Effect of Single Total Irradiation on the Reproductive System and Vitamin Content in Rat Progeny

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Reduction in the epididymis weight and in the spermatozoid concentration together with marked morphological changes in the seminiferous tubules were observed in the progeny of irradiated rats. Vitamin E content in the testes increased 2.8-fold, while that of vitamin B_2 decreased 1.6-fold.

Key Words: *irradiation; rats; progeny; spermatogenesis; vitamins*

Now the generation grown against the background of increased radiation after the Chernobyl disaster enters the pre- and puberty period. Unfortunately, little attention has been paid on the remote effects of constant irradiation on the population of the contaminated territories. Since the background radiation continues to increase in many regions of the country [2] as well as the number of couples in which one or both spouses have been exposed to radiation, the problems associated with reproduction of human population in these regions are relevant.

In the present study we examined the effect of radiation on the reproductive system and some internal organs of rat progeny.

MATERIALS AND METHODS

Wistar rats aged 1.5 years at the beginning of the study were used. Experimental group included the first-generation males which were obtained from intact males and females exposed to a single radiation dose of 0.5 Gy (power 0.003 Gy/sec). The rats were irradiated in a Lutch apparatus using ^{60}Co as the source of ionizing radiation. The males were mated with intact females 2 weeks after irradiation, i.e., the eggs were fertilized by spermatozoa irradiated at the spermatid stage, when they are sensitive to the radiation-induced damage [14,15]. Control group consisted of 10 male offspring of intact rats. All the animals (both parents and the offspring) lived in the same vivarium and received the same diet (standard dry fodder without meat and fat) throughout the entire experiment.

The method of spermatozoid counting was described previously [1]. The riboflavin content in the testes and liver was determined by the titration with riboflavin-binding protein [4]. The vitamin B_6 content was determined by serum concentration of pyridoxal coenzymes (pyridoxal-phosphate and pyridoxal), which was measured by high-performance liquid chromatography [9]. The contents of vitamins A and E in serum and testes were determined by high-performance liquid chromatography [10]. Morphological analysis of the testes was performed by routine techniques [7]. For evaluation of the state of spermatogenic epithelium, 100 transverse sections of convoluted seminiferous tubules were analyzed, and the tubules with three and/or four layers of developing germ cells (spermatogones, spermatocytes, spermatids and spermatozoa), two layers (spermatogones and spermatocytes), one layer (spermatogones) as well as tubules with collapsed and without sperm cells were counted. The

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TABLE 1. The State of Seminiferous Tubules in the Progeny offsprings of Irradiated Male Rats *(M±m)*

Group of animals (*n*=6)	Number of seminiferous tubules containing spermatogenic cells at different stages of development (in 100 tubules)					Spermato-genesis index
	III-IV stage	II stage	I stage	declinestage	desquamation	
Control	97.8±0.17	0.67±0.21	0	1.5±0.22	3.3±0.8	3.92±0.005
Experimental	95.3±0.42**	2.0±0.25**	1.3±0.21***	1.17±0.65*	11.2±1.9**	3.85±0.015**

Note. Here and in Table 2: *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with the control.

proportion of tubules of each type and the spermatogenesis index — (the total number of all layers of spermatogonia in 100 tubules):100 [7,12] — were calculated.

RESULTS

A statistically significant decrease in the epididymis weight (847±145 *vs.* 1044±180 g in the control) and in the spermatozoid concentration (21±13 *vs.* 36±4 mln cells/ml in the control) was observed in the offspring of irradiated rats. The production of spermatozoa decreased against the background of morphological changes in seminiferous tubules (Table 1). The rats were irradiated when their spermatozoa were at the stage of spermatids. Spermatogonia have the highest sensitivity to ionizing radiation. The sensitivity of primary spermatocytes is defined by the stage of the prophase of the first meiotic division. Two stages of preleptotenal transformations of chromosomes were defined in human and mammalian gametogenesis [6,8]; their high sensitivity to damaging factors and manifestations of the damage in the gametes of the progeny have been demonstrated [3,11]. Although spermatids are less sensitive to damaging factors than the preleptotenal stage cells, the synthesis of the components which are necessary for their differentiation into spermatozoa is disrupted [13]. In our experiments a significant decrease in the intensity of spermatogenesis resulted from a reduction in the number of tubules containing all four types of germ cells and intense desquamation of cells into the lumen of tubules (Table 1), i.e., in the progeny of irradiated males massive loss of spermatocytes leads to reduction in the number of spermatids and spermatozoa and in the fertilizing potency.

In the first generation of irradiated rats serum and blood contents of tocopherol, retinol and pyridoxal coenzymes did not differ from those in the control (Table 2), while vitamin E content in the testes increased significantly by 2.8 fold. Since *in vitro* vitamin E protects spermatozoa from peroxidation, which is a potent factor impairing the function of germ cells [11], it was suggested that the elevation of tocopherol concentration in the testes of the progeny of irradiated rats has protective effect against peroxidation and a damaging effect which manifests itself in the structure of seminiferous tubules.

There were no alterations in the vitamin B metabolism in control rats, judging from the total vitamin B_2 liver content. The content of vitamin B_2 in the liver and testes of the offspring of irradiated males was lower 1.6 fold than in control rats. Although both control and irradiated rats received the same diet, pronounced vitamin B_2deficiency was observed in the experimental group. The concentration of this vitamin in the liver of experimental rats corresponded to that observed when dietary consumption of the vitamin was equal to $^1/_5$ of physiological norm. This may be due to reduced absorption of riboflavin in the small intestine or its increased excretion with urine. We have shown that the excretion of vitamin B_2 is increased in rats on the second day after a single whole-body high-dose (4 Gy) irradiation. Bearing the mind that numerous physiological processes in the body, for example, oxidation, detoxification of xenobiotics, metabolism of other vitamins, regeneration of glutathione and hemoglobin, etc., are vitamin B_2-dependent, it can be concluded that ionizing radiation causes metabolic disorders not only in irradiated animals but also in their progeny. This is consistent with the concept on the integral consequences of irradiation of the gametes of both parents [5].

Since the extrapolation of experimental results on the people living on radiologically contaminated terri-

TABLE 2. Content of Vitamins in The First Generation Progeny of Intact and Irradiated Rats *(M±m)*

Vitamin		Control (n=6-10)	Experiment (n=6-7)
A	serum, μg/dl	9.5±3.0	8.9±3.8
E	serum, μg/dl	0.05±0.02	0.05±0.03
	testis, μg/g	2.5±1.0	6.9±1.4*
B_2	testis, μg/g	19.1±2.8	11.3±2.0*
	liver, μg/g	36.8±5.2	22.5±1.2*
B_6	serum, nmol/ml	160±18	165±18

tories is relevant, unfavorable consequences of radiation on reproductive potential of these people could be expected.

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