

CHANGES IN THE SENSITIVITY OF MOUSE
EMBRYOS TO THE CARCINOGENIC ACTION
OF URETHANE AT DIFFERENT AGES

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A differential study of age sensitivity of line A mouse embryos to the carcinogenic action of urethane showed that the sensitivity of embryonic lung tissue does not change appreciably during the last 6 days before birth, i.e., during the entire fetal period.

Previous investigations revealed the high sensitivity of embryonic lung tissue of mice to the carcinogenic action of urethane in experiments both in vivo and in vitro [2, 3].

Later work in the authors' laboratory demonstrated the high sensitivity of embryonic tissues to the transplacental action of some carcinogenic hydrocarbons, nitroso-compounds, o-aminoazotoluene, and other substances in experiments in vitro [1, 7, 8]. In all these investigations, the transplacental action of the substance took place in the last third of pregnancy.

According to one hypothesis, if these agents are administered at this period, i.e., the fetal period, they have a carcinogenic effect, whereas in the first and second thirds of pregnancy they exhibit embryotoxic and teratogenic effects [11].

The young organism is known to be more sensitive to many factors, including carcinogenic agents. However, in a differential study of age sensitivity to the carcinogenic action of urethane in mice aged 1-30 days, animals aged 7-14 days were found to be most sensitive [4].

In experiments with a single brief exposure to the action of teratogenic agents, as a rule the most sensitive stages or critical periods of the anlagen can be distinguished, when administration of the agent is followed by the maximum number of disturbances in a particular system of organs [6, 16].

The problem of transplacental carcinogenesis has recently attracted the attention of many investigators [2, 9, 10, 12, 14, 15]. Individual reports have been published stating that administration of a carcinogenic agent in the later stages of pregnancy gives rise to the maximum number of tumors in the progeny [13]. However, no special investigations of the age sensitivity of the embryo to carcinogenic action have yet been undertaken.

The present investigation was carried out for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on line A mice with a high incidence of spontaneous carcinoma. Pregnant females received a single subcutaneous injection of urethane solution in a dose of 1 mg/g body weight.

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TABLE 1. Incidence of Pulmonary Adenomas in Progeny of Mice Receiving Urethane

Time of administration of urethane (days before birth)	No. of animals investigated	No. of animals with adenomas	Incidence of adenomas (in %)	Index of multiplicity (adenomas/mouse)
6	45	11	24.4	1.0
5	13	6	46.1	1.6
4	10	5	50.0	1.7
3	24	10	41.6	3.4
2-1	58	20	34.3	1.7

Urethane is readily, rapidly, and totally eliminated from the organism within 24 h. For this reason, the duration of its transplacental action on the fetus could be determined accurately. The investigation was concerned with the sensitivity of mouse embryonic lung tissue to the carcinogenic action of urethane. The anlagen of the mouse lung appear on the 12th day of intrauterine development. Exposure to the carcinogen was therefore timed to begin not before this stage. However, a viable progeny could be obtained only if urethane was administered not more than 6 days before birth, i.e., on the 15th day of pregnancy. If urethane was given earlier than this, either abortion or the birth of a stillborn fetus resulted, or the progeny were so weak they died or were eaten by the females during the 1st day.

The mouse progeny were sacrificed at the age of 5 months, before spontaneous adenomas of the lungs could arise. The animals' lungs were fixed in 10% formalin solution. The number of adenomas appearing on the surface was carefully counted, the frequency of onset of adenomas of the lungs was calculated in percent, and the index of multiplicity, i.e., the number of adenomas per mouse among animals with tumors was determined.

EXPERIMENTAL RESULTS

Altogether 150 mice from 57 females receiving urethane 1, 2, 3, 4, 5, and 6 days before parturition survived until the stated time. Since there was no difference in the incidence of adenomas in females and males, the combined results are shown (Table 1). The incidence of pulmonary adenomas varied from 24.4 to 50%.

The impression may be obtained from these results that the greatest carcinogenic effect was produced by administration of the agent 5, 4, and 3 days before birth. However, statistical analysis of the numerical data by the chi-square method showed that the difference in incidence of adenomas in the successive group of animals was not significant. It likewise was not significant when the figures for the 6th and 4th and also for the 6th and 2nd days of administration were compared. Moreover, if the groups of animals receiving urethane on the 5th, 4th, and 3rd days before parturition were combined (groups with the greatest incidence of adenomas), the difference between the combined and extreme groups (the 6th and 2nd days before parturition; the lowest incidence of adenomas) also was not significant.

The sensitivity of mouse embryonic lung tissue to the carcinogenic action of urethane thus shows no appreciable change during the last 6 days before birth, i.e., throughout the fetal period. This must be taken into account when the times of action on the embryo are being chosen in order to study the characteristics of transplacental carcinogenesis whether the experiments be carried out in vivo or in vitro.

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