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EFFECT OF URETHANE ON PROLIFERATIVE ACTIVITY OF THE EPITHELIUM OF EMBRYONIC MOUSE LUNG ORGAN CULTURES

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Previous investigations have shown that under normal conditions, despite the similarity in their morphogenesis, the survival rate of organ cultures of normal embryonic tissues and, in particular, of the lungs, varies considerably depending on the species, strain, and age of the donor animals [3, 11]. Under the influence of transplacental exposure to carcinogens the survival rate of explanted tissues is considerably increased (the growth-stimulating effect), and specific morphological changes develop in the tissue, culminating in tumor formation [2-5, 7-11]. Interlinear differences are observed in the survival rate and sensitivity to carcinogens of embryonic tissues from mice with high and low predisposition to cancer [3-5, 8, 9].

To study the nature of the growth-stimulating effect of carcinogens comparative investigations were undertaken of the proliferative activity of organ cultures of normal embryonic tissues and of tissues exposed to the action of carcinogens. This paper gives the results of a study of the proliferative activity of the epithelium of embryonic lung organ cultures from two lines of mice: one genetically resistant (C57BL) and one predisposed (A) to the development of lung tumors, under normal conditions and after transplacental exposure to urethane, a carcinogen with affinity for the lungs.

EXPERIMENTAL METHOD

Experiments were carried out on organ cultures of the lungs of 21-day embryos of intact and experimental mice of strains A and C57BL. Urethane was injected subcutaneously, in 10% physiological saline, into the pregnant females once or three times in a dose of 1 mg/g body weight per injection. The effect of a single dose of urethane (1 mg/g body weight) on the 18th and 20th days of pregnancy, i.e., 3 days or 1 day before explantation of the embryonic lungs into culture, was studied in mice of the A strain. The effect of three doses of urethane (3 mg/g body weight) on the 18th, 19th, and 20th days of pregnancy was studied on explants of embryonic lungs of mice of both strains. On the 21st day of pregnancy the intact and experimental females were killed and the lungs of the embryos were explanted into organ culture by the method described previously [2], and studied on the 1st, 7th, 15th, and 21st days in culture. Proliferative activity of the epithelium was judged by the fraction of DNA-synthesizing cells in the explants by an autoradiographic method [6]. ^{3}H-Thymidine was added to the nutrient medium of the cultures 24 h before fixation of the explants, in a concentra-

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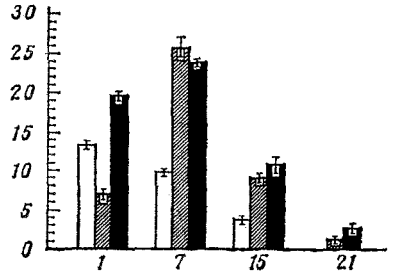

Fig. 1

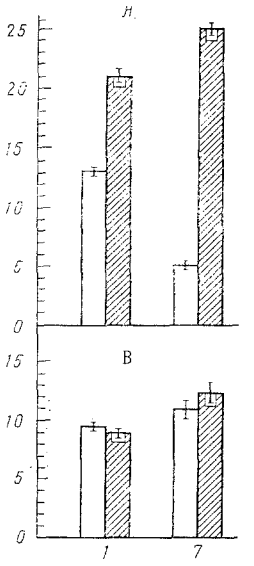

Fig. 2

Fig. 1. LI of epithelium of embryonic lung organ cultures of A mice under normal conditions and after transplacental exposure to a single dose of urethane (1 mg/g body weight of donor). Abscissa, LI (in %); ordinate, duration of culture (in days). Unshaded columns – normal (intact cultures of mouse embryonic lungs), obliquely shaded columns – transplacental exposure to urethane 1 day before explantation of mouse embryonic lungs into culture; black columns – transplacental exposure to urethane for 3 days before explantation of embryonic lungs into culture.

Fig. 2. LI of epithelium of embryonic lung cultures of A and C57BL mice under normal conditions and after transplacental exposure to three doses of urethane (3 mg/g body weight of donor). A) Organ cultures of embryonic lungs of A mice; B) the same, of C57BL mice. Shaded columns – experimental cultures of embryonic lungs of mice exposed to transplacental action of urethane. Remainder of legend as in Fig. 1.

tion of 1 μCi/ml. Dewaxed serial sections 3 μ thick were coated with emulsion and exposed for 4 weeks at 4°C, developed, and stained with hematoxylin. In all series of experiments eight to 10 explants were studied at each time of culture: The labeling index (LI) was determined by counting at least 1000 epithelial cells in each explant. The results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

An autoradiographic study of the course of DNA synthesis in embryonic lung organ cultures from intact A mice showed that during culture there is a gradual decline in proliferative activity of the epithelium. LI, determined 1 day after explantation of the lungs, was 13.3%, falling to 9.9% after 7 days and 3.9% after 15 days; after 21 days no labeled cells were present in the cultures (Fig. 1).

Under the influence of a single transplacental exposure to urethane (1 mg/1 g body weight of donor) the dynamics of proliferation of the epithelium in the explants was significantly altered. It depended on how long elapsed between administration of urethane to the pregnant mouse and explantation of the embryonic lungs into culture. It will be clear from Fig. 1 that LI in the epithelium of the lungs of the experimental embryos explanted 1 day after exposure to urethane (group 1), and studied after 1 day in culture with ^{3}H-thymidine, fell by almost half compared with normal. In the lungs of the experimental embryos exposed to urethane for 3 days before explantation (group 2), and investigated at the same time, LI was higher than normal. By this time inhibition of DNA synthesis in the epithelial cells of the mouse embryonic lungs, due to a single transplacental exposure to urethane, was replaced by stimulation compared with normal. Since incubation of embryonic lungs of normal (intact) and experimental mice with ^{3}H-thymidine began immediately after their explantation into culture, it will be evident that the results reflect changes in proliferative activity of the epithelium developing in the lungs of the experimental embryos in vivo.

LI in the experimental explants after 7 days in culture was higher than its initial level, especially in group 1. As a result, LI in the two groups of experimental explants became approximately equal. Under these circumstances LI in the experimental group was more than 2.6 times higher than in the corresponding control. During further culture LI in the experimental explants fell gradually, but still remained above the control level. Labeled cells after 21 days in culture were found only in the experimental explants (Fig. 1).

Under the influence of a single transplacental exposure to urethane, brief inhibition of proliferative activity of the epithelium of the embryonic lungs of A mice thus took place, and was replaced by a longer period of stimulation compared with normal. Significant changes in LI in the epithelium of the control and experimental explants were detected as early as after 1 day in culture, and they reached a maximum after 7 days. These times accordingly were chosen for a comparative study of the effect of transplacental exposure to three doses of urethane (3 mg/g body weight of donor) on the proliferative activity of the epithelium of the embryonic lungs of A and C57BL mice.

The comparative study of LI of the epithelium in intact explants of embryonic lungs of these strains of mice showed that when conditions were adequate, LI in the A mice 1 day after explantation was not significantly higher than in the C57BL mice (Fig. 2). After 7 days LI in explants of the lungs of A mice was reduced by 60%, whereas LI in explants from C57BL mice under the same conditions was practically unchanged compared with its initial level. As a result LI in explants of embryonic lungs of A mice at this time was only half its value in C57BL mice (Fig. 2).

Judging from the initial value of LI (1st day) the proliferative activity of the epithelium of embryonic lungs of mice of these strains under normal conditions in vivo evidently did not differ significantly. However, under extremal conditions such as during culture in vitro, interlinear differences in this index were clearly manifested: LI was significantly reduced in explants from line A and remained stable in the resistant line C57BL. This may evidently explain the higher rate of survival of the embryonic lung tissue of C57BL mice than of A mice during long-term organotypical culture, as has been observed previously [3, 4].

Under the influence of transplacental exposure to three doses of urethane LI in explants of embryonic lungs of A mice, just as after exposure to a single dose, was significantly higher than normal. LI of the epithelium 1 day after explantation was twice as high as normal, and after 7 days, it was five times higher than normal (see Fig. 2A). Transplacental exposure to embryonic lungs of C57BL mice to the same dose of urethane, but repeated three times, had practically no effect on LI of the epithelium during organotypical culture (Fig. 2B).

Transplacental exposure to urethane thus led to a considerable increase in proliferative activity of the epithelium only in organ cultures of embryonic lung tissue of mice of the sensitive line A. In experimental explants exposed to the action of the above doses of urethane, LI of the epithelium was about equal (see Figs. 1 and 2A). However, comparison with the corresponding control revealed dose dependence. For instance, after a single dose of urethane (1 mg/g body weight) LI of the epithelium after 7 days in culture was 24% compared with 9.9% in the control, but after three doses (3 mg/g body weight) it was 24.8% compared with 5.2% in the control.

As already stated, transplacental exposure to various carcinogens including urethane not only induces pretumor changes and tumors, but also has a growth-stimulating action on organ cultures of embryonic tissues [8, 10, 11]. This was manifested as the faster adaptation of the explanted tissue to conditions of growth in vitro, a decrease in the frequency and intensity of nutritional disturbances in the experimental cultures, and a decrease in their ability to grow for a longer period in vitro compared with intact (normal) cultures. On the basis of the results of this investigation the growth-stimulating effect of urethane and, evidently, of other carcinogens can be explained by an increase in proliferative activity of the experimental cultures. Stimulation of growth in vitro may perhaps also be connected with a reduction in the growth demands of the experimental cultures in the course of tumor evolution. Facts such as these are known for cell cultures of fibroblasts, and recently it has also been demonstrated for epithelial cell and organ cultures exposed to the action of carcinogens in vivo or in vitro [1, 12, 13].

In conclusion, it is important to draw attention, first, to the stability of proliferative activity of the epithelium of the embryonic lungs of mice of strain C57BL, with low predisposition for cancer, when cultured in vitro under normal conditions and during exposure to a carcinogen, by contrast with strain A, with high predisposition for cancer; second, to correlation between the growth-stimulating effect of a pneumotropic carcinogen and a genetic predisposition of the biological object for the development of lung tumors. By virtue of this correlation, the "growth-stimulating effect" can be used as a reliable criterion for primary screening of sub-

stances for carcinogenicity by the use of organ cultures of embryonic target tissues [11]. The use of this criterion is evidently promising also for the detection and quantitative estimation of species, linear, and organ predisposition to the development of tumors.

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EFFECT OF SUBLETHAL HYPERTHERMIA ON PROLIFERATION OF THE CORNEAL EPITHELIUM IN ALBINO RATS

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Processes of cell proliferation in eukaryotes in vitro take place over a wide range of above-zero temperatures [7, 11]. Data on the effect of high temperatures on cell division in vivo in homoiothermic animals are few and inconsistent in character [10, 12].

Nevertheless the study of this problem is of considerable practical importance in connection with the use of hyperthermia in the treatment of diseases accompanied by disturbances of cell division [1], and this was the motivation behind the present investigation.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 150-200 g. The animals, which were first adapted to the experimental conditions, were heated in a hot chamber at 42°C and with a relative air humidity of 60-65%. Exposure to a high temperature lasted 1.5 h until the rectal temperature was 41-41.5°C. The normal body temperature of the rats was restored 30-45 min after the end of hyperthermia. Considering the existence of a circadian rhythm of proliferation in the corneal epithelium in response to stressor stimulation [5, 8, 9], the animals were heated in the morning at 6-8 a.m., at midday between 11 a.m. and 1 p.m., and in the evening between 5 and 7 p.m. Mitotic activity was studied 2, 6, and 12 h after the end of exposure to heat. The number of animals in the experiment was 280. To assess the reproducibility of the results, all series of experiments were repeated twice. Total preparations were obtained, the mitotic index (MI) and level of patho-

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