.М., Саякенов Н.Б. и др. Влияние радиационного излучения на иммунную систему // Международный журнал прикладных и фундаментальных исследований. – 2016, – № 8 (4). – С. 538-541.
10 Kataoka T. Study of antioxidative effects and anti-inflammatory effects in mice due to low-dose X-irradiation or radon inhalation // J. Radiat. Res. – 2013. – Vol. 54. – P. 587–596.
11 Kataoka T., Sakoda A., Yoshimoto M. et al. Studies on possibility for alleviation of lifestyle diseases by low-dose irradiation or radon inhalation // Radiat. Prot. Dosim. – 2011. – Vol. 146. – P. 360–363.
12 Kataoka T., Yoshimoto M., Nakagawa S. et al. Basic study on active changes in biological function of mouse liver graft in cold storage after low-dose X-irradiation // J. Clin. Biochem. Nutr. – 2009. – Vol. 45. – P. 219–226.
13 Kataoka T., Mizuguchi Y., Yoshimoto M. et al. Inhibitory effects of prior low-dose X-irradiation on ischemia-reperfusion injury in mouse paw // J. Radiat. Res. – 2007. – Vol. 48. – P. 505–513.
14 Van der Flier L.G., Clevers H. Stem cells, self–renewal, and differentiation in the intestinal epithelium // Annu Rev Physiol. – 2009. – Vol. 71. – P. 241–260.
15 Bardak Y., Ozerturk Y., Ozguner F. et al. Effect of melatonin against oxidative stress in ultraviolet–B exposed rat lens // Curr Eye Res. – 2000. – Vol. 20. – P. 225–230.
16 Song L.H., Yan H.L., Cai D.L. Protective Effects of Soybean Isoflavone against Gamma–Irradiation Induced Damages in Mice // J. Radiat. Res. – 2006. – Vol. 47. – P. 157–165.
17 Steinert M., Weiss M., Gottlöber P., Belyi D., Gergel O., Bebeshko V., Nadejina N., Galstian I. et al. Delayed effects of accidental cutaneous radiation exposure: fifteen years follow–up after the Chernobyl accident // J. Am. Acad. Dermatol. – 2003. – Vol. 49, № 3. – P. 417–423.
18 Gultekin F.A., Bakkal B.H., Guven B., Tasdoven I. et al. Effects of ozone oxidative preconditioning on radiation–induced organ damage in rats // J. Radiat. Res. – 2013. – Vol. 54. – P. 36–44.
19 Jones D.P. Radical–free biology of oxidative stress / Am. J. Physiol. // Cell Physiol. – 2008. – Vol. 295. – P. 849–868.
20 Koylu H., Mollaoglu H., Ozguner F., Naziroglu M., Delibas N. Melatonin modulates 900 Mhz microwaveinduced lipid peroxidation changes in rat brain // Toxicol. Ind. Health. – 2006. – Vol. 22, № 5. – P. 211–216.
21 Koc M., Taysi S., Emin Buyukokuroglu M., Bakan N. The effect of melatonin against oxidative damage during total–body irradiation in rats // Radiat. Res. – 2003. – Vol. 160, № 2. – P. 251–255.
22 Maurya D.K., Devasagayam T.P., Nair C.K., Some novel approaches for radioprotection and the beneficial effect of natural products // Indian J. Exp. Biol. – 2006. – Vol. 44. – P. 93–114.
23 Sharma N.K. Modulation of radiation–induced and mitomycin C–induced chromosome damage by apigenin in human lymphocytes in vitro // J. Radiat. Res. – 2013. – Vol. 54. – P. 789–797.
24 Prasad N.R., Menon V.P., Vasudev V. et al. Radioprotective effect of sesamol on gamma–radiation induced DNA damage, lipid peroxidation and antioxidants levels in cultured human lymphocytes // Toxicology. – 2005. – Vol. 209. – P. 225–235.
25 Moskovitz J., Yim M.B., Chock P.B. Free radicals and disease // Arch. Biochem. Biophys. – 2002. – Vol. 397, № 2. – P. 354–359.
26 Collodei G., Moretti E. Sperm morphology and aneuploidies: defects of supposed genetic origin // Andrologia. – 2006. – Vol. 38, № 6. – P. 208–215.
27 Tamminga J., Koturbash I., Baker M., Kutanzi K., Kathiria P., Pogribny I.P. et al. Paternal cranial irradiation induces distant bystander DNA damage in the germline and leads to epigenetic alterations in the offspring // Cell Cycle. – 2008. – Vol. 7, № 9. – P. 1238–1245.
28 Aghajanyan A. Transgenerational genomic instability in children of irradiated parents as a result of the Chernobyl Nuclear Accident // Mutat. Res. – 2009. – Vol. 1, № 2. – P. 52–57.

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СӘУЛЕЛЕНГЕН ЕГЕУҚҰЙРЫҚТАР ҰРПАҚТАРЫНЫҢ ЖІҢІШКЕ ІШЕГІНДЕГІ ЛИПИДТЕРДІҢ АСҚЫН ТОТЫҒУЫНЫҢ САЛЫСТЫРМАЛЫ СИПАТТАМАСЫ

Түйін: Иондағыш радиацияның шағын дозасы зерттеуге алынған сәуле әсеріне ұшыраған егеуқұйрықтардың 10 айлық ұрпақтарының жіңішке ішек лимфа түйіндерінде ДК мен МДА концентрациясының нақты түрде жоғарлағанымен сипатталатыны анықталған, ал жасы 2,5 айлықтағы тәжірибелік егеуқұйрықтарда зерттеуге алынған өнімдер нақты өзгерістерге түспеген.
Түйінді сөздер: радиацияның шағын дозасы, егеуқұйрықтар ұрпақтары, жіңішке ішек, липидтердің асқын тотығуы

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СРАВНИТЕЛЬНАЯ ХАРАКТЕРИСТИКА ПЕРЕКИСНОГО ОКИСЛЕНИЯ ЛИПИДОВ В ТОНКОМ КИШЕЧНИКЕ ПОТОМКОВ ОБЛУЧЕННЫХ КРЫС

Резюме: Установлено, что влияние малой дозы ионизирующей радиации проявляется у 10-ти месячных потомков облученных крыс достоверным повышением концентраций ДК и МДА в исследованных лимфатических узлах тонкого кишечника, тогда как у 2 и 5-ти месячных потомков облученных животных уровень исследованных продуктов не претерпевает достоверных изменений.
Ключевые слова: малые дозы радиации, потомство крыс, тонкий кишечник, перекисное окисление липидов