place on account of migration of the recipient's hematopoietic cells. The findings indicating a small number of cells repopulating extensive areas of the hematopoietic system and a small scale of renewal of the hematopoietic clones on account of hematopoietic stem cells arriving from the blood during steady-state hematopoiesis are in full agreement with results [6] obtained recently in experiments with radiation chimeras.

As Table 1 shows, the character of repopulation by the recipient's hematopoietic cells was the same in the syngeneic and semisyngeneic systems. At all times of investigation no increase in the fraction of the donor's hematopoietic cells could be found in the heterotopic focus. Hence, it follows that hematopoietic cells of the C57BL/6 genotype have no selective advantage over the cells of mice with a T6 chromosome in the territory of the hematopoietic focus formed by stromal precursors of the C57BL/6 genotype. On the other hand, the absence of any decrease in their fraction in the focus with the passage of time indicates that the phenomenon of hybrid resistance is not involved in this case [3, 5]. Hence it follows that hematopoietic cells of the (C57BL × CBA)F_1 hybrid capable of repopulation are not responsible for the phenomenon of hybrid resistance, as has been suggested [2].

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ACTION OF X-RAY RADIATION ON DNA SYNTHESIS IN THE EPITHELIUM OF THE UTERINE GLANDS AND ITS DEPENDENCE ON THE PHASE OF THE MITOTIC CYCLE

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The action of x-ray irradiation on DNA synthesis in the epithelium of the uterine glands of ovariectomized mice stimulated by dihydrostilbestrol and the dependence of its action on the phase of the mitotic cycle were studied by autoradiography with thymidine-^{3}H. After local irradiation of the region of the uterus in a dose of 400 rad the decrease in the index of labeled nuclei was found to differ depending on the phase of the mitotic cycle in which most cells were.

KEY WORDS: x-ray irradiation; epithelium of uterine glands; DNA synthesis.

During irradiation a decrease in the number of labeled cells is observed in the reproductive organs and its degree depends on the state of the cells at the moment of irradiation [2, 4-6, 8].

The object of this investigation was to study the action of x-ray irradiation on the entry of the epithelial cells of the uterine glands into the phase of DNA synthesis and the dependence of this action on the phase of the mitotic cycle in which most of the cells were.

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TABLE 1. Changes in ILN in Epithelium of Uterine Glands after Local Irradiation of Ovariectomized Mice in a Dose of 400 R at Various Times after Injection of 0.05 μg Dihydrostilbestrol (M ± m)

Interval between injection of hormone and irradiation	Time after irradiation	Interval between injection of hormone and sacrifice	ILN, %		P
			control	experiment	
h					
4	6	10	27,5±2,0	4,1±0,8	<0,001
4	10	14	45,9±2,8	8,7±1,2	<0,001
4	20	24	13,8±0,7	11,5±1,3	>0,05
8	6	14	45,9±2,8	18,9±2,6	<0,001
14	6	20	17,6±1,1	8,2±1,2	<0,001
18	6	24	13,5±0,8	9,6±0,9	<0,001

EXPERIMENTAL METHOD

Experiments were carried out on 120 female (CBA × C57BL)F_1 mice weighing 18-20 g. Before the beginning of the experiment ovariectomy was performed on all the animals. An oily solution of dihydrostilbestrol in a dose of 0.05 μg was used in the experiment; doses of dihydrostilbestrol of 0.05 and 0.01 μg are optimal for the epithelium of the uterine glands [1]. Local irradiation of the mice in the region of the uterus was given with x rays in a single dose of 400 R by means of the RUM-11 apparatus (190 kV, 15 mA, filters: 0.5 mm Cu + 1 mm Al, dose rate 63 R/min). The animals were divided into three groups.

The mice of group 1 were killed at various times (eight animals at each time) after injection of the hormone (2, 4, 8, 10, 12, 14, 18, 20, and 24 h). The animals of the second group were irradiated 4, 8, 14, and 20 h (eight mice at each time) after injection of dihydrostilbestrol; each batch of mice was killed 6 h after irradiation. The mice of group 3 were irradiated 4 h after injection of the hormone and were killed at two times: 10 and 20 h after irradiation. The mice of all three groups were given a single injection of thymidine-^{3}H in a dose of 0.7 μCi/g body weight 1 h before sacrifice. The mice of group 1 acted as the control. The epithelium of the uterine glands was investigated. 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, exposed in a refrigerator for 16 days, and then stained with Carrazzi's hematoxylin. In each case 3000 cells were counted. The index of labeled nuclei (ILN, in %) was determined in the resulting autoradiographs.

EXPERIMENTAL RESULTS

The experimental results showed that a single injection of dihydrostilbestrol in a dose of 0.05 μg into the ovariectomized mice caused an increase in the number of cells synthesizing DNA in the epithelium of the uterine glands. The first morphological signs of the action of the hormone, revealed as an increase in the number of cells entering the phase of DNA synthesis, were found 4 h after injection of the hormone (ILN = 3.8± 0.33%). The ILN rose gradually and 12 h after injection of dihydrostilbestrol it reached 27.68 ± 2.3%. The maximal increase in ILN was observed 14 h after injection of the hormone (ILN = 45.9 ± 2.8%). DNA synthesis then gradually diminished in intensity, so that 24 h after injection of the hormone ILN was 13.5 ± 0.8%. Confirmation that the conditions of these experiments were well standardized was given by the agreement between the control values and those given by other workers [3, 7].

Local irradiation of the ovariectomized mice receiving dihydrostilbestrol in the region of the uterus caused a sharp decrease in DNA synthesis in the epithelium of the uterine glands (Table 1). The animals of group 2 were irradiated 6 h before sacrifice. Since the mice were killed at successive periods after injection of the hormone, irradiation occurred in different phases of the proliferation wave induced by injection of the hormone. As Table 1 shows, in the mice of all the experimental groups ILN at all times of the investigation was significantly lower than in the control animals. However, this decrease was irregular and depended on the phase of the proliferative wave at the moment of irradiation. It was most marked at the 10th hour of the experiment, i.e., in mice irradiated 4 h after injection of the hormone (ILN was 14.9% relative to the control). It can be concluded from the control data that in the period of irradiation the overwhelming majority of epithelial cells of the uterine glands had still to enter the phase of DNA synthesis. Presumably, however, most of

the cells at this time had already passed from the G_0 period into the G_1 period and were engaged on the synthetic processes essential for subsequent transition into the period of DNA synthesis. This hypothesis is based, as shown by the results obtained with the mice of the third group, on the fact that in mice irradiated during this period and killed 10 h after irradiation, entry of the cells into the period of DNA synthesis was sharply retarded. However, 20 h after irradiation ILN of the animals of the third group was close to the control value and amounted to 83.3% of it.

The results confirm those of the writers' previous experiments which showed that after irradiation of mice 6 h after injection of dihydrostilbestrol, i.e., before most of the cells had started the period of DNA synthesis, and if the animals were sacrificed 16 h after injection of the hormone, hormonal stimulation of DNA synthesis in the epithelial cells of the mouse uterus was considerably inhibited. If the mice of this group were killed 20 h after irradiation, ILN was considerably higher [2]. These results indicate that the response of the cells to irradiation under the experimental conditions used is manifested as delay of the beginning of DNA synthesis rather than as its constant inhibition.

As Table 1 shows, irradiation of the mice 8 h after injection of the hormone inhibited the entry of the cells into the period of DNA synthesis, and ILN was 41% of the control value. In the epithelial cells of the uterus of the ovariectomized mice stimulated by dihydrostilbestrol in a dose of 1.0 μg, irradiation at a time when ILN was somewhat increased could no longer prevent the subsequent entry of most cells into the period of DNA synthesis [2]. This suggests that the radiosensitive G_1 period lasts longer in the cells of the uterine glands.

Irradiation of the mice 14 h after injection of dihydrostilbestrol, when about 46.0% of the cells were in the period of DNA synthesis, was again sharply reflected in proliferative activity, and at the 20th hour of the experiments ILN of the irradiated mice was only 46.5% of its value in the unirradiated mice. If irradiation was given when the proliferation wave was falling its effect was weaker and at the 24th hour of the experiment ILN of the irradiated mice was 71.7%. The inhibitory action of the same dose of irradiation on the number of DNA-synthesizing cells in these experiments thus depended on the phase of the mitotic cycle in which most of the cells of the uterine glands were at the time of irradiation. The greatest decrease in ILN was caused by irradiation of the mice at a time 10 h before the maximal increase in ILN.

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