Comparison of the dynamics of the numbers of mast cells and eosinophils in the mammary gland tissue of the unirradiated rats revealed a certain parallel in the change in their numbers. Irradiation disturbed this parallel by increasing the number of mast cells in the early stages and delaying the increase in the number of eosinophils at the same time. These differences in the response of the mast cells and eosinophils to irradiation were evidently due to the fact that these cells perform different functions in the mammary gland tissue. The content of eosinophils in the stroma of the mammary gland, as can be deduced from data in the literature [4], may perhaps reflect the presence of estrogenic hormones in them.

The results of these experiments thus showed that an important role in the genesis of radiation tumors of the mammary glands in rats receiving a single dose of irradiation in the early period of postnatal development is played by a disturbance of the dynamics of the number of eosinophils, which may probably be evidence of the accumulation of estrogenic hormones in the tissues of the mammary gland, and by a change in the response of the mast cells, as the cellular endocrine system of the body.

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ACTION OF CELL EXTRACT OF EHRLICH'S MOUSE ASCITES TUMOR ON MITOTIC ACTIVITY AND DNA SYNTHESIS IN THAT TUMOR

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An extract of cells of Ehrlich's ascites tumor inhibits the proliferative activity of its cells tissue specifically. The effect is expressed as a marked decrease in the number of dividing and DNA-synthesizing cells after injection of the extract. The mitotic index falls considerably as early as 2 h after the injection, reaches a minimum after 4-5 h, and returns to the control level again after 9-12 h. The radioactive index is on the whole uniformly low during the 18 h of the experiment.

KEY WORDS: *chalone; Erhlich's ascites tumor; mitotic index; index of labeled nuclei.*

Starting in 1964 [2], considerable attention has been paid to the study of tissue-specific inhibitors of cell division, or chalones. An essential factor in these investigations

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has been the analysis of the effect of the chalone on individual indices of proliferation (mitotic activity and number of DNA-synthesizing cells). Tissue-specific inhibitors of tumor cells from this point of view have been inadequately studied.

The object of this investigation was to isolate the chalone of Ehrlich's ascites tumor and to investigate its action on mitotic activity and on the number of DNA-synthesizing cells of this tumor for a short period after a single injection of the chalone.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino mice aged 1.5-2 months. A diploid strain of Ehrlich's ascites tumor was transplanted by intraperitoneal injection of 0.2 ml of ascites fluid. The extract was prepared by the method described previously [1] and injected at noon into mice with a five-day-old tumor (intraperitoneally, 1 ml). The animals were then sacrificed 2, 3, 4, 5, 6, 9, 12, 15, and 18 h later. Control animals received injections of physiological saline. At each time during the experiment five control and five experimental animals were used. All the animals were given an intraperitoneal injection of [^{3}H]thymidine in a dose of 1 μCi/g body weight 1 h before sacrifice. The ascites fluid was collected from the animals, and the ascites fluid and part of the small intestine were removed from animals killed 4 and 9 h after injection of the extract or physiological saline. Preparations for cytological and autoradiographic analysis were made from the material thus obtained. In preparations of Ehrlich's ascites tumor 5000 cells were analyzed and the mitotic (MI) and radioactive (RI) indices calculated in promille. The cells in preparations from the intestine were examined in 50 crypts cut longitudinally, and MI and RI were calculated in promille.

EXPERIMENTAL RESULTS

As Fig. 1 shows, MI 2 h after injection of the extract was reduced by 72.3% compared with the control ($P = 0.003$). The degree of depression of cellular proliferation reached a maximum (by 94.4%; $P = 0.0001$) 4 h after injection. Similar inhibition was obtained in the previous investigation [1] when undiluted extract was used. A high level of inhibition continued for the next 2 h. Extract of Ehrlich's ascites tumor cells thus has the ability to depress mitotic activity in the tumor. Since the effect of depression of mitotic activity was manifested during the first few hours after injection of the extract, presumably passage of the cells through the mitotic cycle was blocked in the G_2 phase. Starting with the ninth hour of the experiment, differences between the experimental and control animals were no longer statistically significant. Consequently, in the interval between 4 and 9-12 h after injection of the extract gradual recovery of mitotic activity up to the level of the control animals was observed. The action of the extract on proliferation of Ehrlich's ascites cells is thus characterized by its brevity.

A previous investigation [1] showed that the extract has a synchronizing action on the division of Ehrlich's tumor cells. This effect was found 8 h after injection of the extract. In the present investigation no synchronization of cell division was observed. Possibly the synchronizing effect is detectable only if the extract is injected at a definite phase of the diurnal rhythm of mitotic activity. This problem requires further experimental investigation.

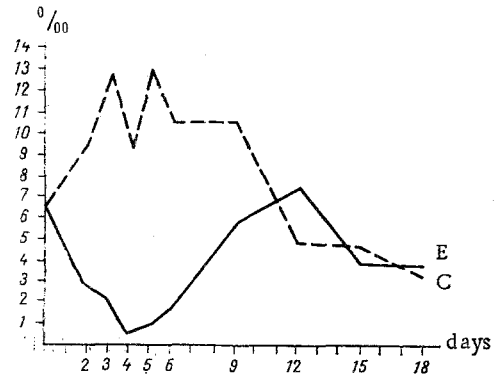

Fig. 1. MI in Ehrlich's ascites tumor cells during 18 h after injection of extract. Here and in Figs. 2 and 3: C) control; E) experiment. Abscissa, time (in days); ordinate, MI (in promille).

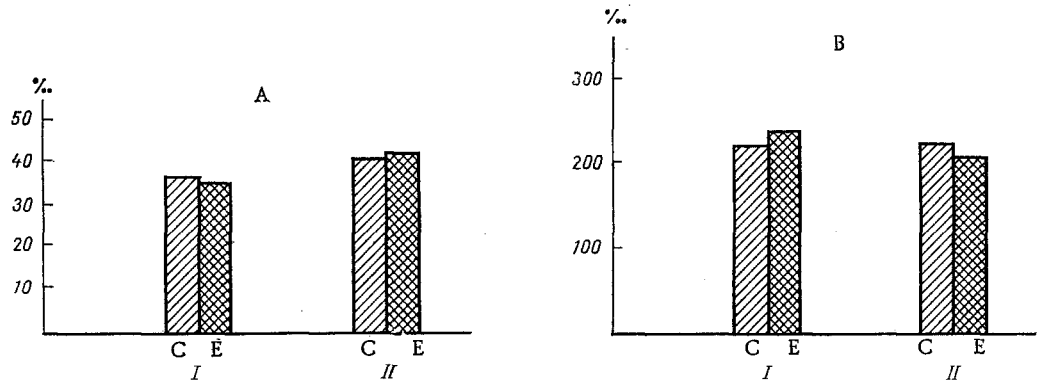

Fig. 2. MI (A) and RI (B) in epithelium of small intestine after injection of extract: I) 4 h after injection of extract; II) 9 h after injection of extract. Ordinate, in A: MI (in promille), in B: RI (in promille).

MI in the epithelium of the small intestine 4 and 9 h after injection of the extract was virtually indistinguishable from the control (Fig. 2A). Consequently, the action of the extract on mitotic activity is tissue specific, in agreement with earlier observations [1]. The presence of a brief specific action of this extract on mitotic activity suggests that it is a chalone.

The number of DNA-synthesizing cells in the tumor (Fig. 3) 2 h after injection of the extract was reduced by 51.1% ($P = 0.02$), after 3 h by 32% ($P = 0.05$), after 4 h by 51.4% ($P = 0.003$), after 5 h by 66.1% ($P = 0.002$), after 6 h by 34% ($P = 0.05$), after 15 h by 80% ($P = 0.0001$), and after 18 h by 55.4% ($P = 0.05$). No significant differences between the value of RI in the control and experimental animals could be found 9 and 12 h after the beginning of the experiment, although RI in the experimental groups was lower than in the control by 38 and 30%, respectively. Determination of RI in the epithelium of the small inestine revealed no difference (Fig. 2B). It can be concluded from these results that the action of the extract on the number of DNA-synthesizing cells is tissue specific, evidence that it blocks the passage of the cells from the G_1 into the S period of the mitotic cycle.

Comparison of the curves (experimental) in Figs. 1 and 3 leads to the conclusion that the character of action of the extract on cells in the G_1 and G_2 phases differs significantly. Under the influence of the extract MI fell and then recovered, whereas RI in general was uniformly low throughout the experiment. These results may provide indirect evidence for the existence of G_1 and G_2 inhibitors of cellular proliferation in the extract of Ehrlich's ascites cells. The greater intensity of the effect of inhibition of the passage of the cells from the G_2 period of the cell cycle into mitosis compared with the passage from the G_1 to the S period may indicate a higher content or greater activity of the G_2 than of the G_1 inhibitor.

There is information in the literature on the existence of two inhibitors of cell division, which differ from each other substantially in molecular weight [3]. Further investigations are evidently required in order to solve this problem.

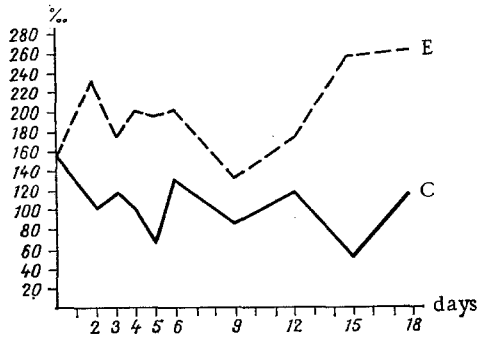

Fig. 3. RI in Ehrlich's ascites tumor cells during 18 h after injection of extract. Abscissa, time (in days); ordinate, RI (in promille).

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TRANSPLACENTAL ACTION OF BENZO(a)PYRENE AND PYRENE

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The transplacental and direct action of benzo(a)pyrene (BP) on mice of strains A and C57BL and of their progeny was studied. BP was found to represent a carcinogenic risk for the progeny. The greatest carcinogenic effect in progeny of strain A mice was exhibited by BP in a dose of 6 mg: The frequency of development of lung tumors was 76.8% compared with 12.3% in the control ($P < 0.001$). Liver tumors were found in the progeny of the C57BL mice (chiefly in males). Their frequency after a dose of 12 mg of BP was 31.6% in males and 9.1% in females, compared with only 1.2% in males in the control. No tumors of the liver were observed in females in the control. Pyrene, the noncarcinogenic analog of BP, had no carcinogenic effect.

KEY WORDS: *transplacental action; benzo(a)pyrene; carcinogen.*

Among carcinogenic agents polluting the external environment benzo(a)pyrene (BP) is particularly important. It can serve as an indicator of environmental pollution with polycyclic aromatic hydrocarbons (PAH) [2].

The carcinogenic action of BP on man has been proved by numerous observations on occupational cancer. The wide distribution of this substance in the environment presents the risk of its entering the human body and, in particular, the pregnant woman.

Nowadays the possibility of a transplacental carcinogenic action has been demonstrated for several substances, including BP [1, 3, 5-7]. The writers previously investigated the transplacental action of BP using the method of organ culture [4]. This paper gives the results of a study of the action of BP and pyrene on pregnant mice of strains A (with high risk of cancer) and C57BL (low risk) and their progeny.

EXPERIMENTAL METHOD

On the 18th to 19th day of pregnancy BP in 0.2 ml sunflower oil was injected subcutaneously into the experimental animals as a single dose of 4 and 6 mg or as two separate injections each of 6 mg, i.e., 12 mg per mouse. The action of pyrene was investigated in the maximal dose of 12 mg in strain A mice only. The young mice were weaned at the age of 4-5 weeks. The experimental mothers and their progeny were investigated 1 year later. Intact mice of both strains and mice of strain A receiving subcutaneous injections of pure sunflower oil in a dose of 0.4 ml (two injections, each of 0.2 ml), served as the control. The index of multiplicity was calculated for adenomas of the lungs and the numerical results were subjected to statistical analysis by Student's method.

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