ACTION OF X-RAY IRRADIATION ON DNA SYNTHESIS AND MITOTIC ACTIVITY IN UTERINE EPITHELIAL CELLS AT DIFFERENT STAGES OF THE SEX CYCLE

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The action of x-ray irradiation on DNA synthesis and mitotic activity of the uterine epithelium was studied by autoradiography with thymidine-H^3 at different stages of the sex cycle. In response to local irradiation in a dose of 400 R the decrease in the index of labeled nuclei and in the mitotic index differed depending on the stage of the sex cycle at which irradiation was given.

KEY WORDS: autoradiography; x-ray irradiation; DNA synthesis; mitotic activity; estrous cycle.

The results of autoradiographic studies using thymidine-H^3 on ovariectomized animals treated with estrogen show that x-ray irradiation disturbs DNA synthesis in the epithelium of the reproductive organs and inhibits the entry of the cells into mitosis [5-8]. However, these investigations shed no light on the question of what changes the irradiation induces in the different stages of the sex cycle, i.e., during normal ovarian function. It is well known that when the secretion of estrogens by the ovaries is increased or decreased, the stages of intensive proliferation of the epithelium in the uterus and vagina of sexually mature mice are replaced by periods of low mitotic activity. For these reasons the estrous cycle in mice and its various stages constitute a convenient model with which to study processes concerning cell division.

The object of this investigation was to study the action of x-ray irradiation on proliferation of the epithelium lining the uterine cavity of mice at different stages of the estrous cycle.

EXPERIMENTAL METHOD

By examining vaginal smears daily for 3 weeks 144 virgin sexually mature female (CBA $\times$C57BL/6j)F_1 hybrid mice weighing 18-20 g at different stages of the estrous cycle were selected. To study changes in the number of cells synthesizing DNA the mice were given a single injection of thymidine-H^3 (USSR product) with specific activity of 1.4 Ci/mmole in a dose of 0.7 μCi/g body weight. Local irradiation was given to the uterine cornua, so that the morphological changes arising in them in response to the direct action of x-rays on the organ could be studied. Local irradiation of the uterine cornua, with the other parts of the body shielded, was given by the RUM-7 apparatus (filter 3.57 mm Al, half-attenuation layer 2 mm, focal distance from anode to uterine cornua 10.5 cm, total dose 400 R per animal, dose rate 63 R/min). Depending on the stage of the sex cycle (proestrus, 1st, 2nd, and 3rd days of estrus, metestrus, 1st, 2nd, and 3rd days of diestrus) all the mice were divided into 8 groups with 18 animals in each group. Nine mice from each group were irradiated in a dose of 400 R. These animals were sacrificed 6 h after irradiation (by decapitation) along with the 9 unirradiated mice of the same group, which acted as the control. Thymidine-H^3 was injected into both the irradiated and the unirradiated mice 1 h before sacrifice. The epithelium of the uterine cavity was used as the test object. The uterus was fixed in Carnoy's fluid and embedded in paraffin wax, and sections were cut to a thickness of 5 μ. The dewaxed sections were coated with type M radiosensitive emulsion,

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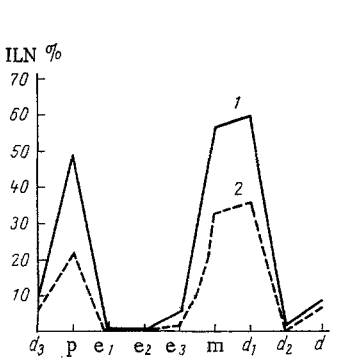

Fig. 1

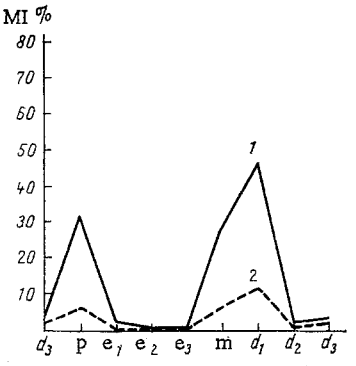

Fig. 2

Fig. 1. Changes in ILN in uterine epithelium depending on stage of sex cycle with irradiation in a dose of 400 R: 1) control; 2) experiment. Abscissa, stages of the sex cycle: d_3) 3rd day of diestrus; p) proestrus; e_1) 1st day of estrus; e_2) 2nd day of estrus; e_3) 3rd day of estrus; m) metestrus; d_1) 1st day of diestrus; d_2) 2nd day of diestrus; ordinate, ILN (in percent).

Fig. 2. Changes in MI in uterine epithelium depending on stage of sex cycle with x-ray irradiation in dose of 400 R: 1) control; 2) experiment. Abscissa, stages of sex cycle (Fig. 1); ordinate, MI (in percent).

exposed in a refrigerator for 2 weeks, developed with amidol developer, and stained with Carazzi's hematoxylin. In each case 3000 epithelial cells were counted. Nuclei were regarded as labeled if there were not less than 3 grains of silver above them. The index of labeled nuclei (ILN) in percent and the mitotic index (MI) per thousand cells were determined.

EXPERIMENTAL RESULTS

The results of the determination of ILN and MI in the uterine epithelial cells at the different stages of the estrous cycle in the control mice are given in Fig. 1, curve 1 and Fig. 2, curve 1. In the course of the sex cycle in mice two periods of intensive DNA synthesis and high mitotic activity were found in the epithelium of the uterine cavity: proestrus and metestrus – 1st day of diestrus. The cells synthesizing DNA in proestrus divide in the same stage of the estrous cycle. The same can also be suggested for metestrus and the 1st day of diestrus. These findings are in agreement with those described by Epifanova [2, 3], Laguchevа [4], and Budik [1].

Curves reflecting changes in ILN and MI in the uterine epithelium after local irradiation in a dose of 400 R are given in Fig. 1, curve 2 and Fig. 2, curve 2. The curves for ILN and MI of the experimental animals are considerably lower than those for the controls. The decrease varied depending on the stage of the sex cycle in which the animals were irradiated. If the mice were irradiated in proestrus, metestrus, and the 1st day of diestrus ILN was 43.2, 58.4, and 59.6% of the control, respectively (Fig. 1, curve 2). On the 3rd day of estrus ILN was 39.4% of the control. If the animals were irradiated on the 2nd-3rd day of diestrus and the 1st-2nd day of estrus ILN was 28, 25, 36, and 32% lower than for the unirradiated animals. The greatest decrease in ILN was observed on the 3rd day of estrus and in proestrus. DNA synthesis in the uterine epithelium is evidently more sharply inhibited by irradiation in the stage of the sex cycle in which mass entry of the cells into the period of DNA synthesis is observed.

Inhibition of mitotic activity (Fig. 2, curve 2) was observed after irradiation at all stages of the sex cycle. The greatest decrease was found in proestrus, the first day of diestrus, and metestrus, when MI was 18, 24.6, and 20.8% of the control, respectively. On the 1st-2nd day of estrus MI was close to zero. On the 2nd-3rd day of diestrus and on the 3rd day of estrus MI was 32, 36.4, and 29.8% of the control, respectively.

The inhibitory action of the same dose of irradiation was thus manifested differently depending on the stage of the sex cycle. Besides the decrease in ILN, MI also was reduced.

The results agree with data described in the literature. They show that the decrease in DNA synthesis

and mitotic activity depends on the dose of the hormone present at the time of irradiation and the pulse of mitotic cycle in which most cells of the epithelium of the reproductive organs were at the time of irradiation [5-8].

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